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Prevalence of Multidrug Resistant Shiga Toxin-Producing *Escherichia coli* in Broiler Chicken

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ABSTRACT

Rising in virulent, multidrug-resistant (MDR), avian pathogenic *Escherichia coli* (APEC) is concerned a great hazard to poultry industry. In order to better understand the occurrence and antimicrobial resistance patterns of MDR *E. coli* isolates, as well as their virulence gene profile and related resistance genes, the current study was examined 200 samples by standard methods for isolation and identification, the result showed presence of *E. coli* among commercial broiler farms in rate 55% which apparently health and freshly died from Sharkia governorate in Egypt during March to October 2022. Serological identification of *E. coli* isolates was O157:H7 (12.7%), O26:H11 (21.8%), O127:H6 (17.2%), O128:H- (10%), O111:H4 and O103:H2 (9%) each. Antibiogram pattern test indicated high resistant rate against oxytetracycline, ampicillin, doxycycline, streptomycin, colistin, ceftriaxone, kanamycin, gentamicin, sulfamethoxazole, and norofloxacin which were 74.54%, 70.90%, 67.27%, 65.45%, 54.54%, 45.45%, 29.09%, 18.18%, 12.72% and 9.09%, respectively of tested isolates by using disc diffusion method. Uniplex PCR examination for *integrase*, *bla*SHV, and *tet*B genes were detected in all examined MDR *E. coli* isolates 5/5 (100%), while *stx*1 gene detected in one isolate only 1/5 (20%) on plasmid. We concluded that the broilers in the study area frequently have multidrug-resistant *E. coli* which may pose a public health risk if it enters the food chain. The presence of bacteria should be strongly advised as harmful to health, and risk factors should be avoided.

1. INTRODUCTION

Escherichia coli belongs to family *Enterobacteriaceae* which is Gram-negative, rod-shaped and facultative anaerobic. *E. coli* is widely found in the gastrointestinal systems of birds (Runa et al., 2018), it serves to prevent the colonization of pathogenic bacteria in the intestine, aids in digestion, and can benefit their hosts by creating trace amounts of vitamins B12 and K2, it is a harmful to both humans and poultry to some level (Hudault et al., 2001). Although the majority of *E. coli* isolates are not harmful, their presence indicates that feces have contaminated food. Around 10-15% of serotypes of coliforms, which are pathogenic and opportunistic, are present in the colon. Along with poultry, it also affects immune-compromised hosts and causes a range of lesions (Barnes and Gross, 1997). There are six subgroups of *E. coli* sero-groups that can cause illness and food poisonings: enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enteroinvasive *E. coli* (EIEC), enterhemorrhagic *E. coli* (EHEC), enteroadherent *E. coli* (EAEC), and diffusely adherent *E. coli* (DAEC). The EHEC bacteria are a subset of the patient-isolated Shiga toxin-producing *E. coli* (STEC) strains (Holko et al., 2006), STEC was recently detected in broiler chickens (Mamun et al., 2016), the primary source of infections in humans is STEC-contaminated food (Hussein et al., 2022). STEC causes two serious clinical symptoms, hemorrhagic colitis (HC) and the possibly fatal hemolytic uremic syndrome (HUS) (Karch et al., 2005). Avian infectious with the ability to induce colibacillosis, an invasive systemic disease, *E. coli* serotypes are suspected to be responsible for a wide spectrum of poultry diseases (Younis et al., 2017). The disease known as avian colibacillosis, which affects poultry globally and results in significant financial losses, is brought on by APEC (Barnes et al., 2008). The use of antibiotics may have facilitated the emergence and spread of resistant E. coli, which can enter humans through food or direct contact with infected animals. These resistant organisms might be extremely important in the spread of antibiotic resistance among human infections (Schroeder et al., 2002). The resistance is a fast-expanding global public health issue. The rate of infections due to antibiotic-resistant bacteria has increased, and certain diseases are now resistant to several different classes of antibiotics. Antimicrobial resistance (AMR) is thought to be responsible for about 500,000 human deaths annually, according to estimates from the FAO (Reardon, 2014). PCR has also been widely employed in a molecular biology to help scientists clone and sequence genes to find mutations. It is a new method for microbial agent detection that just needs a little sample to be analyzed. The PCR process takes four to eight hours, which is about three times quicker than cultures (Liu et al., 2019).

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The current study objectives were to isolate *E. coli* with determine the antibiogram profile in addition to molecular detection of Integrase, stx1, blaSHV and tetB genes from broilers.

2. MATERIAL AND METHODS

Ethical Approval

The study was done according to an approved protocol by the Ethical Committee, Faculty of Veterinary Medicine, Benha University (Approval number BUFVTM 17-03-23).

2.1. Collection of Samples

Two hundred samples of visceral organs [liver (70), heart (70) and lungs (60)] from apparently health (100) and freshly dead (100) broiler chicken were collected from different commercial farms at Sharkia governorate, Egypt during the period from March to October 2022, the samples were tested at (RLQP) Sharkia branch and stored at 4ºC to 8ºC.

