



Antibiotic Resistance Patterns of *Escherichia coli* Isolated From Broiler Chickens with Colibacillosis in Duhok Province

Waffa G. Jaseem¹ and Aqeel M. Shareef²

¹University of Duhok College of Veterinary Medicine, Duhok, Kurdistan region, Iraq.

²University of Mosul College of Veterinary Medicine, Mosul, Iraq.



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THE study was aimed to describe the Prevalence, bacteriological and antimicrobial resistance of *Escherichia coli* isolated from broilers flocks in Duhok province during the period from October 2021 till January 2022. Fifty commercial broiler flocks at 14 different regions of Duhok province were included. Bacteriological swab samples were collected from broilers aged 2 to 6 weeks showing clinical signs and necropsy findings of suspected colibacillosis. Two hundred and fifty pericardial swab samples were taken from necropsied live and recently died chickens. Standard bacteriological methods represented by culturing on MacConkey and EMB agar followed by biochemical tests were performed to detect *Escherichia coli*. Qualitative disc spread method was used to assay antibiotic sensitivity to 19 types of antibiotics. The results showed that out of 250 samples, 162 (64.8%) were positive to *E.coli* isolation. Antibiotic sensitivity test of 50 *E.coli* isolates showed high resistance (>50%) to Neomycin, Lincomycin, Azithromycin, Cephalexin, sulphamethaxazole-Trimethoprim, Spiramycin, Florfenicol, Norfloxacin, Doxycycline, Enrofloxacin, Phosphomycin, erythromycin, Tetracycline, Tylosin and Amoxicillin, while a moderate resistance (20-50%) was observed with Colistin, Ciprofloxacin, Levofloxacin, and Gentamycin. Twelve resistance patterns (MDR) were recorded with *E. coli* isolates. Measures must be taken to avoid the development of resistance to *E. coli* in chicken farms through applying antibiotics sensitivity test before drug administration coupled with biosecurity and good hygienic practice.

Keywords: Broilers, Colibacillosis, Antibiotics sensitivity.

Introduction

Escherichia coli is one of the most common pathogens responsible for localized or systemic colibacillosis in poultry with major health concern [1, 2]. Colibacillosis is the main reason of cost-effective losses among poultry production around the world, as it causes poor performance and high death rates [3, 4]. In poultry, *E.coli* causes chronic respiratory illness, omphalitis, synovitis, coligranulomatosis, and salpingitis, all of which are categorized as diseases caused by avian pathogenic *E.coli* [5, 6]. If colibacillosis is suspected, the condition is diagnosed primarily

through clinical symptoms, polyserocytis lesions, and pathogen isolation and identification [6].

Colibacillosis is a major cause of death in chickens of all ages [7]. Although birds of all ages are susceptible, younger birds have a more severe type of sickness than older birds. Stress, a weakened immune system, and infection are all predisposing factors. [3, 8]. Environmental, Physiological, and dietary factors [9]. are linked to the development of clinical illness. The disease has high mortality in birds of various ages [7].

The infection is found all over the world and is one of the most serious problems in both

commercial and backyard poultry [8, 1]. Mostly due to delayed development of the affected birds, resulting in irregular batches [11]. Rearing of birds of different age group together, exposure to bacterial disease such as *Mycoplasma* and poor ventilation, overcrowding, excessive dust and ammonia levels are the main predisposing factors for *E.coli* to invade the respiratory system [12, 13]. Colibacillosis can occur as secondary infection of some primary respiratory tissue illness, for example Newcastle disease (including vaccine strains), infectious bronchitis, mycoplasmosis and pasteurellosis [14]. The nature of the exudate covering the heart and peritoneum is fibrinous type and the respiratory tract involvement may cause signs such as respiratory rate, sneezing, cough and gasping for air [14]. The septicemic form of *E.coli* infection may lead to osteomyelitis, tenosynovitis. The postmortem findings representing by, presence of yellowish cheesy exudate covering the heart and liver in the form of fibrinous pericarditis and perihepatitis is evident in severe cases. In mild cases airsacculitis with some cheesy yellowish plaque formation and clouding of air sacs is evident [15].

