Journal of Current Veterinary Research

ISSN: 2636-4026

Journal homepage: http://www.jcvr.journals.ekb.eg

Animal health & Zoonotic disease

Prevalence of Extended Spectrum B-Lactamase (ESBL) Producing *Escherichia coli* and Molecular Characterization of ESBL, Carbapenemases, and Blacmy2 Genes in Broilers and Humans at Menoufia Governorate, Egypt

Ahmed Bayoumi[1]; Sherif Zidan[1]; Moustafa A. Sakr[2]; Adel ElMashtouly[1*] and Ghada Hadad[1]

(1)Department of Animal Hygiene and Zoonoses, Faculty of Veterinary Medicine, University of Sadat City, Egypt.

(2)Department of Molecular Diagnostics and Therapeutics Genetics, Engineering and Biotechnology Research Institute (GEBRI), University of Sadat City, Egypt.

[*]Corresponding author: adel.mohamed@vet.usc.edu.eg *Received: 25/6/2023 Accepted: 30/7/2023*

ABSTRACT

Antibiotic resistance is a serious worldwide problem threatening human health and life. Poultry, especially broilers, represent a major source of ESBL-producing *E. coli* due to the extensive use of antibiotics during their production. This study investigated the prevalence of ESBL-EC in broilers, the surrounding environment, and humans, as well as the occurrence of ESBLs, carbapenemases, and bla_{CMY2} genes. 249 samples were included in that study: 130 broiler cloacal swabs, 54 environmental samples, and 65 human samples. All samples were grown on Macconkey broth, then cultured on EMB containing cefotaxime sodium. Suspected *E. coli* isolates were identified and confirmed biochemically by IMVIC tests. The disc diffusion method was used to determine the antibiotic susceptibility pattern of *E. coli* isolates grown on EMB with cefotaxime. PCR was performed for the amplification of ESBLs, carbapenemases, and bla_{CMY2} genes. Our results revealed that ESBL-EC was isolated from 180 (72.3%) of the 249 collected samples. The prevalence of ESBL-producing *E. coli* was as follows: 85.4%, 46.3%, and 67.7% in broiler cloacal swabs, environmental samples, and human samples, respectively. Among 188 *E. coli* isolates grown on EMB containing cefotaxime, 187 were resistant to CTX, 170 to CAZ, 166 to FEP, and 146 to FOX. PCR amplification showed that the predominant gene was bla_{TEM}, which was detected in 98% of the amplified isolates, followed by bla_{CTX-M} (88%). Bla_{KPC} wasn't detected in any isolates. Many gene combinations were detected, and the most common one was bla_{TEM} with bla_{CTX-M}, which was detected in 24 (48%) of the 50 isolates.

Keywords: Broiler**,** Carbapenemases, *E. coli*, ESBLs and Human**.**

INTRODUCTION

Antibiotics are prescribed mainly for the treatment of bacterial infections and prophylaxis of diseases (FAO/WHO/OIE, 2008). However, antibiotics use may be a main factor in developing and spreading of antibiotic resistance in normal bacterial flora and target pathogens. Consequently, antibiotic-resistant bacteria have an increasing surge, globally (EFSA, 2009). Zoonotic antimicrobial-resistant bacteria such as *E. coli* have many consequences, such as failure to treat human infections and prolongation of treatment periods more than usual. WHO identifies cephalosporins and fluoroquinolones as essential

antibiotics for the treatment of human infections. Additionally, improper abuse and/or misuse in the agricultural and animal production sectors should not compromise the effectiveness of critical antibiotics for use in treating humans (FAO/WHO/OIE, 2008). But among bacteria of zoonotic importance, the rate of resistance to therapeutically significant antibiotics including carbapenems, Extended spectrum-lactams, aminoglycosides, and fluoroquinolones has increased (Bitrus et al., 2019).

Poultry industry is a vast global food industry. Chickens dominate global farmed animal production, producing over 90 billion tons annually (FAO, 2017). The extensive use of antibiotics in broiler production enhances the evolution of bacteria resistant to antibiotics (Diarra & Malouin, 2014). In addition, poultry has been implicated as a possible source of ESBL-producing bacteria that can infect humans and cause intestinal colonization and serious illness when transmitted to humans via direct contact or ingestion of contaminated meat and meat products (Lazarus et al., 2015).

E. coli is regularly found in food, water, and soil due to faecal contamination or contamination during the slaughter of food animals. It is also frequently found in the gastrointestinal tracts of both humans and animals. Several *E. coli* strains cause colibacillosis in poultry, and some of which are responsible for severe diseases in humans like hemorrhagic colitis and hemolytic uremic syndrome (Ferens & Hovde, 2011). *E. coli* is present in both humans and animals as a part of intestinal commensal flora. *E. coli* and other bacteria found in the commensal flora can act as a reservoir for antibiotic resistance genes spreading them to other bacterial species, including those that can infect both people and animals. The consequences of antibiotic usage and changes in the prevalence of antimicrobial resistance in food-producing animals should be studied more thoroughly in these indicator bacteria rather than in food-borne pathogens (EFSA, 2008).

Extended-spectrum β-lactamases (ESBLs) are a group of enzymes carried on plasmid and provide resistance to cephalosporins, and inhibited by clavulanic acid (Bush & Jacoby, 2010). ESBLs are regarded as troublesome on a global scale and are becoming more common in various regions of the world (Ghafourian et al., 2015). A variety of Gram-negative bacteria have the intrinsic beta-lactamase enzyme AmpC on their chromosomes. With the exception of carbapenem and fourth-generation cephalosporins, they are effective against cefoxitin, penicillin, cephalosporins, cephamycin, and β-lactam/inhibitor combination (Carattoli, 2008). Carbapenemases are β-lactamases with a variety of hydrolytic capabilities. Penicillins, cephalosporins, monobactams, and carbapenems can all be destroyed by them. Bacteria that produce these β-lactamases can cause harmful infections in which the action of numerous β-lactams renders them useless (Queenan & Bush, 2007).

ESBL-EC colonization was found to be enhanced in both healthy humans and animals globally due to the haphazard use of antibiotics in both populations. It was proposed that animals, particularly poultry, might transmit bacterial genetic components carrying ESBL-encoding genes and resistant plasmids to humans (Nguyen et al., 2019).

In Egypt, a study made on poultry and poultry products concluded that all *E. coli* isolates had ESBL encoding genes. PCR revealed that bla_{TEM} was found in 100% of the *E. coli* isolates tested but bla_{SHV} was only found in 10% of them. All of the isolates were devoid of the blaCTX-M gene (Kamel et al., 2021). Furthermore, in another study

carried out on humans with infections of urinary tract, a total of 80 (59.7%) out of 134 uropathogenic *E. coli* (UPEC) investigated were reported to produce ESBLs. Sixty-two percent (62%) of ESBL producers were multi-drug resistance (MDR). They were all sensitive to meropenem. The Most prevalent genes were bla_{TEM}(60%) and bla_{CTX-M} (45%) (Hassuna et al., 2020).

Broiler faecal matter contain *E. coli* strains that can grow and integrate into a model of the human digestive system. Plasmids bearing ESBL genes can simultaneously spread from the *E. coli* poultry strain to strains of *E. coli* with human origin (Smet et al., 2011).