2.2. E. coli isolation and identification (Cruickshank et al., 1975*)*

The organs surfaces were serialized with a hot spatula before being inoculated onto buffered peptone broth and then incubated aerobically at 37˚C. Subsequently, the cultures were isolated on MacConkey (HiMedia M081) and EMB (HiMedia M317) agar plates and identified using conventional techniques (Quinn et al., 2002). Furthermore, the pathogenicity of *E. coli* positive samples was evaluated onto a Congo red agar medium. Each isolate was streaked on a sterile separate plate and kept at 37°C for 24 hrs. The cultures were kept at room temperature for 48 hours. After 48 hours in room temperature, Congo red positive pathogenic *E. coli* isolates appeared with brick red color while non-pathogenic ones were colorless (Sharma et al., 2006).

2.3. Serological identification of E. coli

The probable *E. coli* isolates were chosen at random and examined serologically using the slide agglutination test (Edward and Ewing, 1972).

2.4. Antimicrobial susceptibility

In accordance with Clinical and Laboratory Standards Institute (CLSI, 2021) recommendations, antimicrobial resistance (AMR) was assessed for 10 antimicrobials, for selected strains, using the disc diffusion method using *E. coli*-ATCC 25,922 as a reference strain (Animal health research institute). There were the following antimicrobial discs used: oxytetracycline (T) (30 µg), streptomycin (S) (10 µg), doxycycline (Do) (30 µg), colistin (CT) (10 µg), ceftriaxone (CTR) (30 µg), sulfamethoxazole (SXT) (25 µg), ampicillin (AM) (10 µg), norofloxacin (NOR) (10 µg), kanamycin (K) (30 µg) and gentamicin (GEN) (10 µg) (HiMedia, Mumbai, India). An inoculum of each strain was streaked on Mueller-Hinton agar (Himedia, Mumbai, India), and the impregnated discs were placed on the agar surface. The Multiple Antibiotic Resistance (MAR) Index, a tool for assessing risks to wellbeing and health, was looked at. When there is resistance to more than three medicines, this indicator is helpful for monitoring the spread of bacterial resistance in a specific population (Christopher et al., 2013). The MAR index is calculated as the ratio between the number of antibiotics that an isolate is resistant to the total number of antibiotics tested. A high estimated MAR index (greater than 0.2) suggests a potentially contaminated environment with a high risk of antibiotic use.

2.5. Molecular detection of virulence and antibiotic resistance genes

Plasmid DNA extraction from samples were performed using the QIAprep Spin Miniprep Kits. (Qiagen, Germany, GmbH). The *integrase*, *stx*1, *bla*SHV and *tet*B genes were designed and amplified as showed in Table (1 & 2). In a 25-µl reaction, the primers were used along with 12.5 µl of EmeraldAmp Max PCR Master Mix (Takara, Japan), 1 µl of each primer at a concentration of 20 pmol, 5.5 µl of water, and 5 µl of the template. An Applied Biosystems 2720 thermal cycler was used to carry out the reactions. The PCR products were separated by electrophoresis employing gradients of 5V/cm in 1x TBE buffer at room temperature on a 1.5% agarose gel (Applichem, Germany, GmbH). Twenty µl of the products were placed in each gel slot prior to gel analysis. The fragment sizes were calculated using a generuler 100 bp ladder (Fermentas, Germany). Gel documentation equipment (Alpha Innotech, Biometra) was used to take pictures of the gel, and computer software was used to analyze the results.

Table 1 Oligonucleotide primer sequences of different genes.

Target gene	Primer's sequence (5'-3')	Reference
*stx*1	ACACTGGATGATCTCAGTGG CTGAATCCCCCTCCATTATG	Dipineto et al. (2006)
*bla*SHV	AGGATTGACTGCCTTTTTG ATTTGCTGATTTCGCTCG	Colom et al. (2003)
*tet*B	CCTTATCATGCCAGTCTTGC ACTGCCGTTTTTTCGCC	Sabarinath et al. (2011)
Integrase	TGCGGGTYAARGATBTKGATTT CARCACATGCGTRTARAT	White et al. (2000)

Table 2 Cycling condition and PCR product sizes of different genes.

Target gene	Initial Denaturation ˚C/min	Amplification (35 cycles) ˚C/sec			Final extension ˚C/min	Amplified segment (bp)
		Denaturation	Annealing	Extension		
*stx*1	94/5	94/30	58/40	72/45	72/10	614
*bla*SHV	94/5	94/30	54/40	72/40	72/10	392
*tet*B	94/5	94/30	50/40	72/45	72/10	773
Integrase	94/5	94/30	55/40	72/45	72/10	491

3. RESULTS

3.1. Bacteriological and serotyping identification

In the present study, out of 200 samples, one hundred and ten isolates were positive for *E. coli* (55%), On MacConky's agar, all *E. coli* isolates produced pink colonies, while on EMB agar, all of the colonies had the recognizable green metallic sheen. The *E. coli* isolates were also Gram negative, medium sized bacilli, positive for indole, negative for citrate, positive for urease, and produced yellow slant and butt with gas generation but not H2S on TSI agar media.