Many antimicrobial drugs are utilized in the poultry production sector in order to reduce colibacillosis morbidity and mortality. However, Overuse of pharmaceuticals as infection preventative and therapeutic agents, as well as growth boosters in poultry, has resulted in the appearance and transmission of resistant genes, as well as an increase in antibiotic resistance, resulting in lower efficiency and making treatment harder [16, 17] and increased prophylaxis and treatment costs [18]. Adhesions, poisons, iron acquisition factors, lipopolysaccharides, polysaccharide capsules, and invasions are only a few of the virulence factors found in *Escherichia coli* [19]. But, the occurrence of some virulence factors is needed for their pathogenicity, which grants them to have power to induce avian diseases [8].

Many factors of virulence have been proven to have important impact in the pathogenicity of the *Escherichia coli* strain [20]. Diagnosis of the condition can be made by combining clinical and pathological characteristics, as well as isolating and identifying the etiological agent [21]. Furthermore, as one of the most common illnesses diagnosed in poultry production, colibacillosis is one of the key difficulties for poultry productivity. Kurdistan's poultry sector has recently attempted

to make significant headway in the rapid development of the local chicken business has not been without consequence, notably in terms of concerns about the introduction of infectious diseases such as colibacillosis. Therefore, the aim of this study was to describe the Prevalence, bacteriological, antimicrobial resistance, of colibacillosis in broilers of Duhok province.

Material and Methods

Collection of Samples

From October 2021 to January 2022, 50 commercial broiler flocks (2-6 weeks old) from fourteen different regions of Duhok province were evaluated in Duhok College of Veterinary Medicine for clinical signs and necropsy findings of colibacillosis. Two hundred and fifty pericardial swab samples were taken from necropsied live and recently died chickens (5 samples / flock). Swab samples were transported in icebox within an hour of collection to the Microbiological Laboratory at the College of Veterinary Medicine in Duhok, where they were processed. Number and regions of broiler flocks distributed in Duhok province used in samples collection were including the following: Sheladize (7 Flocks), Kani Golan (2 Flocks), Zakho (5 Flocks), Sindore (2 Flocks), Misurike (3 Flocks), Bade (3 Flocks), Bagera (2 Flock), Bardarashe (5 Flocks), Sumel (5 Flocks), Sarsink (3 Flocks), Pirumara (2 flocks), Kevla (1 flock) Mangeshke (5 Flocks), Gilbeshe (5 Flocks). (Fig. 1).

E.coli Isolation and identification.

Under aseptic technique, *E.coli* isolation techniques (Fig. 2), were performed using standard bacteriological methods on MacConky agar and Eosin methylene blue agar followed by biochemical tests for confirmation of *E.coli*. Collected swabs were incubated with MacConky broth overnight at 37°C. A loopful of colony of the cultured bacteria was streaked on top of MacConky agar and further incubated at 37°C for twenty four hours [21].

Gram's stain was used to define the proven positive cultured *E.coli* of bright pink colonies on MacConkey agar and unique metallic sheen colonies on EMB agar. *E.coli* colonies that are positive were transferred onto nutrient agar and biochemical assays were used to confirm their identity. For additional analysis, triple sugar iron (TSI) agar was utilized, observation of yellow slant, yellow butt, presence of gas bubbles, and absence of black precipitate in the

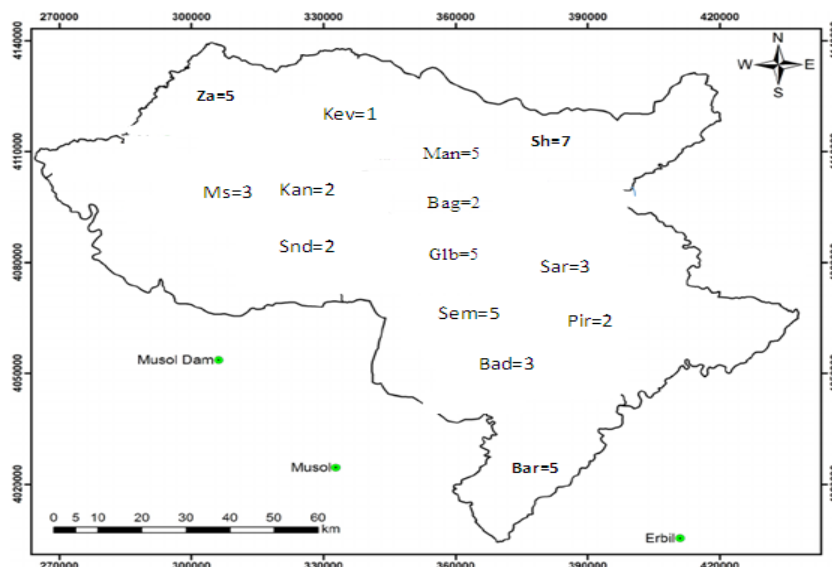


Fig. 1. Sampled broiler flocks in Duhock governorate.

