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Molecular study of resistance genes in *Escherichia coli* isolated from chronic respiratory disease cases in broilers

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Abstract

Chronic respiratory disease is famous in poultry farming, mainly in broiler farms. The disease is caused by Mycoplasma species in participation with *E. coli*. Our study was conducted on CRD of broiler chickens to isolate and determine the resistance of *Escherichia coli* Seventy-four swabs of the internal organs of broilers (severe respiratory signs) were collected from different areas of Mosul from September 2021 to March 2022. MacConkey agar was used with cefotaxime (1 µg/ml) to grow the isolates, and they were incubated at 37 °C for 24 hours. Colonies were identified according to standard bacteriological methods. The cefotaxime-resistant *E. coli* isolates underwent DNA extraction. The polymerase chain reaction of *Escherichia coli* isolates was used for confirmation. The results of the existing study revealed that 61 samples appeared positive for bacterial isolation from 74 (82.4%). All isolates were resistant to an arsenal of antibiotics when testing their sensitivity to antibiotics, including azithromycin, levofloxacin, gentamycin, chloramphenicol…ext. Molecular detection of resistance genes showed that all isolates contained the CTX-M gene by 100%. In comparison, *the TEM* gene appeared in 52 isolates (85.25%), and only 9 (14.75%) isolates showed the *SHV* gene. In conclusion, our results shed light on the serious problem in poultry fields, which showed that *Escherichia coli* isolates contain genes with high resistance to antibiotics, which are the most widely used in the treatment of bacterial infections in these farms, which means the demand for introducing more novel antibiotics in the cure of poultry health problems.

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Introduction

Avian pathogenic *E. coli* is the main pathogen in poultry. It causes many infections and is accompanied by viral and other infections (1,2). Colibacillosis has economic importance in poultry through its effect on reducing bird productivity, increased mortality, contamination of infected carcasses at slaughter, and the cost of prevention and treatment (3,4). To control and protect against infection in poultry, antimicrobials are used in feed during risky durations of bacterial infections, as prophylaxis, or as a growth stimulator (5). Bacterial resistance to antibiotics is increasing, and its relationship with the wrong use of antibiotics in controlling diseases in humans and animals has been noted (6,7). There must be vectors that transfer antibiotic-resistant bacteria isolated from chicken meat to humans after consuming poultry meat and animal products (8). Beta-lactam antibiotics have remained the prime selection for treating *E. coli* infections (9,10). The *E. coli* resistance to cephalosporins through its weapons enzymes (ESBLs) limits therapeutic options against *E. coli* infections (11). Beta-lactamases are enzymes produced by *E. coli* that cause hydrolyzing of beta-lactam ring in penicillin and cephalosporin; thus, these antibiotics lose their therapeutic ability to give bacteria the character of resistance (12,13). One of the beta-lactamase enzymes includes extended-

spectrum beta-lactamases (ESBLs) like *TEM*, *SHV*, *CTX-M*, and *OXA* (14,15). The *blaCTX−M* genes are swiftly pervasion and were recorded in *E. coli* (16,17). Plasmids encode these enzymes and are transmitted to consumers through the nourishment chain and animal contact (18,19). The goal of the existing study was to isolate Escherichia coli strains from the CRD in broiler chickens and determine their responsible genes for antibiotic resistance.

Materials and methods

Ethical approve

The endorsement certificate with the number UM.VET.2021.075 on 18/8/2021was granted by the Commission of scientific morals, which also provided the moral consent to carry out this methodical activity in the College of Veterinary Medicine.

Sampling

Seventy-four swabs from internal organs (air sacs, heart, lung, liver) were collected from broiler chickens with severe respiratory signs in Mosul City from September 2021 to March 2022. Specimens were placed in a sterile screw-capped. Specimens were conveyed to the microbiology laboratory of the College of Veterinary Medicine, University of Mosul.

Isolation and identification of *E. coli*

Swabs were placed on MacConkey agar containing foxime 500mg (20,21) and incubated at 37ºC for 24h. Pink fermented colonies were chosen and re-cultured on BHI (brain heart infusion) agar for purification. Colonies were classified biochemically using standard bacteriological methods (22).

Antimicrobial susceptibility testing

The disc diffusion method was used to test the sensitivity of the isolates to eleven antimicrobial agents (23). The isolates that exhibited multi-drug resistance by the disc diffusion method were selected for antimicrobial resistance gene amplification using PCR.

Extraction of DNA

Sixty-one isolates of cefotaxime-resistance *E. coli* underwent DNA extraction using DNA preparation Cat NO. PP-206 (Jena Bioscience, Germany). The extraction was done by following the manufacturer's instructions and depending on (24,25). Keep the extracted DNA at -20°C till use.