Sadly, Egypt lacks legislation regulating antibiotic use, with some antibiotics like tetracycline, quinolones, and beta-lactams still used for other purposes rather than therapeutic ones (WHO, 2014). Improper antimicrobial use in poultry leads to high evolvement of multi-resistant *Enterobacteriaceae* strains (Moawad et al., 2017).

Based on clinical microbiology techniques, many ESBL detection approaches have been proposed. These methods comprise preliminary screening for ESBL generation followed by confirmation testing. Disk diffusion methods are frequently used for screening, whereas phenotypic confirmatory methods primarily rely on the enhancement of the inhibitory zone caused by beta-lactamase inhibitors such as clavulanic acid and tazobactam (Salihu et al., 2020).

It is still advised to use carbapenems (imipenem, meropenem, ertapenem, doripenem) for the treatment of severe infections caused by ESBL-EC and *K. pneumoniae*. According to reports, over 98% of ESBL-EC respond to these medications (Perez et al., 2007).

So, this study inspected the prevalence of ESBL-EC in broilers, farms and retail poultry shop workers, and community humans, and identified ESBLs, carbapenemases, and CMY2 encoding genes in resistant *E. coli* strains.

MATERIAL AND METHODS

Study site and sample collection:

The study was made in El Shuhada district, Menofia governorate, for six months, from June 2022 to November 2022. Samples were collected from 3 broiler farms, 10 retail poultry shops, and 3 human clinical laboratories. A total No. of 249 samples were collected, including 130 broiler cloacal swabs (80 from broiler farms and 50 from retail poultry shops), environmental samples (54), and human samples (65). The age of broilers at the time of sample collection was between 30 and 35 days at the end of the fattening period. Different types of environmental samples were collected as follows: 12 litter samples, 12 feeding trough swabs, and 12 watering trough swabs from broiler farms, and 18 working stage swabs from retail poultry shops. Collected human samples were divided into hand swabs (18) obtained from broiler farms and retail poultry shop workers and stool swabs (47) obtained from human clinical laboratories. Swab samples were collected using sterile cotton swabs, inserted into saline tubes, and transferred to the laboratory for examination. Litter samples were collected by taking suitable amounts of litter from different sites inside the broiler house, preserved in a cooling ice box, and transferred immediately to the laboratory for examination.

Isolation and identification of ESBL producing E. coli:

Firstly, all samples were grown on MacConkey broth (Oxoid, UK) for

enrichment. Yellow colouration and gas formation in macConkey broth tubes indicate the Presence of coliforms. Then, a loopful from yellow MacConkey broth tubes were streaked onto eosin methylene blue agar (EMB) (TM MEDIA, India) containing 2 mg/l cefotaxime sodium (MERCK, Germany) and incubated at 37°c for 18:24 hrs. Suspected *E. coli* colonies were confirmed biochemically using indole, methyl red, vogues Proskauer, citrate utilization, and triple sugar iron tests. Typical *E. coli* colonies were subcultured on glycerol broth 20% and stored at -20°c for subsequent study.

Antimicrobial susceptibility testing:

Kirby-Bauer disk diffusion method was used for determination of antimicrobial susceptibility pattern of potential ESBLs producing *E. coli* isolates using several antibiotic discs including cefotaxime (CTX, 30 µg), ceftazidime (CAZ, 30 µg), cefepime (FEP, 30 µg), cefoxitin (FOX, 30 µg), meropenem (MEM, 10 µg), and imipenem (IPM, 10 µg) (Bioanalyse, Turkey).The test was carried out according to EUCAST Disk Diffusion Method for Antimicrobial Susceptibility Testing. The measured zone diameters were recorded, and compared to guideline zone diameters recommended by CLSI.

Phenotypic confirmation of ESBL producers:

ESBL production was confirmed by a combination disc test (CDT), recommended by CLSI. Cefotaxime and ceftazidime with and without clavulanic acid discs were applied on inoculated MHA surface with a 20 mm distance between the discs and then plates incubated at 37ºc for 18 hrs. ESBL-positive isolates were identified by a zone diameter increase of 5mm or more for either antimicrobial agents combined with clavulanic acid in comparison to zone diameter when tested alone (CLSI, 2022).

Molecular detection of ESBLs genes, carbapenemases genes and bla$_{CMY2}$ gene:

A No. of 5o resistant isolates were selected for detection of genes responsible for resistance. G-spin Total DNA Extraction Kit (iNtRON Biotechnology, Korea) was used for DNA extraction and the protocol was carried out according to the kit manual. PCR amplification was performed by certain oligonucleotide primers to detect resistance-encoding genes; TEM, SHV, CTX-M, OXA48, NDM, KPC, and CMY2 (Table 1). Amplification of target genes was done using Easy Taq DNA polymerase (TRANSGEN biotech, China) where each isolate was subjected to three PCR reactions as shown in (Table 2). Agarose gel electrophoresis was used to determine the size of amplified genes using a 100 bp plus DNA ladder (TRANSGEN biotech, China).

Table 1: Oligonucleotide primers used for PCR amplification of β-lactamase genes.

Gene	Sequence 5`-3`	Product size, pb	Reference	Company
bla$_{TEM}$	TCGCCGCATACACTATTCTCAGAATGA	445	**Monstein et al. (2007)**	Metabion internatiol AG (Germany)
	ACGCTCACCGGCTCCAGATTTAT			
bla$_{SHV}$	ATGCGTTATATTCGCCTGTG	747		
	TGCTTTGTTATTCGGGCCAA			

bla$_{CTX-M}$	ATGTGCAGYACCAGTAARGTKATGGC	593	
	TGGGTRAARTARGTSACCAGAAYCAGCGG		
bla$_{CMY2}$	AGCGATCCGGTCACGAAATA	695	**Kim et al. (2009)**
	CCCGTTTTATGCACCCATGA		
bla$_{OXA48}$	GCGTGGTTAAGGATGAACAC	438	**Poirel et al. (2011)**
	CATCAAGTTCAACCCAACCG		
bla$_{NDM}$	GGTTTGGCGATCTGGTTTTC	621	
	CGGAATGGCTCATCACGATC		
bla$_{KPC}$	CGTCTAGTTCTGCTGTCTTG	798	
	CTTGTCATCCTTGTTAGGCG		

Table 2: Amplification conditions of each PCR reaction:

	Target resistance genes		
	TEM, CTX-M, SHV[(1)]	**OXA48, NDM, KPC[(2)]**	**CMY2[(1)]**
Final volume	25 µl	25 µl	25 µl
Initial denaturation	95°C (15 min)	94°C(10 min)	94°C (5 min)
Denaturation	94°C (30 s)	94°C(30 s)	94°C (1 s)
Annealing	60°C (30 s)	52°C(40 s)	61°C (1 s)
Extension	72°C (2 min)	72°C(50 s)	72°C (1 min)
Final extension	72°C (10 min)	72°C (5 min)	72°C (5 min)

(1) 30 cycles of denaturation, annealing, and extension; **(2)** 36 cycles of denaturation, annealing, and extension

RESULTS

ESBL-EC was determined in 180 (72.3%) of the 249 collected samples. The prevalence of ESBL-EC obtained from broiler cloacal swabs was 85.4%. Regarding farms, the within-farm prevalence in cloacal swabs was; 96% for farm 1, 80% for farm 2 and 90% for farm 3 (Table3).