Sh:Sheladiz (7 Flocks), Kan: Kani Golan (2 Flocks), Za: Zakho (5 Flocks), Snd: Sndore (2 Flocks) Ms: Msirik (3 Flocks), Bad: Bad: Bade (3 Flocks), Bag: Bagera (2 Flock), Bar: Bardarash (5 Flocks), Sem: Semel (5 Flocks), Sar: Sarsink (3 Flocks), Pir: Piromara (2 flocks) Kev: Kevla (1 flock) Mangeshke (5 Flocks), Glbeshe (5 Flocks).



Fig. 2. Swab sampling from pericardial sac of affected broiler chicken.

butt was considered as potentially *E.coli* isolate. The isolates were then put through a battery of biochemical tests, including indol production, Voges-Proskauer, methyl red and citrate utilization (IMVIC).

Antibiotic sensitivity assay

Qualitative of disc spread method by Kirby Bauer process was utilized to detect resistancy of antibiotic pattern of 50 isolates with 19 antibiotics

usually used in the Poultry Industry [16] according to the guidelines of the Laboratory and Clinical Standards Institution (CLSI) [22]. In this investigation, antibiotic discs were employed (in micrograms concentration). Each isolated bacterial was cultured on MacConkey agar for twenty four hours at 37°C. to obtain 0.5 McFarland turbidity, colonies of the MacConkey agar medium were transferred to a test tube containing TSB (Tryptic Soy Broth) and incubated at 37°C for 3 hours.

Using a sterile cotton swab, the sterile swabs from the half-McFarland bacterial suspension were swabbed on pre-incubated Mueller-Hinton agar (MHA) plates and left to dry for ten to fifteen minutes. After that, standard antibiotic disks (Oxoid Ltd., U.K.) were placed on MHA plates with sterile forceps, and aerobic incubation was carried out at 35°C for 18 hours [14]. According to CLSI standards, the organisms were classed as “resistant,” “intermediate,” or “sensitive” following incubation based on the diameter of their zone of inhibition [23]. The antibiotics chosen for this study include the following compounds: Amoxicillin (AX 25 µg); Cephalexin (CL.30 µg); Colistin (CT 10µg); Norfloxacin (NOR 10µg); Ciprofloxacin (CIP, 10µg); Levofloxacin (LEV 5µg); Enrofloxacin (ENR 10 µg); Doxycycline (DO 10µg); Tetracycline (TE 30µg); Gentamycin (CN 30µg); Neomycin (N 30µg); Florfenicol (FFC 30µg); Phosphomycin (FF 10µg); Lincomycin (L 10µg); Spiramycin (SP 30µg); Tylosin (TYL 15µg); Azithromycin (AZM 30µg); Erythromycin (E 30µg); and sulphamethazole-Trimethoprim (SXT 25µg). The prevalence of resistance against specific antimicrobials in this trial was classified as either high (>50%), medium (21-50%), or low (0-20%).

Results

Total of 162 *E. coli* isolated out of 250 samples were detected, which were positive to lactose fermentation, pink colored colonies on MaConky, unique green metallic sheen colonies on Eosin methylene blue agar, gram-negative, non-spore-forming bacilli after their staining with Gram's stain. Figures 3 and 4 show the positive cultural characteristics of *E. coli* isolates from broiler chickens.



Fig. 3. Colonies of *E. coli* on MaConky agar isolated from affected broilers

Incidence of colibacillosis

Fig. (5) and (6), show that *E. coli* was isolated in 162 cases (64.8 %) of the 250 samples of broilers suspected of being infected with colibacillosis in Duhok province, while it was not cultivated in 88 cases (35.2 %).