Sixty-eight (61.8%) isolates were confirmed to be EHEC positive, which revealed five serotypes O157:H7 (12.7%), O26:H11 (21.8%), O128:H- (10%), O111:H4 (9%) and O103:H2 (9%), while (30.9%) of isolates were EPEC with high prevalence of O127:H6 (17.2%), as shown in Table (3).

Table 3 Prevalence of different serotype in *E. coli* isolates

Types of *E. coli* strains	Serotypes	% (No.) of strains	
	O127:H6	17.2% (19)	
EPEC	O1:H7	7.2 % (8)	30.9% (34)
	O2:H-	6.4% (7)	
	O103:H2	8.2% (9)	
	O26:H11	21.8 % (24)	
EHEC	O157:H7	12.7% (14)	61.8% (68)
	O128:H-	10% (11)	
	O111:H4	9 % (10)	
Untypable			7.3 % (8)
Total			100% (110)

3.2. Resistant Pattern of antimicrobial E. coli broiler specimens.

The obtained results in Table (4) showed that the descending manner in resistant of 110 isolated *E. coli* to oxytetracycline, ampicillin, doxycycline, streptomycin, colistin, ceftriaxone, kanamycin, gentamicin, sulfamethoxazole and norofloxacin were 74.54%, 70.90%, 67.27%, 65.45%, 54.54%, 45.45%, 29.09%, 18.18%, 12.72% and 9.09%, respectively. Meanwhile, the sensitivity in descending order were 65.45%, 60%, 56.36% and 54.54% for kanamycin, gentamicin, sulfamethoxazole and norofloxacin, respectively.

The obtained results in Table (5) declared that ten isolates were resistant all tested antibiotics. In addition to, four, six, twelve and eighteen isolates were resistant to nine, eight, seven and six of selected antibiotics.

Table 4 Resistance profile of antimicrobial in *E. coli* isolates (n = 110).

Antimicrobial	Sensitive	Intermediate	Resistant
Oxytetracycline (T)	8 (7.27%)	20 (18.18%)	82 (74.54%)
Ampicillin (AM)	10 (9.09%)	22 (20%)	78 (70.90%)
Doxycycline (Do)	12 (10.90%)	24 (21.81%)	74 (67.27%)
Streptomycin (S)	14 (12.27%)	24 (21.81%)	72 (65.45%)
Colistin (CT)	32 (29.09%)	18 (16.36%)	60 (54.54%)
Ceftriaxone (CTR)	50 (45.45%)	10 (9.09%)	50 (45.45%)
Kanamycin (K)	60 (54.54%)	18 (16.63%)	32 (29.09%)
Gentamicin (GEN)	62 (56.36%)	28 (25.24%)	20 (18.18%)
sulfamethoxazole – trimethoprim (SXT)	66 (60%)	30 (27.27%)	14 (12.72%)
Norofloxacin (NOR)	72 (65.45%)	28 (25.45%)	10 (9.09%)

Table 5 Resistant index of multiple antibiotic (MAR) in *E. coli* isolates (n = 110) and antimicrobial resistance profile.

Resistance Pattern	Resistance Profile	No. of Isolates	No. of Antibiotics	MAR
I	T, AM, Do, S, CT, CTR, K, GEN, SXT, NOR	10	10	1
II	T, AM, Do, S, CT, CTR, K, GEN, SXT	4	9	0.9
III	T, AM, Do, S, CT, CTR, K, GEN	6	8	0.8
IV	T, AM, Do, S, CT, CTR, K	12	7	0.7
V	T, AM, Do, S, CT, CTR	18	6	0.6
VI	T, AM, Do, S, CT	10	5	0.5
VII	T, AM, Do, S	12	4	0.4
VIII	T, AM, Do	2	3	0.3
IX	T, AM	4	2	0.2
X	T	4	1	0.1

3.3. PCR investigations of virulence and resistant attributes of the recovered isolates

The Shiga toxin virulence gene (*stx*1) carried on plasmid were assessed in only one isolate (20%) of five multi drug resistant (MDR) *E. coli* isolates at 614 bp Figure (1.A). The recorded data in Figure (1.B) revealed that *integrase* gene detected in all examined MDR isolates 5/5 (100%) at 491bp. The presence of *bla*SHV and *tet*B resistance genes on plasmids in all tested *E. coli* strains which explained the phenotypic resistance of *E. coli* isolates to ampicillin and oxytetracycline 5/5 (100%) for each, Figure (1.C).