Table 3: Prevalence of ESBL-EC in broiler cloacal swabs collected from both farms and retail poultry shops.

	No. of examined samples	**Positive ESBL-EC**	
		No.	%

Farm 1	25	24	96
Farm 2	25	20	80
Farm 3	30	27	90
Retail poultry shops	50	40	80
Total	130	111	85.4

Moreover, the prevalence of ESBL-EC in different environmental samples was 46.3%which is described as follows; 58.3%, 50%, 41.7, and 38.9% in litter, feeding trough swabs, watering trough swabs and working stage swabs, respectively (Table 4).

Table 4: Prevalence of ESBL-EC in different environmental samples.

	No. of examined samples	**Positive ESBL-EC**	
		No.	%
Litter	12	7	58.3
Feeding trough swabs	12	6	50
Watering trough swabs	12	5	41.7
Working stage swabs	18	7	38.9
Total	54	25	46.3

With reference to human samples, ESBL-EC was isolated from 44 (67.6%) of 65 collected samples. Two forms of human samples were collected; hand swabs and stool swabs having a prevalence of 38.9% and 78.7%, respectively (Table5).

Table 5: Prevalence of ESBL-EC in human swab samples.

	No. of examined samples	**Positive ESBL-EC**	
		No.	%
Hand swabs	18	7	38.9
Stool swabs	47	37	78.7
Total	65	44	67.7

Among 188 *E. coli* isolates growing on EMB containing cefotaxime, all *E. coli* isolates from different samples had a more or less similar resistance trend, where 187 isolates were resistant to CTX, 170 to CAZ, 166 to FEP, 146 to FOX, 1 to MEM, and 1 to IPM (Table6). The resistant isolate to meropenem and imipenem was obtained from human stool sample.

Table 6: Antimicrobial resistance pattern of the 188 *E. coli* isolates growing on EMB containing cefotaxime.

Antimicrobial agent	**Cloacal swabs (n=115) No. (%)[1]**	**Environmental samples (n=27) No. (%)[1]**	**Human samples (n=46) No. (%)[1]**	**Total (n=188) No. (%)[1]**
Cefotaxime (CTX) 30 µg	115 (100%)	27 (100%)	45 (97.8%)	187 (99.5%)
Ceftazidime (CAZ) 30µg	101 (87.8%)	25 (92.6%)	44 (95.7%)	170 (90.4%)
Cefepime (FEP) 30 µg	105 (91.3%)	22 (81.5%)	39 (84.8%)	166

				(88.3%)
Cefoxitin (FOX) 30 µg	87 (75.7%)	20 (74%)	39 (84.8%)	146 (77.7%)
Meropenem (MEM) 10 µg	0 (0%)	0 (0%)	1 (2.2%)	1 (0.5%)
Imipenem (IPM) 10 µg	0 (0%)	0 (0%)	1 (2.2%)	1 (0.5%)

[1]Percentage of resistant isolates.

Combination disc test revealed that 180 *E. coli* isolates out of 188 potential ESBL producing strains, were ESBL producers, while the remaining 8 strains were non-ESBL producers. All the eight non-ESBL producing confirmed isolates were resistant to cefoxitin.

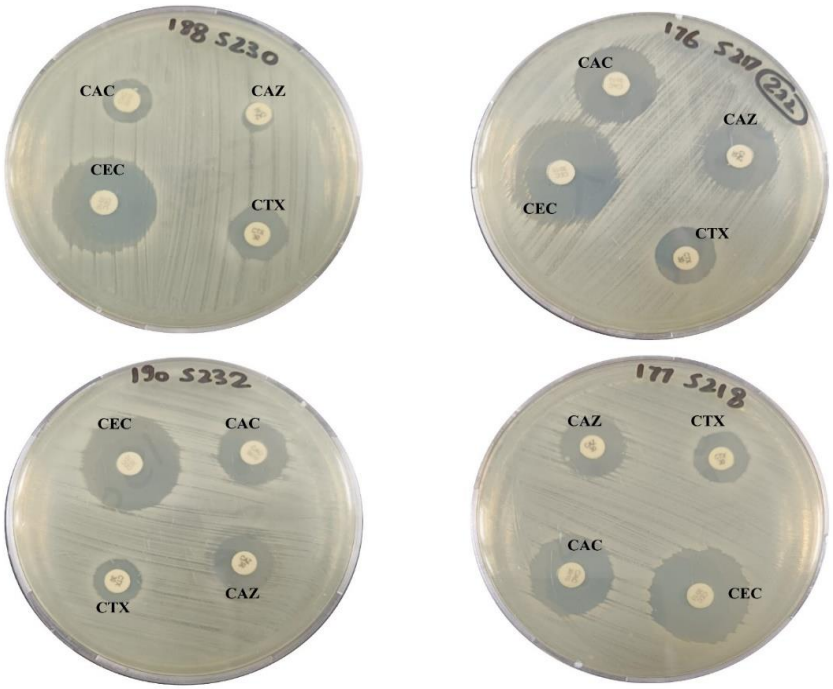

Figure 1: Combination disc test (CDT) for confirmation of ESBL-EC isolates: CTX: Cefotaxime, CAZ: Ceftazidime, CAC: Ceftazidime + Clavulanic acid, CEC: Cefotaxime + Clavulanic acid.

Among the 50 *E. coli* isolates subjected to PCR amplification, bla_{TEM} was detected in 49 isolates, bla_{CTX-M} in 44 isolates, bla_{SHV} in 8 isolates, bla_{CMY2} in 13 isolates, bla_{OXA48} in 10 isolates, and bla_{NDM} was detected only in one isolate obtained from human stool (Table7). No isolate was containing bla_{KPC} gene. Many gene combinations were detected and the most common one was bla_{TEM} with bla_{CTX-M} which occur in 24 (48%) of the 50 isolates (Figure 2).

Table 7: Occurrence of ESBL genes, carbapenemases genes and bla_{CMY2} gene in genotypically screened *E. coli* isolates.

Detected gene	**Cloacal swabs (n=26) No. (%)[1]**	**Environmental samples (n=7) No. (%)[1]**	**Human samples (n=17) No. (%)[1]**	**Total (n=50) No. (%)[1]**

TEM	26 (100%)	7 (100%)	16 (94.1%)	49 (98%)
CTX-M	24 (92.3%)	6 (85.7%)	14 (82.4%)	44 (88%)
SHV	5 (19.2%)	1 (14.3%)	2 (11.8%)	8 (16%)
CMY2	9 (34.6%)	3 (42.9%)	1 (5.9%)	13 (26%)
OXA48	5 (19.2%)	1 (14.3%)	4 (23.5%)	10 (20%)
NDM	0 (0%)	0 (0%)	1 (5.9%)	1 (2%)
KPC	0 (0%)	0 (0%)	0 (0%)	0 (0%)

[1]Percentage of positive isolates.