Antibiotic sensitivity test

The result of antibiotic sensitivity test are illustrated in Table 1 and Fig. 7. From these table and figure, it is evident that they were distributed into highly sensitive, intermediates while the others were highly resistant. The resistance to 19 different antibiotics in a descending manner were as follows Amoxicillin (94%), Tylosin (82%), Tetracycline (80%), Erythromycin (76%), Phosphomycin (74%) and Enrofloxacin (74%). Medium level resistance to Norfloxacin (68%), Doxycycline (68%), Spiramycin (66%), Florfenicol (66%), sulphamethazole-Trimethoprim (64%), Cephalexin (62%), Azithromycin (58%), Lincomycin (58%), Neomycin (54%). Moderate resistance to antibiotics was recorded with Gentamycin (50%), Levofloxacin (44%), Ciprofloxacin (30%), and Colistin (22%).

Multiple drug resistance (MDR)

Isolated *E. coli* were tested for having multiple antibiotic resistance to at least one antibiotic of 3 or more of the following antimicrobial classes β -Lactam antibiotics Penicillins (Amoxicillin), Cephalosporins (Cephalexin), Peptide Antibiotic (Polymyxin, Colistin), Fluoroquinolones (Norfloxacin, Ciprofloxacin, Levofloxacin, Enrofloxacin), Tetracycline (Doxycycline, Tetracycline), Aminoglycosides (Gentamycin, Neomycin), Amphiphenicols (Florphenicol), Phosphonic acid (Phosphomycin), Lincosamides (Lincomycin), Macrolide



Fig. 4. *E. coli* Colonies on Eosin methylene blue (EMB) Agar

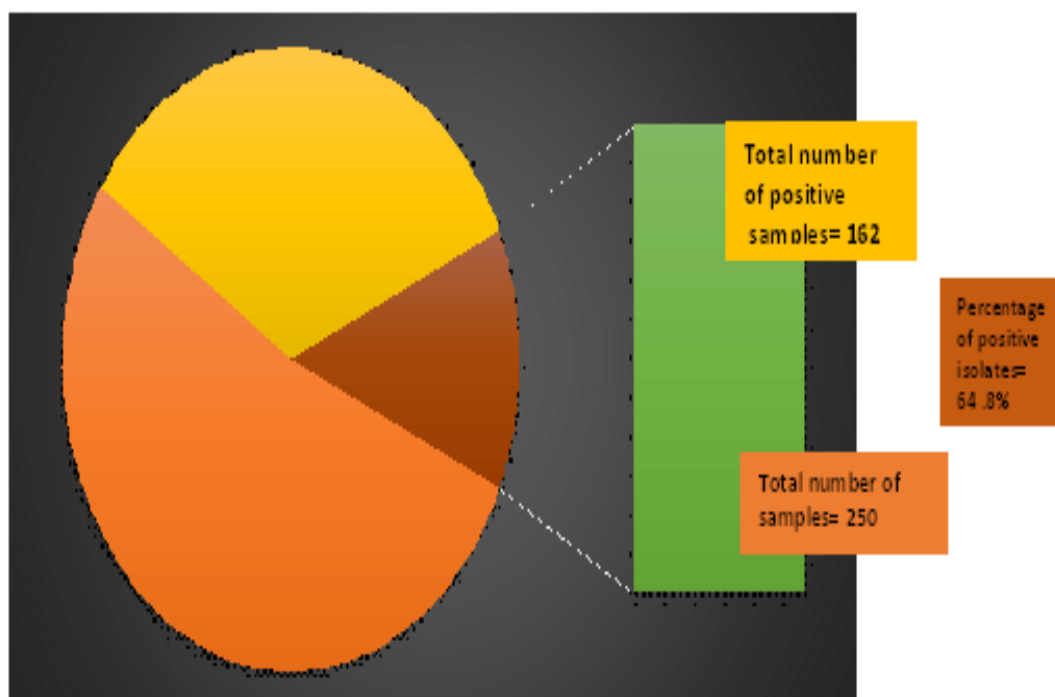


Fig. 5. Overall Percentage of *E.coli* positive samples.