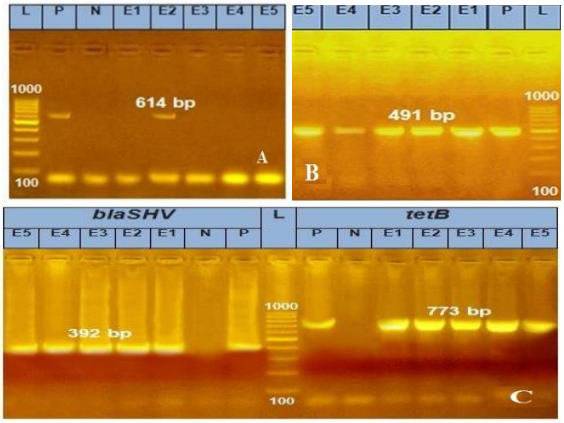

Fig (1) Electrophoresis of PCR amplified products of (A): *stx*1 virulence gene, (B): *Integrase* gene, (C) *bla*SHV and *tet*B resistant genes, Lane L: Ladder, lanes E1 to E5: *E. coli* isolates, P: positive control and N: negative control.

4- DISCUSSION

Escherichia coli is the main infectious pathogens in poultry. According to their virulence traits and clinical symptoms, pathogenic *E. coli* strains can be divided into intestinal and extra-intestinal *E. coli*. The most severe diseases that affects poultry was colibacillosis which causes considerable economic losses due to mortality, weight loss, carcass condemnations, and expenditures associated with treatment and preventive measures. Moreover, it has been determined that Avian Pathogenic *E. coli* (APEC), the organism that causes colibacillosis, is a zoonotic pathogen (Dhaouadi *et al*., 2020). In this study, out of 200 examined samples obtained from freshly dead and apparently healthy broilers revealed (55%) *E. coli* isolates. The obtained results were comparable the finding of Abd El Tawab et al. (2016) who recorded that 52% freshly slaughtered chickens contained *E. coli* and Hussein et al. (2022) who found 59.3% of

broiler chickens with postmortem colibacillosis harbored *E. coli*. The higher recovery rates 90% (Abd El-Aziz et al., 2007) 75.5% (Samanta et al., 2014) and 70% (Khalaf et al., 2020). Lower isolation rate was obtained by Ammar *et al.* (2015) 20%, (Younis et al., 2017) 36.5%, (Amer et al., 2018) 35%, (Abd El Tawab et al., 2015) 37.1% and (Ramadan et al. 2016) 29%. In this investigation, the lung had the greatest *E. coli* isolation rate (67%) followed by the liver (57%) and the heart (44%). The fact that an infection often begins in the respiratory tract before spreading to other internal organs and becoming systemic may help to explain the high rate of isolation from the lungs (Barnes et al., 1999). In the current study sixty-eight (61.8%) isolates were confirmed to be EHEC positive, which revealed five serotypes O157:H7 (12.7%), O26:H11 (21.8%), O128:H (10%), O111:H4 (9%) and O103:H2 (9%), while (30.9%) of isolates were EPEC with high prevalence of O127:H6 (17.2%). The obtained results are in parallel with those obtained by Kumar et al., (2003) who recorded that that the most common *E. coli* serogroups were O78 (23.5%) and Jin et al., (2008) who found that the most popular serotype of *E. coli* in avian diseases was O78 and O26. In addition, Hussein et al., (2022) recorded O127 in 15.7% of examined APEC from broiler affected with colibacillosis. These differences demonstrated that *E. coli* serotypes are national specific, which would be essential in the order to prepare of vaccine, which must be specific to the dominant serotypes. Medication relies heavily on antimicrobials to treat a variety of infections in both people and animals. Antimicrobial resistance (AMR), which is a major risk to both human and animal health, is an important global problem. Antimicrobial misuse and/or abuse in people and animals places a high selection pressure on microbial populations to develop resistance characteristics. Antimicrobials have thus lost some of their effectiveness over time, resulting in treatment failures and problems as well as higher healthcare expenses for both humans and animals. People, animals, and the environment are all exposed to resistant microbes. This spread between nations is facilitated by globalization and international travel (Gray et al., 2021). Fifty-five *E. coli* isolates were tested for their antibiotic susceptibility against 10 antibiotics using the disc diffusion method to determine. The results revealed showed that 74.54%, 70.90%, 67.27%, 65.45%, 54.54%, 45.45% and 29.09% were resistant to oxytetracycline and ampicillin, doxycycline, streptomycin, colistin, ceftriaxone and kanamycin, respectively. Meanwhile, the highest susceptibility recorded for norofloxacin (65.45%), sulfamethoxazole (60%), gentamicin (56.36%) and kanamycin (54.54%) and ceftriaxone (45.45%). The obtained results were comparable to the finding of Abd El Tawab et al. (2016) who reported that 45% and 40% of examined *E. coli* isolates were resistant to colistin and gentamicin, respectively. However, different resistant pattern for *E. coli* isolated from broiler as sensitivity for gentamicin and norofloxacin was 100% and 64.7%, respectively (Miles et al., 2006). In addition, the sensitivity for norofloxacin was 27% for *E. coli* isolated from broilers suffered from colibacillosis (Hussein et al., 2022). The differences in antibiotic use in each location and time period, as well as the variables in study methods, may be reflected in these disparities in resistance rates (Temmerman et al., 2020).