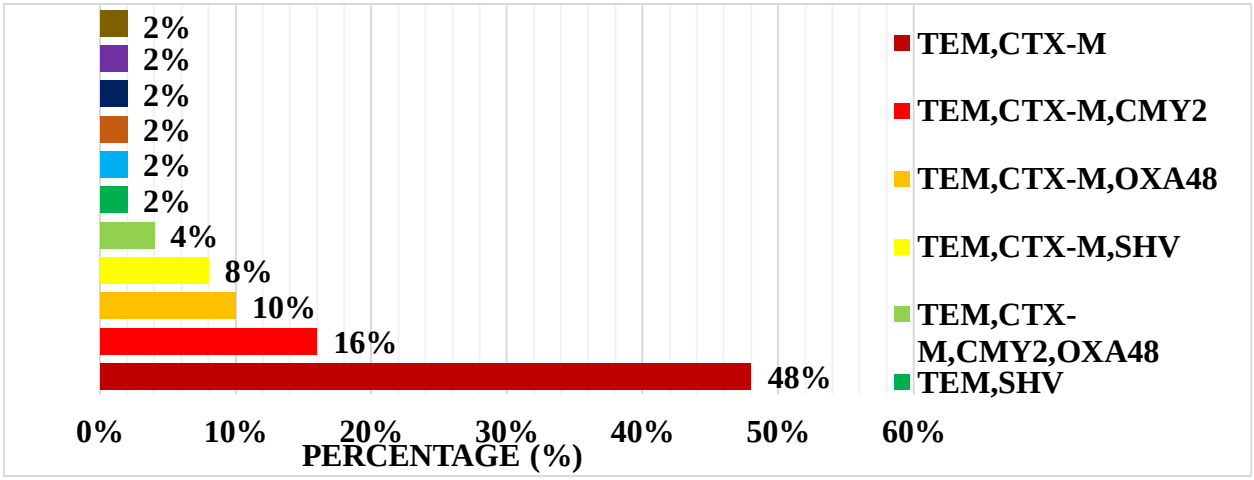

Figure 2: Occurrence of ESBL genes, carbapenemases genes and bla$_{CMY2}$ gene combinations in genotypically screened *E. coli* isolates.

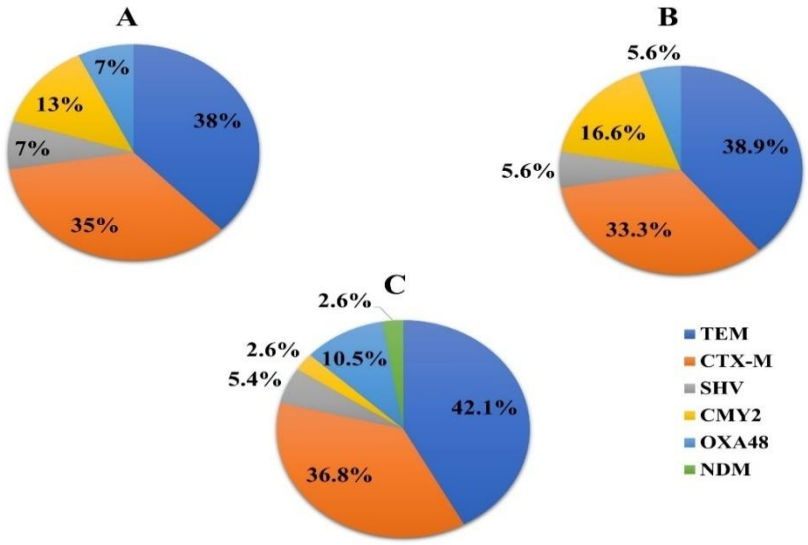

Figure 3: Prevalence of resistance genes in *E. coli* obtained from cloacal swabs and environmental samples in comparison to isolates from humans: A, cloacal swabs; B, environmental samples; C, humans.

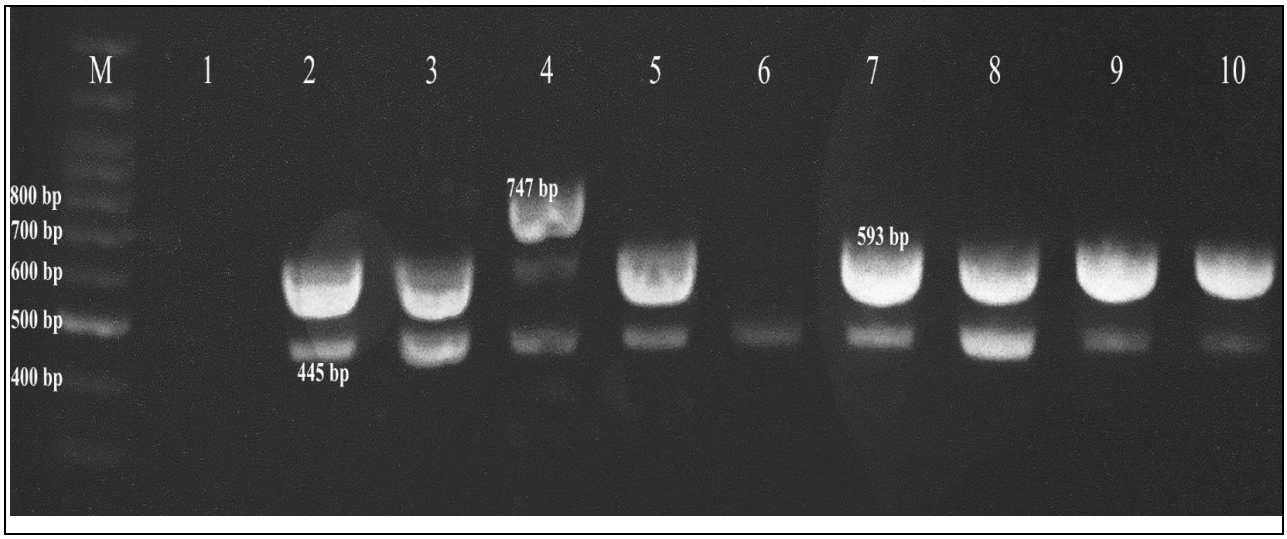

Figure 4: Agarose gel electrophoresis of multiplex PCR of ESBL genes in *E. coli* isolates; Lane M: 100bp plus DNA Marker (100:1500bp); Lane No. 4 is positive for bla_{TEM}, bla_{SHV} and bla_{CTX-M}; Lanes No. 2, 3, 5, 7, 8, 9 and 10 are positive for bla_{TEM} and bla_{CTX-M}; Lane No. 6 is positive for bla_{TEM}; Lane No. 1 is negative.