Amplification of DNA

The primers and their information are explained in table 1. A confirmatory genetic assay was conducted to diagnose *E. coli* using a specific primer for the 16S rRNA gene (ECOL200-F and ECOL400-R) (26). whereas detection of the *CTX-M*, *TEM*, and *SHV* genes (24-28).

Results

Escherichia coli isolates were isolated from chronic respiratory disease infections in poultry with a percentage of 82.4% (61 out of 74 samples). All isolates showed absolute resistance to the tetracyclines, sulphamethoxazole, chloramphenicol, and levofloxacin, while all isolates were sensitive to imipenem. They also showed resistance in varying proportions to azithromycin, tobramycin, gentamycin, streptomycin, and ciprofloxacin (Table 2). All 61 isolates were molecularly detected as *E. coli* (232bp) (Figure 1). *CTX-M* gene was found in all isolates of *E. coli* 100%, which appeared at 550bp (Figure 2), while 52 (85.25%) isolates appeared positive for the *TEM* gene with the band at 822bp (Figure 3). Finally, only 9 (14.75%) isolates showed the *SHV* gene with an expected band size of 753bp (Figure 4).

Table 1: Sequence of primers

Primer	Sequence 5`-3`	Tm	Product size (bp)	References
ECOL200-F	ATC AAC CGA GAT TCC CCC AGT	55	232	15
ECOL400-R	TCA CTA TCG GTC AGT CAG GAG			
CTX-M-Uni-F	CGC TTT GCG ATG TGC AG	54	550	15
CTX-M-Uni-R	ACC GCG ATA TCG TTG GT			
SHV-F	ATG CGT TAT ATT CGC CTG TG	45	753	16
SHV-R	TGC TTT GTT ATT CGG GCC AA			
TEM-F	AAA CGC TGG TGA AAG TA	45	822	17 and 18
TEM-R	AGC GAT CTG TCT AT			

Table 2: Antibacterial resistance of *E. coli* isolated from chronic respiratory disease

Antimicrobials		Concentration (µg)	Disc diffusion (mm)		Number (%) resistant isolates
			R	S	
β-lactams	Imipenem (IPM)	10	≤19	≥23	0 (0%)
Macrolides	Azithromycin (AZM)	30	≤22	≥28	59 (96.7%)
Tetracycline	Tetracyclines (OX)	30	≤14	≥19	61 (100%)
Aminoglycoside	Tobramycin (TOB)	10	≤12	≥15	43 (70.5%)
	Gentamycin (CN)	10	≤12	≥15	53 (86.89%)
	Streptomycin (S)	25	≤12	≥13	43 (70.5%)
Sulfonamides	Trimethoprim/ Sulphamethoxazole (COT)	25	≤13	≥17	61 (100%)
Miscellaneous	Chloramphenicol (C)	30	≤12	≥18	61 (100%)
Quinolone	Ciprofloxacin (CIP)	5	≤15	≥21	40 (65.57%)
	Levofloxacin (LEV)	5	≤13	≥17	61 (100%)
Nitrofuran	Nitrofurantoin (F)	300	≤14	≥17	0 (0%)

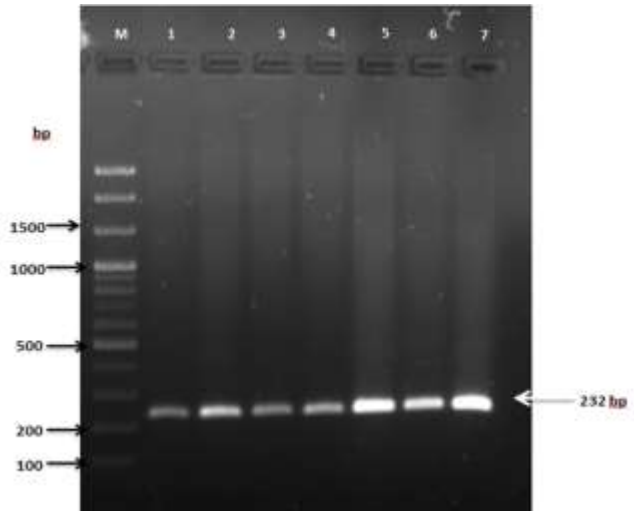

Figure 1: Molecular confirmation of *E. coli* isolates according to the sequence of 16S rRNA (M: Ladder, 1-7 positive for *E. coli* at 232 bp).