Fig. 6. Three to six weeks broilers affected by colibacillosis showing myocarditis and inflamed, cloudy, edematous, adhesive with whitish yellowish exudative to caseous pericarditis. The pericardial sac is adherent to the epicardium which undergoes fibrosis organization. Affected Liver characterized by fibrinous perihepatitis (Hepatic serosis). Extensive inflammation and exudation in peritoneal surface is also seen.

TABLE 1. Number and percentage of sensitive, intermediate and resistant *E. coli* isolates from broiler chickens to 19 different antibiotics.

Class of Antibiotics	Antibiotic	Sensitive No. (%)	Intermediate No. (%)	Resistant No. (%)
B-Lactam antibiotics				
Penicillins	Amoxicillin	2/50 (4%)	1/50 (2%)	47/50 (94%)
Cephalosporins	Cephalexin	12/50 (24%)	7/50 (14%)	31/50 (62%)
Peptide Antibiotic (Polymyxin)	Colistin	26/50 (52%)	13/50 (26%)	11/50 (22%)
Quinolone and Fluoroquinolone				
2nd generation	Norfloxacin	7/50 (14%)	9/50 (18%)	34/50 (68%)
3rd generation	Ciprofloxacin	19/50 (38%)	16/50 (32%)	15/50 (30%)
4th generation	Levofloxacin	18/50 (36%)	10/50 (20%)	22/50 (44%)
	Enrofloxacin	4/50 (8%)	9/50 (18%)	37/50 (74%)
Tetracycline				
Long acting	Doxycycline	4/50 (8%)	12/50 (24%)	34/50 (68%)
Short acting	Tetracycline	3/50 (6%)	7/50 (14%)	40/50 (80%)
Aminoglycosides	Gentamycin	12/50 (24%)	13/50 (26%)	25/50 (50%)
	Neomycin	9/50 (18%)	14/50 (28%)	27/50 (54%)
Amphiphenicols	Florphenicol	4/50 (8%)	13/50 (26%)	33/50 (66%)
Phosphonic acid	Phosphomycin	3/50 (6%)	10/50 (20%)	37/50 (74%)
Lincosamides	Lincomycin	10/50 (20%)	11/50 (22%)	29/50 (58%)
Macrolides	Spiramycin	4/10(40%)	10/50 (14%)	33/50 (66%)
	Azithromycin	10/50 (20%)	11/50 (20%)	29/50 (58%)
	Tylosin	3/50 (6%)	6/50 (12%)	41/50 (82%)
	Erythromycin	2/50 (4%)	10/50 (20%)	38/50 (76%)
Sulphonamides,Intermediate acting and Trimethoprim	Sulphamethoxazole-Trimethoprim	8/50 (16%)	10/50 (20%)	32/50 (64%)

(Spiramycin, Azithromycin, Erythromycin and Tylosin), Sulphonamides And Trimethoprim (Sulphamethoxazole-Trimethoprim). In *E.coli* isolates, 12 distinct MDR patterns were detected (Table 2). Multiple resistance was found among the resistant isolates, with 2.43% to 5 resistant patterns; 4.87% to 2 resistant patterns; 7.31% to 2 resistant patterns; 19.51% to 2 resistant patterns and 26.82% to 1 resistant pattern (Table 2) [24].

Discussion

Colibacillosis stays one of the most common economic cost-effective bacterial contagion in the poultry industry, particularly among broilers, both globally and in Duhok province. Nowadays, the fight against colibacillosis not only relies on the application of strict biosecurity but we forced to the abuse of antimicrobial chemicals and the widespread usage of antimicrobs, which led to

Development of resistance to some bacterial isolates and a huge economic loss due to treatment failure and high mortalities [12, 17].

In the present study, high bacterial resistance (> 50%) was recorded in 15 out of the 19 total antibiotics tested, they include Neomycin, Lincomycin, Azithromycin, Cephalexin, sulphamethaxazole-Trimethoprim, Spiramycin, Florfenicol, Norfloxacin, Doxycycline, Enrofloxacin, Phosphomycin, Erythromycin, Tetracycline, Tylosin, Amoxicillin. Only four antibiotics show moderate resistance (21-50%) and were Colistin, Ciprofloxacin, Levofloxacin and Gentamycin. In this study antibiotic resistance were resemble to large extent those reported by many authors [17, 25] who reported greatest amount of resistance to nalidixic acid (100%) and a maximum level of sensitivity to Colistin