Escherichia coli (STEC) that produce the Shiga toxin is evidence that these germs constitute a harm to the general public's health (Nataro and Kaper, 1998). These are frequently seen in animals raised for food and have been linked to serious gastrointestinal and systemic illnesses such and hemolytic uremic syndrome (HUS), hemorrhagic colitis (HC) which cause diarrhea, particularly in children in underdeveloped nations. Shiga toxins, also known as *Stx*1 and *Stx*2, are one or both of the two main types of toxins produced by STEC strains (Dutta et al., 2011). While serogroups are crucial for identifying potential pathogens, the presence of virulence traits and the stx1 gene were crucial indicators of the strains' increased pathogenicity. This isolates' virulence factor was connected to plasmids, which could be found using PCR. As a result, they are likely to be involved in interspecies horizontal gene transfer as evidenced by the spread of plasmids (Magwedere et al., 2013). The obtained results by PCR revealed presence of *stx*1 in (20%) of examined *E. coli*. Nearly similar results obtained from Bangladesh, (10.20%) were positive for *stx*1 gene in live healthy broiler chickens (Mamun *et al*.,2016). In addition, (45%) of poultry isolates were positive for *stx*1 in Egypt (Nasef et al., 2017). Meanwhile, all examined *E. coli* negative for *stx*1 in Iran (Ghanbarpour et al., 2011) and Egypt (Abd El Tawab et al., 2015).

The integrons act as reservoirs of antimicrobial resistance genes within microbial populations and are recognized to be the principal source of transferrable resistance genes. (Ochman et al., 2000; Collis et al., 2002). In current, study the *integrase* gene detected in all examined samples (100%). The *integrase* gene detected in previous studies worldwide in Italy from avian source examined (49.8%) (Cavicchio et al., 2015), in Iran (50%) of *E. coli* chicken isolates were positive for integrase gene (Kheiri and Akhtari, 2016). Comparatively, a high incidence of integron-bearing *E. coli* isolates (75%) showed positive results in Poland (Racewicz et al., 2022). One AMR problem that has grown globally and affects both humans and animals is *E. coli* that produces extended-spectrum beta lactamases (ESBL). (Chong et al., 2018). Due primarily to the synthesis of SHV -lactamase, which is encoded by the bla_{SHV} genes, the bacteria are resistant to cephalosporins. This gene can be expressed chromosomally or via plasmids (Michael et al., 2015). Public health issues are brought up by the presence of ESBL-producing *E. coli* (ESBL-EC) in food animal production systems because germs can infect people through the food chain (Tekiner and Özpınar, 2016). In this study bla_{SHV} obtained in (100%) of *E. coli.* The detection limit was lower in Philippians, where (20.29%) of PCR tested *E. coli* from broiler cloacal swabs contained bla_{SHV} (Gundran et al., 2019). Furthermore, in Algeria, (16.5%) (Chabou et al., 2018). Genes of tetracycline resistance are typically encoded in plasmids and transposons and are passed from one species to another. But in some isolates, the necessary genes can also be located on plasmid (Oppegaard et al., 2001). The main mechanisms of tetracycline resistance that occur from the acquisition of tet genes are efflux pumps, ribosome protection, and enzymatic deactivation. Mutations can lead to

antibiotic resistance as well (Koo and Woo, 2011). The *tet*A, *tet*B, *tet*C, *tet*D, and *tet*G genes code for efflux pumps, which are related to the *tet* genes most frequently found in gram-negative bacteria (Schwaiger et al., 2010). In current study, *tet*B detected in (100%) of examined *E. coli.* Lower incidence of *tet*B gene were reported in Turkey (1.9%) (Sandalli et al., 2010). Furthermore, in Korea, Koo and Woo (2011) declared that (1.6%) of *E. coli* isolates contained *tet*B, also in Iran, *tet*B found in (38.3%) of colibacillosis *E. coli* broilers isolates (Jahantigh et al., 2020).

5. CONCLUSION

The APEC strains isolated from broiler chickens are multidrug resistant and contain antibiotic resistant genes such as *integrase*, bla_{SHV}, and *tet*B. Due to the potential transmission of these resistant isolates and their determinants to humans via direct and indirect contact with the birds, the existence of multidrug-resistant APEC constitutes a danger to public health. In order to decrease the overuse of antibiotics, it is necessary to put into place all preventive measures, infection control guidelines, immunizations, high-standard biosecurity practices, and hygiene procedures.