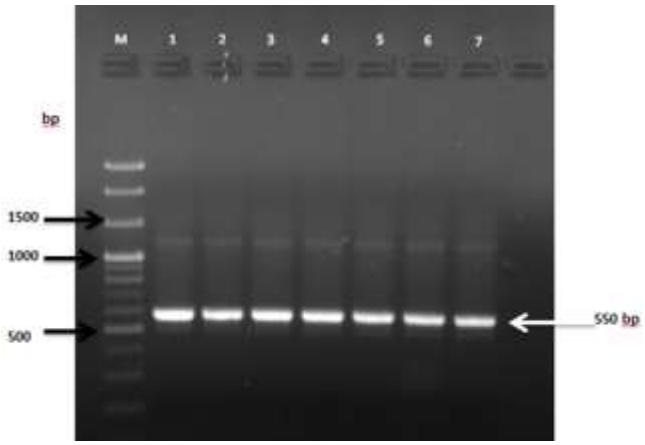

Figure 2: Affirmation of *CTX-M* gene in *E. coli* isolates. M: ladder, lanes 1-7 positive *CTX-M* gene (550 bp).

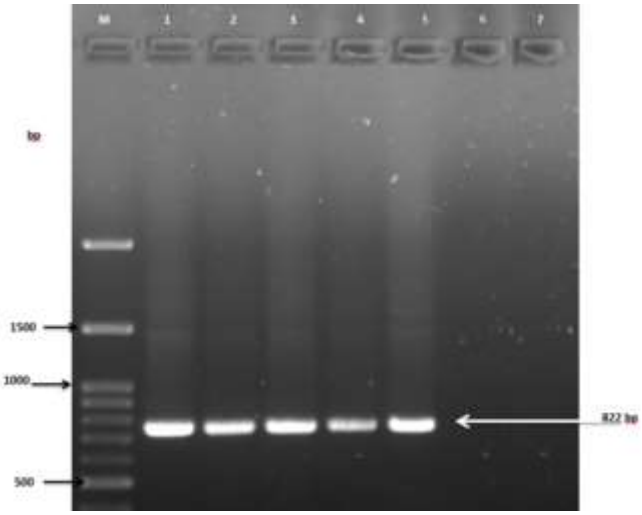

Figure 3: Detection of *TEM* gene. M: Ladder, lanes 1-5 positive *TEM* gene (822 bp), lanes 6- 7 are negative.

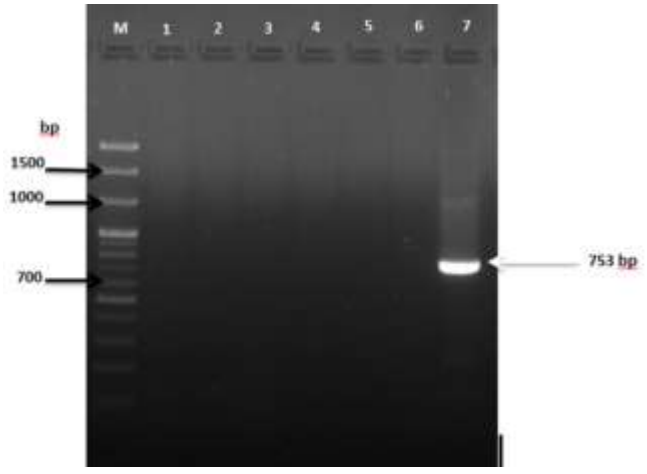

Figure 4: PCR products of *SHV* gene. M: Ladder, lane 7 positive *SHV* gene (753 bp), lanes 1-6 are negative.

Discussion

Although chicken meat is considered a primary food source in many countries, the broiler is a persistent source of bacterial spreading that causes many diseases between humans and animals. Raising chickens and keeping them in contact with humans facilitates the transmission of bacteria that cause diseases of animal origin to humans (29). Chicken meat contains some *E. coli* strains considered more pathogenic than others and responsible for most deaths recognized as avian pathogenic *E. coli* (APEC) live outside the intestines and is endemic to the respiratory system and some organs, and causes systemic diseases and major fatalities (30). The present study recorded that the isolation rate of APEC reached 82.4% from 74 samples suffering from different clinical cases such as septicemia, peritonitis, and airsacculitis. This result is close to what was obtained by researchers in India, where they recorded a rate of isolation of 67% (31), in addition to the results of other researchers in Egypt, where the isolation rate was 75.4% from cases of chronic respiratory infections (32). The isolated bacteria appeared highly resistant to azithromycin, levofloxacin, gentamicin, tetracycline, and chloramphenicol. This has been confirmed in many articles and studies (31,33). This resistance may be attributed to the wrong utilization of antibiotics without testing the sensitivity of isolates to antibiotics, as well most poultry breeders' resort to adding antibiotics to stimulate growth and obtain high production (34). Also, one of the causes of antibiotic resistance like tetracycline is having genes that code for excluding this antibiotic from the cell leading to reduced concentration and the protection of *E. coli* ribosomes (35). One of the most plasmid mechanisms is the transfer and spreading of resistance genes among the bacterial population (36). Resistance was also recorded in the β-lactam group in the study due to β-lactamase production by *E. coli* (37). All isolated *E. coli* (61 isolates) was carried out with the *CTX-M* gene encoding ESBL (100%). This result was consistent with recent studies with similar proportions, and the gene was prevalent in isolates taken from the broilers' farm (38). The *CTX* enzymes have hydrolytic effects on cephalosporins (39). The plasmids carry the ESBL genes and are also easily transmitted between commensal and pathogenic bacteria in poultry (40). The animal-human transference of ESBL genes leads to the difficulty and failure of treatment in both cases and the economic losses they cause (41). In the existing results, the *TEM gene*, responsible for releasing an enzyme that awards resistance to β-lactam, has appeared *in* 52 *E. coli* isolates. This gene can cause ESBL by mutation altering the sequence of one amino acid nearby the active site of β-lactamases (42,43). The *CTX-M* gene was the most predominant in isolates of *E. coli*, after the *TEM* and *SHV* genes. It was consistent with what was obtained by researchers in poultry farms in America, where the highest percentage was 33.6% of gene *CTX-M* followed by a low frequency of *TEM* and *SHV* genes (18,42).