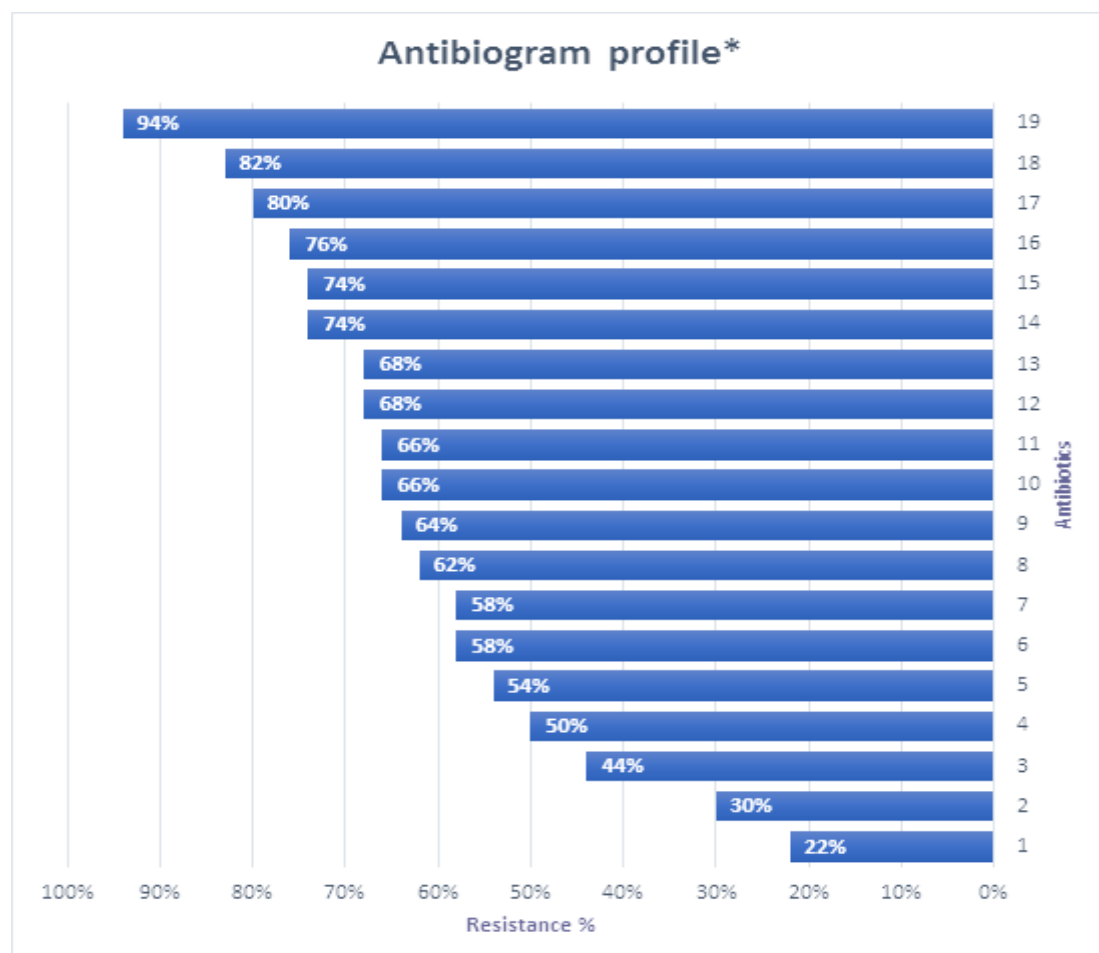


Fig. 7 . Percentage of resistant *E. coli* isolates to different antibiotics.

*: 19= Amoxicillin (AX 25 µg); 18= Tylosin (TYL 15µg); 17= Tetracycline (TE 30µg); 16= Erythromycin (E 30µg); 15=Phosphomycin (FF 10µg); 14= Enrofloxacin (ENR 10 µg); 13= Doxycycline (DO 10µg); 12= Norfloxacin (NOR 10µg); 11= Spiramycin (SP 30µg); 10= Florfenicol (FFC 30µg); 9= sulphamethaxazole-Trimethoprim (SXT 25µg); 8= Cephalexin (CL.30 µg); 7= Azithromycin (AZM 30µg); 6= Lincomycin (L 10µg); 5= Neomycin(N 30µg); 4= Gentamycin (CN 30µg); 3= Levofloxacin(LEV 5µg);; 2 Ciprofloxacin (CIP, 10µg); 1= Colistin (CT 10µg).