6. REFERENCES

1. Abd El Tawab, A., Abd El-Aal, S., mazied, E., El-Morsy, D. 2015. Prevalence of E. Coli in broiler chickens in winter and summer seasons by application of PCR with its antibiogram pattern. Benha Vet. Med. J., 29, 253–261.
2. Abd El Tawab, A.A., El-Hofy, F.I., Ammar, A.M., Abdel Hakeem, M., Abdel Galil, N.M. 2016. Preliminary studies on *E. coli* implicated in avian coli bacillosis with reference to their antibiotic resistance profiles. Benha Veterinary Medical Journal, 30(1), 68-77.
3. Abd El-Aziz, E.E., Hassan, A.M., Badr, J.M. 2007. In vitro efficacy of some cases of chronic respiratory disease in broilers in Egypt. Zag. Vet. J., 35(3), 40-49.
4. Amer, M.M., Mekky, H.M., Amer, A.M., Fedawy, H.S. 2018. Antimicrobial resistance genes in pathogenic *Escherichia coli* isolated from diseased broiler chickens in Egypt and their relationship with the phenotypic resistance characteristics. Vet. World, 11, 1082–1088.
5. Ammar, A.M., Abd El-Hamid, M.I., Eid, S.E.A., El Oksh, A.S. 2015. Insights into antimicrobial resistance and virulence genes of emergent multidrug resistant avian pathogenic *Escherichia coli* in Egypt: How closely related are they? Rev. Med. Vet. (Toulouse), 166, 304–314.
6. Barnes, H.J. & Gross, W.B. 1999. Colibacillosis. In: Diseases of poultry, Calnek, B.W., Beard, C.W., McDougald, L.M. Saifin, Y.M. (eds), Ames: Iowa State University Press; 131–141.
7. Barnes, H.J. and Gross, W.B. 1997. Colibacillosis. In; Diseases of Poultry. 10th ed., Calnek, B.W. (ed.), London, UK: Mosby-Wolf Publication Ltd., pp. 131–139.
8. Barnes, H.J., Nolan, l.k., vaillancourt, J.A. 2008. Colibacillosis. In: Disease of poultry, Saif, Y.M., Fadly, A.M. (Eds.), Blackwell Publishing, Ames, IA PP.691-732.
9. Cavicchio L, Dotto G, Giacomelli M, Giovanardi D, Grilli G, Franciosini MP, Trocino A, Piccirillo A. 2015. Class 1 and class 2 integrons in avian pathogenic Escherichia coli from poultry in Italy. Poult Sci., 94, 1202–1208.
10. Chabou, S., Leulmi, H., Davoust, B., Aouadi, A., Rolain, J. M. 2018. Prevalence of extended-spectrum β-lactamase-and carbapenemase-encoding genes in poultry feces from Algeria and Marseille, France. Journal of Global Antimicrobial Resistance, 13, 28-32.
11. 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.
12. Christopher, A.F., Hora, S., Ali, Z. 2013. Investigation of plasmid profile, antibiotic susceptibility pattern multiple antibiotic resistance index calculation of Escherichia coli isolates obtained from different human clinical specimens at tertiary care hospital in Bareilly India. Ann. Trop. Med. Public Health, 6, 285-289.
13. CLSI, Clinical and Laboratory Standards Institute. 2021. Performance Standards for Antimicrobial Susceptibility Testing; Clinical and Laboratory Standards Institute: Wayne, PA, USA.
14. Collis, C., Kim, M., Stokes, H., Hall, R. 2002. Integron-encoded IntI integrases preferentially recognize the adjacent cognate attI site in recombination with a 59-be site. Mol. Microbiol., 46(5), 1415–1427.
15. Colom, K., Pèrez, J., Alonso, R., Fernández-Aranguiz, A., Lariño, E., Cisterna, R. 2003. Simple and reliable multiplex PCR assay for detection of blaTEM, blaSHV and blaOXA-1 genes in Enterobacteriaceae. FEMS Microbiology Letters, 223, 147-151.
16. Cruickshank, R., Duguid, J.P., Marmion, B.R., Swain, R.H.A. 1975. Medica Microbiology, 12th Ed., vol.2, Churchill Livingstone, Edinburg, London.
17. Dhaouadi, S., Soufi, L., Hamza, A., Fedida, D., Zied, C., Awadhi, E., Landolsi, R.B. 2020. Co-occurrence of mcr-1 mediated colistin resistance and β-lactamase-encoding genes in multidrug-resistant Escherichia coli from broiler chickens with colibacillosis in Tunisia. Journal of Global Antimicrobial Resistance, 22, 538-545.
18. Dipineto, L., Santaniello, A., Fontanella, M., Lagos, K., Fioretti, A., Menna, L.F. 2006. Presence of Shiga toxin-producing Escherichia coli O157:H7 in living layer hens. Letters in Applied Microbiology, 43, 293–295.
19. Dutta, T.K., Roychoudhury, P., Bandyopadhyay, S., Wani, S.A., Hussain, I. 2011. Detection & characterization of Shiga toxin producing Escherichia coli (STEC) & enteropathogenic Escherichia coli (EPEC) in poultry birds with diarrhoea. The Indian journal of medical research, 133(5), 541–545.
20. Edward, P.R. & Ewing, W.H. 1972. Edward and Ewing's identification of Enterobacteriacae, 3rd Ed. Burgess, Minneapolis.
21. Ghanbarpour, R., Sami, M., Salehi, M., Ouromiei, M. 2011. Phylogenetic background and virulence genes of Escherichia coli isolates from colisepticemic and healthy broiler chickens in Iran. Tropical animal health and production, 43(1), 153-157.
22. Gray, P., Jenner, R., Norris, J., Page, S., Browning, G. 2021. Antimicrobial prescribing guidelines for poultry. Australian veterinary journal, 99(6), 181–235.
23. Gundran, R.S., Cardenio, P.A., Villanueva, M.A., Sison, F.B., Benigno, C.C., Kreausukon, K., Punyapornwithaya, V. 2019. Prevalence and distribution of blaCTX-M, blaSHV, blaTEM genes in extended-spectrum β-lactamase-producing E. coli isolates from broiler farms in the Philippines. BMC veterinary research, 15(1), 1-8.
24. Holko, I., Bisova, T., Holkova, Z., Kmet, V. 2006. Virulence markers of Escherichia coli strains isolated from traditional cheeses made from unpasteurized sheep milk in Slovakia. Food Control, 17, 393–396.
25. Hudault, S., Guignot, J., Servin, A.L. 2001. Escherichia coli strains colonizing the gastrointestinal tract protect germfree mice against Salmonella typhimurium infection. Gut, 49(1), 47-55.
26. Hussein, M.S.S., Fahmy, H.A., Abd El Tawab, A.A. 2022. Characteristics of Fluoroquinolone-Resistant