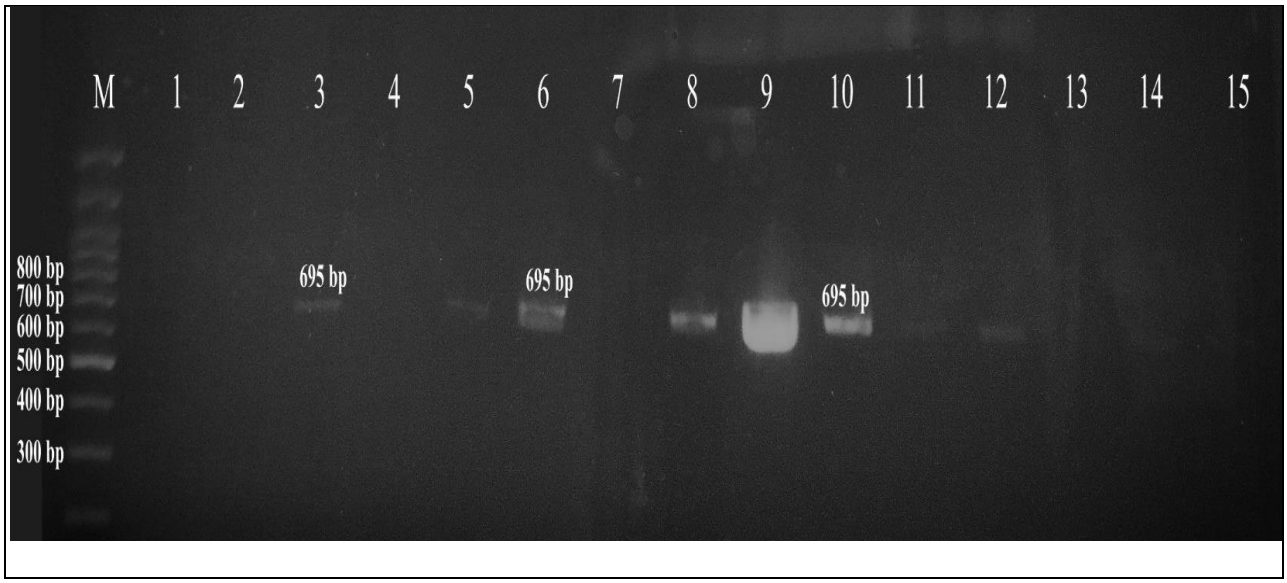

Figure 5: Agarose gel electrophoresis of uniplex PCR of CMY2 gene in *E. coli* isolates; Lane M: 100bp plus DNA Marker (100:1500bp); Lanes No. 3, 5, 6, 8, 9, 10, 11 and 12 are positive for bla_{CMY2}; Lanes No. 1, 2, 4, 7, 13, 14 and 15 are negative.

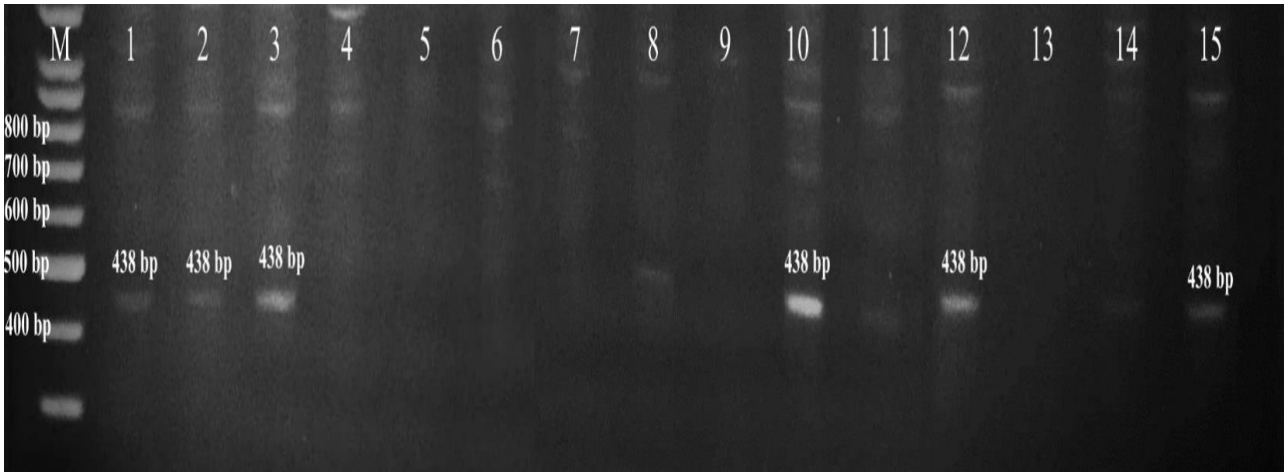

Figure 6: Agarose gel electrophoresis of multiplex PCR of carbapenemases gene (bla_{OXA48}, bla_{NDM}, bla_{KPC}) in *E. coli* isolates; Lane M: 100bp plus DNA Marker (100:1500bp); Lanes No. 1, 2, 3, 10, 12 and 15 are positive for bla_{OXA48}; Lanes No. 4, 5, 6, 7, 8, 9, 11, 13 and 14 are negative.

DISCUSSION

ESBL-EC in poultry poses a public health concern as it makes treatment difficult with commonly used β-lactam antibiotics, increasing the risk of death and preventing proper treatment. Although cephalosporins and penicillin/β-lactamase inhibitors may be effective, they are rarely used as first-choice therapy (Chong et al., 2018).

In the present study, ESBL-EC was detected in 180 (72.3%) of the 249 collected samples. This high prevalence may be attributed to many factors such as the overuse of antibiotics during the rearing period of broilers as co-selection under antibiotic pressure was discussed as a potential reason for the enrichment of ESBL/AmpC-producing *E. coli* on broiler fattening farms (Costa et al., 2009; Dierikx et al., 2013; Smet et al., 2010). Also, the lack of hygienic practices and biosecurity measures inside the investigated farms as well as the persistence of resistant bacteria inside poultry houses due to inadequate cleaning and disinfection which also was reported by Hiroi et al. (2012). Moreover, the misuse of antibiotics by humans and bad personal hygiene and many farm workers may work on different farms may be effective factors increasing the prevalence of ESBL-EC.

The prevalence of ESBL-EC in broiler cloacal swabs was 85.4% and that finding is consistent with other studies conducted in Egypt (82.2%) (Ahmed, 2015), Belgium (85%) (De Koster et al., 2021), and China (88.8%) (Li et al., 2016). In a study made in Netherlands, it was found that 85% of examined broiler farms had a prevalence of 80% or higher (Dierikx et al., 2013). Also, a prevalence of 96.4% of ESBL-EC was found in Dutch broiler farms (Huijbers et al., 2014). In a study conducted on broiler farms and hospitalized children in Ghana, the prevalence of ESBL-EC in broilers was 29% (Falgenhauer et al., 2019). Moreover, a study made in Luzon, Philippines reported that the prevalence of ESBL-EC in pooled broiler cloacal swabs was 60.26% (Gundran et al., 2019).This clearly demonstrates that the prevalence of ESBL-producing *E. coli* varies by country.

In environmental samples, the overall prevalence of ESBL-EC was 46.3%; 58.3% in litter samples, 50% in feeding trough swabs, 41.7% in watering trough swabs, and 38.9% in working stage swabs. In a previous study made at broiler chicken fattening farms, ESBL-EC was detected in all examined environmental samples with a prevalence of 54.2% in all environmental swab samples and 100% in litter samples

(Laube et al., 2013). The detection of ESBL-EC in the environment in this study suggests the potential spread of the pathogen in the environment, which may have a role in the transmission to farm workers, retail poultry shop workers, and the general public, as formerly reported (Bui et al., 2018; Huijbers et al., 2014).