(100%) and gentamicin (93%) [13]. According to researchers that tested the sensitivity of 101 *E. coli* isolates from Algerian chicken herds, they showed the highest tetracycline resistance, though resistance to sulfamethoxazole + trimethoprim, ampicillin, and neomycin was next. Moreover the findings of this study were similar to those reported by Ozawa et al.[26] in Thai to tetracycline (77.8%) and chloramphenicol (50%) but lower in Enrofloxacin (9.3 %). The findings reported here also matched the resistance of those reported with Ampicillin (77.1%), charted by oxytetracycline (74.9%), trimethoprim (25.3%), Enrofloxacin (21.7%), and fluorophenicol (0.6%) [27]. Resistance to Ampicillin (89%), enrofloxacin (43%), tetracycline (33%), and fluorophenicol

(33%) were all shown to be associated to *E. coli* isolates in slaughtered fowl [28]. Different percentage of resistance than those reported in this study was observed in Bangladesh by Wakawa et al.[29] of 100% and 93.3% to erythromycin and tetracycline respectively. In Nigeria, there was a low resistance to chloramphenicol (18%), tetracycline (5%), Neomycin (9%), sulfamethoxazole (5%), enrofloxacin (3%), ampicillin (9%), and erythromycin (5%)[30]. Many researches conducted in Iran[31], yielded results relatively close to the resistance reported in this study of 86.2 %, 84.5 %, 39 %, and 27.2 %, to doxycycline, oxytetracycline, enrofloxacin, and chloramphenicol respectively. In an investigation of *E. coli* isolates from around Iran [32] showed

TABLE 2. Multidrug-resistant *E. coli*. (n=50) isolated from colibacillosis cases of broiler chickens.

Antibiotic classes	Number of MDR isolates*	(%)
B-LAC, FLU, TET, MAC**	1/41	2.43
B-LAC, FLU, TET, AMP, MAC	1/41	2.43
B-LAC, FLU, TET, AMP, PHO, MAC, SULPH-TRI	1/41	2.43
B-LAC, FLU, TET, AMI, PHO, MAC, SULPH-TRI	1/41	2.43
B-LAC, TET, AMP, MAC	1/41	2.43
B-LAC, CEP, FLU, TET, AMP, PHO, MAC, LIN, SULPH-TRI	2/41	4.87
B-LAC, CEP, FLU, TET, AMP, PHO, MAC, SULPH-TRI	2/41	4.87
B-LAC, TET, MA	3/41	7.31
B-LAC, FLU, TET, AMP, MAC	3/41	7.31
B-LAC, CEP, FLU, TET, AMI, AMP, PHO, MAC, LIN, SULPH-TRI	8/41	19.51
B-LAC, CEP, FLU, TET, AMI, AMP, PHO, MAC, LIN, SULPH-TRI	8/41	19.51
B-LAC, CEP, PEP, FLU, TET, AMI, AMP, PHO, MAC, LIN, SULPH-TRI	11/41	26.82

* MDR=Multidrug-resistant

**B-LAC=Beta-lactams, FLU = Fluroquinolones, TET = Tetracyclines, MAC = Macrolides, AMP = Florfenicol, AMI = Aminoglycosides PHO= Phosphomycin, SUL-TRI = Sulphonamides-Trimethoprim, CEPH = Cephalosporins, POI = Polyxins, LIN = Lincosamides, PEN =Penicillins.

that Erythromycin resistance was found in 99 % of commercial farms across Iran, followed by tetracycline (96 %), neomycin (87 %), Linco-Spectin (79 %), difloxacin (78 %), enrofloxacin (76 %), ampicillin (49 %), and fluorophenicol (39 %).

These findings could reflect the widely, haphazard prolonged and misuse of antibiotics, resulting in bacterial resistance in some strains. Many scenarios are related to the improvement resistance of bacteria to different classes of antimicrobials, of these the dependence on enzymatic drug degradation, alteration in permeability of bacteria, alterations in bacterial receptors for drugs, changes in the cell wall of bacterial structure, gene mutations or plasmid transferring drug-inhibited sub metabolic reactions [33].