Escherichia Coli from Broiler Chickens with Colibacillosis. Benha Veterinary Medical Journal, 42(1), 46-50.

27. Jahantigh, M., Samadi, K., Dizaji, R.E., Salari, S. 2020. Antimicrobial resistance and prevalence of tetracycline resistance genes in Escherichia coli isolated from lesions of colibacillosis in broiler chickens in Sistan, Iran. BMC veterinary research, 16(1), 1-6.
28. Jin, W., Zheng, Z., Zhang, Y. Qin, A., Shao H. Liu, Y., Wang, J. Wang, Q. 2008. Distribution of virulence associated genes of avian pathogenic Escherichia coli isolates in China. Agricultural Sciences in China, 7(12), 1511-1515.
29. Karch, H., Tarr, P.I., Bielaszewska, M. 2005. Enterohaemorrhagic Escherichia coli in human medicine. International Journal of Medical Microbiology, 295(6-7), 405-418.
30. Khalaf, H.A., Aml, B., Awd, A. 2020. Antimicrobial Resistance Genes of E. coli Isolated from Broiler Chickens in Upper Egypt. Anim. Vet. Sci., 9(1), 19-28.
31. Kheiri, R. and Akhtari, L. 2016. Antimicrobial resistance and integron gene cassette arrays in commensal Escherichia coli from human and animal sources in IRI. Gut pathogens, 8(1), 1-10.
32. Koo, H.J. and Woo, G.J. 2011. Distribution and transferability of tetracycline resistance determinants in Escherichia coli isolated from meat and meat products. Int J Food Microbiol., 145(2–3), 407–13.
33. Kumar, A.D., Sarma, B.J., Rao, A.S., Mishra, S.K. 2003. Serogroups of Escherichia coli isolates from chickens and their antibiogram. Indian Journal of Poultry science, 38 (3), 270-273.
34. Liu, H.Y., Hopping, G.C., Vaidyanathan, U., Ronquillo, Y.C., Hoopes, P.C., Moshirfar, M. 2019. Polymerase Chain Reaction and Its Application in the Diagnosis of Infectious Keratitis. Medical hypothesis, discovery & innovation ophthalmology journal, 8(3), 152–155.
35. Magwedere, K., Dang, H.A., Mills, E.W., Cutter, C.N., Roberts, E.L., DebRoy, C. 2013. Incidence of Shiga toxin–producing Escherichia coli strains in beef, pork, chicken, deer, boar, bison, and rabbit retail meat. Journal of Veterinary Diagnostic Investigation, 25(2), 254–258.
36. Mamun, M.M., Parvej, M.S., Ahamed, S., Hassan, J., Nazir, K.H.M.N.H., Nishikawa, Y., Rahman, M.T. 2016. Prevalence and characterization of shigatoxigenic Escherichia coli in broiler birds in Mymensingh. Bangladesh Journal of Veterinary Medicine, 14(1), 5-8.
37. Michael, G.B., Freitag, C., Wendlandt, S., Eidam, C., Feßler, A.T., Lopes, G.V., Schwarz, S. 2015. Emerging issues in antimicrobial resistance of bacteria from food-producing animals. Future microbiology, 10(3), 427-443.
38. Miles, T.D., McLaughlin, W., Brown, P.D. 2006. Antimicrobial resistance of Escherichia coliisolates from broiler chickens and humans. BMC veterinary research, 2(1), 1-9.
39. Nasef, S.A., EL-Oksh, A.S., Ibrahim, G.A. 2017. Studies on enterohaemorrhagic (EHEC) non O157:H7 strains in chicken with regard to antibiotic resistance gene on plasmid., Assiut Veterinary Medical Journal Assiut Vet. Med. J., 63(153), 157-165.
40. Nataro, J.P. and Kaper, J.B. 1998. Diarrheagenic Escherichia coli. Clinical microbiology reviews, 11(1), 142–201.
41. Ochman, H., Lawrence, J., Groisman, E. 2000. Lateral gene transfer and the nature of bacterial innovation. Nature,18 (405), 299–304.
42. Oppegaard, H., Steinum, T. M., Wasteson, Y. 2001. Horizontal transfer of a multi-drug resistance plasmid between coliform bacteria of human and bovine origin in a farm environment. Appl Environ Microbiol, 67(8), 3732–3734.