With reference to humans, we found a high prevalence (78.7%) of ESBL-EC in stool swabs collected from human clinical laboratories, while in hand swabs from broiler farms and retail poultry shop workers, the prevalence was 38.9%, these findings show that there is a rise in the human faecal ESBL-EC carriage in the Egyptian community from 45.1% (Abdul Rahman & El-Sherif, 2011)to 78.7% in our study which is in harmony with other studies accomplished in Tanzania (76.3%) (Büdel et al., 2019), and Vietnam (75.1%) (Thi Quynh Nhi et al., 2018).

In antimicrobial susceptibility testing, 99.5% of *E. coli* isolates were resistant to cefotaxime followed by 90.4% to ceftazidime, 88.3% to cefepime, and 77.7% to cefoxitin. A study made in China on healthy broilers showed some similarity to our results where 98.6% of isolates were resistant to cefotaxime, 95.1% to cefepime, and in contrast to our results, 32.4% were resistant to ceftazidime (Li et al., 2016). Interestingly and in contrary to our findings, a study performed in Egypt reported resistance percents of 73.8%, 52.3%, and 35.4% to cefotaxime, ceftazidime, and cefepime, respectively (Badr et al., 2022). Regarding carbapenems, all 126 *E. coli* isolates from both cloacal and environmental samples were sensitive to meropenem and imipenem and this was similar to other previous studies in Egypt (Badr et al., 2022), China (Li et al., 2016), as well as Belgium and Netherlands (De Koster et al., 2021).

Among the 50 selected isolates for PCR amplification, blaTEM was the most prevalent gene in 98% of the examined isolates, followed by blaCTX-M in 88%. A study conducted in Egypt on poultry and poultry products agrees with our results, as blaTEM was found in 100% of *E. coli* isolates (Kamel et al., 2021). Also, in contrast to our study, Badr et al. (2022) reported a high incidence of bla_{CTX-M} followed by bla_{TEM} in *E. coli* obtained from chicken farms in Egypt. For humans, it was reported that blaTEM was the predominant gene in studies from the Philippines (Cruz & Hedreyda, 2017), and Saudi Arabia (Hemeg, 2018). Other studies made in Egypt (Badr et al., 2022), Germany (Dahms et al., 2015), Netherlands (Overdevest et al., 2011) mentioned that bla_{CTX-M} gene was the most common gene detected in *E. coli* strains isolated from humans.

Many gene combinations were detected in this study and the most frequent one was bla_{TEM}/bla_{CTX-M} in 24 (48%) from 50 *E. coli* isolates which agrees with previous studies detecting ESBL genes in poultry cloacal swabs (Gundran et al., 2019; Khoshbakht et al., 2016; Li et al., 2016). Alsoin Kenya, it was found that bla_{TEM}/bla_{CTX-M} (69.6%) was the commonly found gene combination in uropathogenic *E. coli* obtained from humans (Muriuki et al., 2022). Multiple ESBL resistance genes may give a chance for the maintenance of β-lactamase resistance although the diminished expression of one or two genes (Gundran et al., 2019).

Interestingly, one *E. coli* isolate was phenotypically confirmed as ESBL producer with no TEM, CTX-M, or SHV gene detected on PCR and that is in harmony with a study made in Germany that confirmed 6 *E. coli* isolates as ESBL producers without TEM, CTX-M, or SHV genes detected in these six isolates (Laube et al., 2013).

In recent study, the most commonly found carbapenemase gene in broiler cloacal swabs and their surrounding environment was bla_{OXA48} while bla_{NDM} and bla_{KPC} weren't detected in any isolate. These findings are consistent with a previous study performed in Malysia on *E. coli* strains harboring carbapenemases genes (Aklilu et al., 2020). Also, in a study made in France and Algeria for detection of carbapenemases genes in broiler feces, no bla_{OXA48}, bla_{NDM}, or bla_{KPC} was detected (Chabou et al., 2018). Regarding humans, bla_{OXA48} was the predominant carbapenemase encoding gene which agrees with a preceding study made in Turkey (Kizilates et al., 2021). Additionally, in contrast to our findings, it was reported that NDM gene was the most common carbapenemase gene in Iran (Hajihasani et al., 2022), and Shanghai (Pan et al., 2019). Co-occurrence of OXA48 gene with ESBL genes was detected especially TEM (9 isolates) and CTX-M (7 isolates) which is in harmony with previous studies stated that OXA-48 was found in ESBL producing strains (Mushi et al., 2014; Potron et al., 2013). It is worth mentioning that eight isolates among ten OXA48 harbouring isolates were sensitive to imipenem and meropenem. This was also reported in a previous study made in Tanzania (Mushi et al., 2014), and in a study carried out by Potron et al. (2013) where 65% of OXA48 harbouring isolates were susceptible to meropenem and imipenem, consequently complicating the detection of OXA-48producing isolates in laboratories.

The bla_{CMY2} gene was detected mainly in broiler cloacal swabs and environmental samples (12 isolate), while in humans was detected only in one isolate. A previous study made on Dutch broiler farms and farm workers agrees with our results, where bla_{CMY2} gene was found in 12, and 5 broiler and farmer isolates, respectively (Dierikx et al., 2013).

CONCLUSIONS

From that study, we concluded that there is a high prevalence of ESBL-EC in broiler farms, and the surrounding environment, as well as in humans either in contact with broilers or other community humans. Seriously, these resistant bacteria can be transmitted zoonotically from broilers and their environment to humans which endangers human health by making the treatment of human infections more difficult with routine antibiotics.

DECLARATION

The authors have no competing interests.

ETHICAL APPROVAL

The study was approved by the Institutional Animal Care and Use Committee (IACUC), Faculty of Veterinary Medicine, University of Sadat City (VUSC-045-1-22).

REFERENCES

Abdul Rahman, E. M., & El-Sherif, R. H. (2011). High rates of intestinal colonization with extended-spectrum lactamase-producing Enterobacteriaceae among healthy individuals. *Journal of Investigative Medicine*, *59*(8), 1284-1286.

Ahmed, M. F. (2015). PREVALENCE AND OCCURRENCE OF ESBL ESCHERICHIA COLI IN EGYPTIAN BROILER FARMS. XVII INTERNATIONAL CONGRESS ON ANIMAL HYGIENE 2015,

Aklilu, E., Harun, A., Singh, K. K. B., Ibrahim, S., & Kamaruzzaman, N. F. (2020). Phylogenetically Diverse Escherichia Coli Strains From Chicken Co-Harbour Multiple Carbapenemase Encoding Genes (blaNDM-blaOXA-blaIMP).

Badr, H., Reda, R. M., Hagag, N. M., Kamel, E., Elnomrosy, S. M., Mansour, A. I., Shahein, M. A., Ali,

S. F., & Ali, H. R. (2022). Multidrug-resistant and genetic characterization of extended-spectrum beta-lactamase-producing E. coli recovered from chickens and humans in Egypt. *Animals*, *12*(3), 346.

Bitrus, A. A., Mshelia, P. A., Kwoji, I. D., Goni, M. D., & Jajere, S. M. (2019). Extended-spectrum beta-lactamase and ampicillin Class C beta-lactamase-producing Escherichia coli from food animals: A review. *Int J One Health*, *5*, 65-75.