Antimicrobial resistance of *E. coli* infecting poultry has increased in recent decades and the form of antibiotic resistance has been changing in different parts of the world [27, 32 and 33].

Antibiotic resistance is a serious problem when

isolated bacteria are resistant to at least three antibiotic classes [28, 34 and 35]. In the previous decade, various nations, have seen significant increases in the incidence of multiple resistance of *E. coli* isolates to antibiotic compounds including Iran, [14, 32] Germany, [35] China, [36] Bangladesh, [37] India, [28] Algeria, [13] Nigeria, [3] and Zimbabwe [26].

From above it is evident that *E. coli* in chicken flocks poses a serious risk in transmission of resistance to humans. This is particularly significant in underdeveloped countries, where antibiotic-treated meat or other animal products should be avoided [31].

Conclusion

According to the current research, Colibacillosis caused by virulent *E. coli* isolates in Duhok province constitute a significant problem for mortality in broiler sector, and the production of ESBL due to continuously use antibiotic result in multidrug resistant and public health concern.

Approval of Ethical Principles

Not applicable.

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Conflict of Interest Disclosure

The authors declare that they have no conflicts of interest.

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انماط مقاومة جرثومة الاشريشييا القولونية للمضادات الحيوية والمعزولة من فروج اللحم في محافظة دهوك

وفاء غانم جاسم^١ و عقيل محمد شريف خضر^٢

^١ فرع الامراض و الاحياء الدقيقة - كلية الطب البيطري - جامعة دهوك - دهوك - العراق.

^٢ فرع الامراض وأمراض الدواجن - كلية الطب البيطري - جامعة الموصل - الموصل - العراق.

هدفت الدراسة إلى وصف انتشار الإشريكية القولونية المعزولة من قطعان فروج اللحم في محافظة دهوك ومقاومتها للمضادات الحيوية خلال الفترة من أكتوبر ٢٠٢١ إلى يناير ٢٠٢٢. شملت الدراسة خمسون من قطعان فروج اللحم موزعة على ١٤ منطقة مختلفة من محافظة دهوك. تم جمع عينات المسحات لعزل جراثيم الإشريكيات القولونية من فروج اللحم بأعمار تراوحت بين ٢ إلى ٦ أسابيع تظهر علامات سريرية وصفة تشريحية يشنبة باصابتها بداء القولونيات. تم أخذ مائتين وخمسين مسحة من شغاف التامور من افراخ حية ونافقة حديثاً. تم اتباع الطرق البكتريولوجية القياسية المتمثلة باستخدام أجار MacConkey و EMB متبوعة باختبارات كيميائية حيوية للكشف عن الإشريكية القولونية. تم استخدام طريقة الانتشار النوعي للأقراص لفحص حساسية الإشريكية القولونية لـ ١٩ نوع من المضادات الحيوية. أظهرت النتائج أنه من بين ٢٥٠ عينة ، كانت ١٦٢ (٦٤,٨٪) إيجابية لعزل الإشريكية القولونية. أظهر اختبار الحساسية للمضادات الحيوية لـ ٥٠ عزلة من الإشريكية القولونية مقاومة عالية (>50%) للنيومايسين ، لينكومايسين ، أزيثروميسين ، سيفاليكسين ، سلفاميثاكسازول-تريميثوبريم ، سبيراميسين ، فلورفينيكول ، نورفلوكساسين ، دوكسيسيكليين ، إينروفلوكساسين ، فوسفوميسين ، فوسفوميسين. بينما لوحظ وجود مقاومة معتدلة (٢٠-٥٠٪) مع كل من الكوليسيتين والسيبروفلوكساسين والليفوفلوكساسين والجنتاميسين. تم تسجيل اثني عشر نمط مقاومة (MDR) من عزلات الإشريكية القولونية. يجب اتخاذ كافة التدابير لتجنب تطور المقاومة للإشريكية القولونية في حقول الدواجن من خلال استخدام اختبار الحساسية للمضادات الحيوية قبل استخدامها كعلاج إلى جانب تطبيق شروط الأمن البيولوجي والممارسات الصحية الجيدة.