Finally, the current results spotlight the serious problem in poultry farms that showed the excessive resistance of E. coli isolates that are prevalent in broilers and appeared proprietor for an arsenal of resistance genes to the most usable antibacterial in the treatment of bacterial infections in these farms, which means the demand for introducing more novel antibiotics in the cure of poultry health problems.

Conclusion

The current results spotlight the serious problem in poultry farms that showed the excessive resistance of *E. coli* isolates that are prevalent in broilers and appeared proprietor for an arsenal of resistance genes to the most usable antibacterial in the treatment of bacterial infections in these farms, which means the demand for introducing more novel antibiotics in the cure of poultry health problems.

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Conflict of interest

The authors declare there are no conflicts of interest or financial ties to any government institutions and that no outside funding was used to carry out this research. We declare that all identified writers have read, reviewed, and approved the work. We have also all agreed on the order in which the authors are listed.

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دراسة جزيئية لجينات المقاومة في جراثيم الإيشريكيا القولونية المعزولة من حالات المرض التنفسي المزمن في فروج اللحم

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الخلاصة

المرض التنفسي المزمن من الأمراض الشائعة في حقول الدواجن وخصوصاً في حقول فروج اللحم. المرض تسببه أنواع المايكوبلازما بالتزامن مع الايشيريكيا القولونية. أجريت دراستنا على الإصابات التنفسية المزمنة في فروج اللحم لعزل وتحديد مقاومة الايشيريكيا القولونية. جمعت أربعة وسبعون مسحة من الأعضاء الداخلية لفروج اللحم (تعاني علامات تنفسية شديدة) من مناطق مختلفة من مدينة الموصل من أيلول ٢٠٢١ ولغاية أذار ٢٠٢٢، م استخدام أكار ماكونكي مع سيفوتاكسيم (١ ميكروغرام/مل) لتنمية العزلات وتم تحضينها عند ٣٧ درجة مئوية لمدة ٢٤ ساعة. تم تشخيص المستعمرات وفقًا للطرق البكتريولوجية القياسية. استخدم فحص تفاعل البلمرة المتسلسل لتشخيص عزلات الإيشريكيا القولونية للتأكيد. وتم استخلاص الحامض النووي لعزلات الإيشريكيا القولونية المقاومة للسيفوتاكسيم (١مايكرو غرام/مل). أظهرت نتائج الدراسة الحالية أن ٦١ عينة كانت إيجابية للعزل الجرثومي من مجموع العينات (٧٤) وبنسبة عزل (٨٢,٤%)، كانت جميع العزلات مقاومة لترسانة من المضادات الحيوية عند اختبار حساسيتها للمضادات الحيوية بما في ذلك الازثرومايسين، الليفوفلوكساسين، الجنتامايسين، الكلورامفنكول وغيرها. أظهر الكشف الجزيئي لجينات المقاومة أن جميع العزلات تحتوي على جين CTX-M وبنسبة ١٠٠٪، بينما كانت ٥٢ (٨٥,٢٥%) عزلة حاملة لجين TEM وكانت فقط ٩ (١٤,٧٥%) عزلات إيجابية لجين SHV. في الختام، سلطت نتائجنا الضوء على المشكلة الخطيرة في حقول الدواجن والتي أظهرت أن عزلات الإيشريكيا القولونية تحتوي على جينات ذات مقاومة عالية للمضادات الحيوية الأكثر استخدامًا في علاج الالتهابات البكتيرية في هذه المزارع، مما يعني الحاجة إلى إدخال المزيد من المضادات الحيوية الجديدة في العلاج مشاكل صحة الدواجن.

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