43. Quinn, P.J., Markey, B.K., Carter, M.E., Donnelly, W.J., Leonard, F.C. 2002. Veterinary Microbiology and Microbial Diseases. 1st ed., Blackwell Science Ltd, Oxford, UK.
44. Racewicz, P., Majewski, M., Biesiada, H., Nowaczewski, S., Wilczyński, J., Wystalska, D., Madeja, Z.E. 2022. Prevalence and characterisation of antimicrobial resistance genes and class 1 and 2 integrons in multiresistant Escherichia coli isolated from poultry production. Scientific Reports, 12(1), 1-13.
45. Ramadan,H., Awad,A.,Ateya,A.2016. Detection of phenotypes, virulence genes and phylotypes of avian pathogenic and human diarrheagenic Escherichia coli in Egypt. J Infect Dev Ctries2016; 10(6):584-591
46. Reardon S. 2014. Phage therapy gets revitalized. Nature, 510(7503), 15–16.
47. Runa, J.A., Lijon, M.B., Rahman, M.A. 2018. Detection of multidrug resistant and shiga toxin producing Escherichia coli (STEC) from apparently healthy broilers in Jessore, Bangladesh. Frontiers in Environmental Microbiology, 4(1), 16-21.
48. Sabarinath, A., Tiwari, K.P., Deallie, C., Belot, G., Vanpee, G., Matthew, V., Sharma, R., Hariharan, H. 2011. Antimicrobial Resistance and Phylogenetic Groups of Commensal Escherichia Coli Isolates from Healthy Pigs in Grenada. Available from: www.webmedcentral.com.
49. Samanta, I., Joardar, S.N., Das, P.K. Das, P., Sar, T.K., Dutta, T.K., Bandyopadhyay, S., Batabyal, S. Isore, D.P. 2014. Virulence repertoire, characterization, and antibiotic resistance pattern analysis of E. coli isolated from backyard layers and their environment in India. Avian Dis. J., 58 (1), 39-45.
50. Sandalli, C., Özgümüş, O. B., Sevim, A. 2010. Characterization of tetracycline resistance genes in tetracycline-resistant Enterobacteriaceae obtained from a coliform collection. World Journal of Microbiology and Biotechnology, 26, 2099-2103.
51. Schroeder, C.M., Zhao, C., DebRoy, C., Torcolini, J., Zhao, S., White, D.G., Meng, J. 2002. Antimicrobial resistance of Escherichia coli O157 isolated from humans, cattle, swine, and food. Applied and environmental microbiology, 68(2), 576-581.
52. Schwaiger, K., Holzel, C., Bauer, J. 2010. Resistance gene patterns of tetracycline resistant Escherichia coli of human and porcine origin. Vet Microbiol., 142(3–4), 329–36
53. Sharma, K. K., Soni, S.S., Meharchandani, S. 2006. Congo red dye agar test as an indicator test for detection of invasive bovine Escherichia coli. Veterinarski Arhiv, 76(4), 363-366.
54. Tekiner, İ.H. and Özpınar, H. 2016. Occurrence and characteristics of extended spectrum beta-lactamases-producing Enterobacteriaceae from foods of animal origin. brazilian journal of microbiology, 47, 444-451.
55. Temmerman, R., Garmyn, A., Antonissen, G., Vanantwerpen, G., Vanrobaeys, M., Haesebrouck, F., Devreese, M., 2020. Evaluation of fluoroquinolone resistance in clinical avian pathogenic Escherichia coli isolates from flanders (Belgium). Antibiotics, 9, 1–14.
56. White, P.A., McIver, C.J., Deng, Y., Rawlinson, W.D. 2000. Characterisation of two new gene cassettes, aadA5 and dfrA17. FEMS Microbiology Letters, 182, 265-269.Younis, G., Awad, A., Mohamed, N. 2017. Phenotypic and genotypic characterization of antimicrobial susceptibility of avian pathogenic Escherichia coli isolated from broiler chickens. Veterinary world, 10 (10), 1167-1172.