Büdel, T., Kuenzli, E., Clément, M., Bernasconi, O. J., Fehr, J., Mohammed, A. H., Hassan, N. K., Zinsstag, J., Hatz, C., & Endimiani, A. (2019). Polyclonal gut colonization with extended-spectrum cephalosporin-and/or colistin-resistant Enterobacteriaceae: a normal status for hotel employees on the island of Zanzibar, Tanzania. *Journal of antimicrobial chemotherapy*, *74*(10), 2880-2890.

Bui, T. K. N., Bui, T. M. H., Ueda, S., Le, D. T., Yamamoto, Y., & Hirai, I. (2018). Potential transmission opportunity of CTX-M-producing Escherichia coli on a large-scale chicken farm in Vietnam. *Journal of Global Antimicrobial Resistance*, *13*, 1-6.

Bush, K., & Jacoby, G. A. (2010). Updated functional classification of β-lactamases. *Antimicrobial agents and chemotherapy*, *54*(3), 969-976.

Carattoli, A. (2008). Animal reservoirs for extended spectrum β-lactamase producers. *Clinical Microbiology and infection*, *14*, 117-123.

Chabou, S., Leulmi, H., Davoust, B., Aouadi, A., & Rolain, J.-M. (2018). Prevalence of extended-spectrum β-lactamase-and carbapenemase-encoding genes in poultry faeces from Algeria and Marseille, France. *Journal of Global Antimicrobial Resistance*, *13*, 28-32.

Chong, Y., Shimoda, S., & Shimono, N. (2018). Current epidemiology, genetic evolution and clinical impact of extended-spectrum β-lactamase-producing Escherichia coli and Klebsiella pneumoniae. *Infection, Genetics and Evolution*, *61*, 185-188.

CLSI. (2022). Performance Standards for Antimicrobial Susceptibility Testing. Clinical and Laboratory Standards Institute 2022. In: CLSI Wayne, PA, USA.

Costa, D., Vinué, L., Poeta, P., Coelho, A. C., Matos, M., Sáenz, Y., Somalo, S., Zarazaga, M., Rodrigues, J., & Torres, C. (2009). Prevalence of extended-spectrum beta-lactamase-producing Escherichia coli isolates in faecal samples of broilers. *Veterinary Microbiology*, *138*(3-4), 339-344.

Cruz, M. C., & Hedreyda, C. T. (2017). Detection of plasmid-borne β-lactamase genes in extended-spectrum β-lactamase (ESBL) and non-ESBL-producing Escherichia coli clinical isolates. *Philippine Journal of Science*, *146*(2), 167-175.

Dahms, C., Hübner, N.-O., Kossow, A., Mellmann, A., Dittmann, K., & Kramer, A. (2015). Occurrence of ESBL-producing Escherichia coli in livestock and farm workers in Mecklenburg-Western Pomerania, Germany. *PloS one*, *10*(11), e0143326.

De Koster, S., Ringenier, M., Lammens, C., Stegeman, A., Tobias, T., Velkers, F., Vernooij, H., Kluytmans-van den Bergh, M., Kluytmans, J., & Dewulf, J. (2021). ESBL-producing, carbapenem-and ciprofloxacin-resistant Escherichia coli in Belgian and Dutch broiler and pig farms: a

cross-sectional and cross-border study. *Antibiotics*, *10*(8), 945.

Diarra, M. S., & Malouin, F. (2014). Antibiotics in Canadian poultry productions and anticipated alternatives. *Frontiers in microbiology*, *5*, 282.

Dierikx, C., van der Goot, J., Fabri, T., van Essen-Zandbergen, A., Smith, H., & Mevius, D. (2013). Extended-spectrum-β-lactamase-and AmpC-β-lactamase-producing Escherichia coli in Dutch broilers and broiler farmers. *Journal of antimicrobial chemotherapy*, *68*(1), 60-67.

EFSA. (2008). Report from the Task Force on Zoonoses Data Collection including guidance for harmonized monitoring and reporting of antimicrobial resistance in commensal Escherichia coli and Enterococcus spp. from food animals. *EFSA Journal*, *6*(4), 141r.

EFSA. (2009). Joint Opinion on antimicrobial resistance (AMR) focused on zoonotic infections. *EFSA Journal*, *7*(11), 1372.

Falgenhauer, L., Imirzalioglu, C., Oppong, K., Akenten, C. W., Hogan, B., Krumkamp, R., Poppert, S., Levermann, V., Schwengers, O., & Sarpong, N. (2019). Detection and characterization of ESBL-producing Escherichia coli from humans and poultry in Ghana. *Frontiers in microbiology*, *9*, 3358.

FAO/WHO/OIE. (2008). WHO/OIE Expert Meeting on Critically Important Antimicrobials: report of the FAO/WHO/OIE Expert Meeting, FAO headquarters, Rome, 26–30 November 2007. *Rome: Food and Agriculture Organization of the United Nations*.

Ferens, W. A., & Hovde, C. J. (2011). Escherichia coli O157: H7: animal reservoir and sources of human infection. *Foodborne pathogens and disease*, *8*(4), 465-487.

Ghafourian, S., Sadeghifard, N., Soheili, S., & Sekawi, Z. (2015). Extended spectrum beta-lactamases: definition, classification and epidemiology. *Current issues in molecular biology*, *17*(1), 11-22.

Gundran, R. S., Cardenio, P. A., Villanueva, M. A., Sison, F. B., Benigno, C. C., Kreausukon, K., Pichpol, D., & Punyapornwithaya, V. (2019). Prevalence and distribution of bla CTX-M, bla SHV, bla TEM genes in extended-spectrum β-lactamase-producing E. coli isolates from broiler farms in the Philippines. *BMC veterinary research*, *15*, 1-8.

Hajihasani, A., Ebrahimi-Rad, M., Rasoulinasab, M., Aslani, M. M., & Shahcheraghi, F. (2022). Prevalence of O25b-ST131 Escherichia coli Clone: Fecal Carriage of Extended-Spectrum β-Lactamase and Carbapenemase—Producing Isolates in Healthy Adults in Tehran, Iran. *Microbial Drug Resistance*, *28*(2), 210-216.

Hassuna, N. A., Khairalla, A. S., Farahat, E. M., Hammad, A. M., & Abdel-Fattah, M. (2020). Molecular characterization of Extended-spectrum β lactamase-producing E. coli recovered from community-acquired urinary tract infections in Upper Egypt. *Scientific reports*, *10*(1), 1-8.

Hemeg, H. A. (2018). Molecular characterization of antibiotic resistant Escherichia coli isolates recovered from food samples and outpatient Clinics, KSA. *Saudi journal of biological sciences*, *25*(5), 928-931.

Hiroi, M., Matsui, S., Kubo, R., Iida, N., Noda, Y., Kanda, T., Sugiyama, K., Hara-Kudo, Y., & Ohashi, N. (2012). Factors for occurrence of extended-spectrum β-lactamase-producing Escherichia coli in broilers. *Journal of Veterinary Medical Science*, *74*(12), 1635-1637.

Huijbers, P. M., Graat, E. A., Haenen, A. P., van Santen, M. G., van Essen-Zandbergen, A., Mevius, D. J., van Duijkeren, E., & van Hoek, A. H. (2014). Extended-spectrum and AmpC β-lactamase-producing Escherichia coli in broilers and people living and/or working on broiler farms: prevalence, risk factors and molecular characteristics. *Journal of antimicrobial chemotherapy*, *69*(10), 2669-2675.

Kamel, N., Farghaly, E., Shawky, H., & Samir, A. (2021). Molecular characterisation of extended-spectrum β-lactamase-producing Escherichia coli and Salmonella isolated from poultry and poultry products in Egypt. *Bulgarian Journal of Veterinary Medicine*, *24*(1), 43-56.

Khoshbakht, R., Seifi, S., & Raeisi, M. (2016). Antibiotic susceptibility and high prevalence of extended spectrum beta-lactamase producing Escherichia coli in iranian broilers. *Revue de Medecine Veterinaire*, *167*(5-6), 133-137.

Kim, J., Jeon, S., Rhie, H., Lee, B., Park, M., Lee, H., Lee, J., & Kim, S. (2009). Rapid detection of extended spectrum β-lactamase (ESBL) for Enterobacteriaceae by use of a multiplex PCR-based method. *Infection and Chemotherapy*, *41*(3), 181-184.

Kizilates, F., Yakupogullari, Y., Berk, H., Oztoprak, N., & Otlu, B. (2021). Risk factors for fecal carriage of extended-spectrum beta-lactamase-producing and carbapenem-resistant Escherichia coli and Klebsiella pneumoniae strains among patients at hospital admission. *American Journal of Infection Control*, *49*(3), 333-339.

Laube, H., Friese, A., Von Salviati, C., Guerra, B., Käsbohrer, A., Kreienbrock, L., & Roesler, U. (2013). Longitudinal monitoring of extended-spectrum-beta-lactamase/AmpC-producing Escherichia coli at German broiler chicken fattening farms. *Applied and environmental microbiology*, *79*(16), 4815-4820.

Lazarus, B., Paterson, D. L., Mollinger, J. L., & Rogers, B. A. (2015). Do human extraintestinal Escherichia coli infections resistant to expanded-spectrum cephalosporins originate from food-producing animals? A systematic review. *Clinical Infectious Diseases*, *60*(3), 439-452.

Li, S., Zhao, M., Liu, J., Zhou, Y., & Miao, Z. (2016). Prevalence and antibiotic resistance profiles of extended-Spectrum β-lactamase–producing Escherichia coli isolated from healthy broilers in Shandong Province, China. *Journal of food protection*, *79*(7), 1169-1173.

Moawad, A. A., Hotzel, H., Awad, O., Tomaso, H., Neubauer, H., Hafez, H. M., & El-Adawy, H. (2017). Occurrence of Salmonella enterica and Escherichia coli in raw chicken and beef meat in northern Egypt and dissemination of their antibiotic resistance markers. *Gut pathogens*, *9*, 1-13.

Monstein, H. J., Östholm-Balkhed, Å., Nilsson, M., Nilsson, M., Dornbusch, K., & Nilsson, L. (2007).

Multiplex PCR amplification assay for the detection of blaSHV, blaTEM and blaCTX-M genes in Enterobacteriaceae. *Apmis*, *115*(12), 1400-1408.

Muriuki, C. W., Ogonda, L. A., Kyanya, C., Matano, D., Masakhwe, C., Odoyo, E., & Musila, L. (2022). Phenotypic and genotypic characteristics of uropathogenic Escherichia coli isolates from Kenya. *Microbial Drug Resistance*, *28*(1), 31-38.

Mushi, M. F., Mshana, S. E., Imirzalioglu, C., & Bwanga, F. (2014). Carbapenemase genes among multidrug resistant gram negative clinical isolates from a tertiary hospital in Mwanza, Tanzania. *BioMed research international*, *2014*.

Nguyen, V. T., Jamrozy, D., Matamoros, S., Carrique-Mas, J. J., Ho, H. M., Thai, Q. H., Nguyen, T. N. M., Wagenaar, J. A., Thwaites, G., & Parkhill, J. (2019). Limited contribution of non-intensive chicken farming to ESBL-producing Escherichia coli colonization in humans in Vietnam: an epidemiological and genomic analysis. *Journal of antimicrobial chemotherapy*, *74*(3), 561-570.

Overdevest, I., Willemsen, I., Rijnsburger, M., Eustace, A., Xu, L., Hawkey, P., Heck, M., Savelkoul, P., Vandenbroucke-Grauls, C., & van der Zwaluw, K. (2011). Extended-spectrum β-lactamase genes of Escherichia coli in chicken meat and humans, The Netherlands. *Emerging infectious diseases*, *17*(7), 1216.

Pan, F., Tian, D., Wang, B., Zhao, W., Qin, H., Zhang, T., & Zhang, H. (2019). Fecal carriage and molecular epidemiology of carbapenem-resistant Enterobacteriaceae from outpatient children in Shanghai. *BMC infectious diseases*, *19*(1), 1-6.

Perez, F., Endimiani, A., Hujer, K. M., & Bonomo, R. A. (2007). The continuing challenge of ESBLs. *Current opinion in pharmacology*, *7*(5), 459-469.

Poirel, L., Walsh, T. R., Cuvillier, V., & Nordmann, P. (2011). Multiplex PCR for detection of acquired carbapenemase genes. *Diagnostic microbiology and infectious disease*, *70*(1), 119-123.

Potron, A., Poirel, L., Rondinaud, E., & Nordmann, P. (2013). Intercontinental spread of OXA-48 beta-lactamase-producing Enterobacteriaceae over a 11-year period, 2001 to 2011. *Eurosurveillance*, *18*(31).

Queenan, A. M., & Bush, K. (2007). Carbapenemases: the versatile β-lactamases. *Clinical microbiology reviews*, *20*(3), 440-458.

Salihu, M., Yarima, A., & Atta, H. (2020). Methods for the Phenotypic Detection of Extended Spectrum Beta Lactamase (ESBL)-Producing Bacteria. *Nigerian Journal of Biotechnology*, *37*(2), 113-125.

Smet, A., Martel, A., Persoons, D., Dewulf, J., Heyndrickx, M., Herman, L., Haesebrouck, F., & Butaye, P. (2010). Broad-spectrum β-lactamases among Enterobacteriaceae of animal origin: molecular aspects, mobility and impact on public health. *FEMS microbiology reviews*, *34*(3), 295-316.

Smet, A., Rasschaert, G., Martel, A., Persoons, D., Dewulf, J., Butaye, P., Catry, B., Haesebrouck, F., Herman, L., & Heyndrickx, M. (2011). In situ ESBL conjugation from avian to human Escherichia coli during

cefotaxime administration. *Journal of applied microbiology, 110*(2), 541-549.

Thi Quynh Nhi, L., Thanh Tuyen, H., Duc Trung, P., Do Hoang Nhu, T., Duy, P. T., Hao, C. T., Thi Thanh Nhan, N., Vi, L. L., Thi Diem Tuyet, H., & Thi Thuy Tien, T. (2018). Excess body weight and age associated with the carriage of fluoroquinolone and third-generation cephalosporin resistance genes in commensal Escherichia coli from a cohort of urban Vietnamese children. *Journal of medical microbiology, 67*(10), 1457-1466.

WHO. (2014). Report on the consultative meeting on antimicrobial resistance for countries in the Eastern Mediterranean Region: from policies to action Sharm el Sheikh, Egypt, 12–14 November 2013.