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Molecular detection of carbapenemases and ESBLs in Gram negative bacilli isolated from broiler chickens infected with respiratory tract infections in Mosul city, Iraq

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عنوان البحث (باللغة العربية)

الكشف الجزيئي عن انزيمات الكاربابنيميز والبيتالاكتاميزواسعة الطيف في العصيات السالبة لصبغة كرام المعزولة من دجاج اللحم المصاب بعدوى الجهاز التنفسي في مدينة الموصل بالعراق

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Abstract

The current study aims to detect the presence of β-lactamase enzymes in Gram-negative bacilli isolated from broiler chickens infected with respiratory tract infections in Mosul city. After cultivation on MacConkey agar, 55 isolates were obtained from 45 samples, they distributed as follows: *Escherichia coli* 56.36%, *Klebsiella pneumoniae* 14.54%, *Pseudomonas aeruginosa*, *Proteus mirabilis* and *Proteus vulgaris* 12.7%, 10.9%, and 5.5%, respectively. Antimicrobial susceptibility testing results showed absolute resistance to cefotaxime and nalidixic acid, high resistance to tetracycline and ciprofloxacin reached 96.4% and 94.6%, and low resistance to meropenem and imipenem with rates of 12.7% and 14.5% respectively. The results showed that all the isolates were Multiple Drug-Resistant (MDR), 96.36% of them were Extensively Drug-Resistant (XDR), while two isolates (3.63%) were Pan Drug-Resistant (PDR). Six most resistant isolates were selected and their identification was further confirmed by molecular method depending on 16s rRNA gene. Phenotypic and molecular detection of carbapenemases and ESBLs was performed for the six isolates. Phenotypically, all isolates were ESBLs producers and five isolates out of six (83.3%) were carbapenemase producers. These detection tests were molecularly confirmed using 14 gene primers (NDM, IMP, KPC, GES, NMC-A, OXA23, OXA48, OXA58, VIM, SPM, SIM, TEM, SHV, CTX-M). The results revealed the presence of VIM and NDM carbapenemase genes at a rates of 83.3% and 33.3%, respectively. TEM and SHV ESBL genes were detected in all isolates (100%), while CTX-M detection rate was 66.6%. Negative detection rates were recorded for the rest of the genes.

Keywords*: carbapenemase, ESBLs, broiler, respiratory infections*

الكشف الجزيئي عن انزيمات الكاربابنيميز والبيتالاكتاميزواسعة الطيف في العصيات السالبة لصبغة كرام المعزولة من دجاج اللحم المصاب

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بعدوى الجهاز التنفسي في مدينة الموصل ، العراق

الخلاصة

تهدف الدراسة الحالية إلى الكشف عن وجود إنزيمات البيتا لاكتاميز بأنواعها في العصيات السالبة الكرام المعزولة من دجاج اللحم المصاب بعدوى الجهاز التنفسي في مدينة الموصل. بعد تلقيح العينات على وسط أكار الماكونكي، تم الحصول على 55 عزلة من 45 عينة، توزعت على *Escherichia coli* ،14.54 *Klebsiella pneumoniae* ، ٪*Pseudomonas aeruginosa* ،*Proteus* النحو التالي: 56.36٪ ٪ ، على التوالي. أظهرت نتائج اختبار حساسية المضادات الحيوية مقاومة مطلقة 5.5,٪ 1210.9٪، *Proteus vulgaris* 7. و *mirabilis* بلغت 96.4%، 94.6% ، ومقاومة ciprofloxacin و tetracycline, ومقاومة عالية لمضادات cefotaximeلمضادات cefotaxime, nalidixic acid بنسب 12.7%، 14.5% . أظهرت النتائج أن جميع العزلات كانت متعددة المقاومة imipenem و meropenem منخفضة لمضادات منها كانت مقاومة للمضادات على نطاق واسع ، بينما كانت عزلتان (3.63%) مقاومة لجميع المضادات . تم اختيار ست 96.36%للمضادات ، . تم إجراء الكشف الظاهري والجزيئي 16s rRNA لجين PCRعزلات الأكثر مقاومة وتم تأكيد هويتها بطريقة جزيئية تعتمد على تقنية وخمس عزلات من أصل ست ESBLs للعزلات الست. ظاهريا ، كانت جميع العزلات منتجة لإنزيمات ESBLsللكاربابنيميز وإنزيمات و GES و KPC و NDM و IMP(83.3٪) كانت منتجة للكاربابنيميز. تم تأكيد اختبارات الكشف هذه جزيئيًا باستخدام 14 بادئًا لجينات ( ). كشفت النتائج عن وجود جينات CTX-M و SHV و TEM و SIM و SPM و VIM و OXA58 و OXA48 و OXA23 و NMC-A في جميع العزلات SHV وTEM بمعدلات 83.3٪ و 33.3٪ على التوالي. تم الكشف عن الجينات واسعة الطيف NDM و VIMالكاربابنيميز ٪ 66.6. بالنسبة لبقية الجينات أعطت نتيجة تحري سالبة. CTX-M (100٪)، بينما كان معدل الكشف عن

Introduction

The poultry industry provides a solution to the problem of nutrient deficiencies that are prevalent in the diets of an increasing number of people by providing an abundant and cost-effective supply of animal protein. This poultry industry also offers a solution to the problem of nutrient deficiencies(1). As far as poultry is concerned, respiratory infections are considered to be among the most dangerous infections that can cause harm to poultry. Specifically, this is the case with regard to chronic respiratory disorders that are common in poultry production, particularly in broiler chicken farms. Not only do they result in tremendous economic losses all around the world, but they also have a terrible effect on the poultry industry (2).

When it comes to poultry, respiratory problems can be brought on by a wide variety of pathogens, which can include a large number of viruses, bacteria, and fungus (3,4). Environmental factors may increase these pathogens as well as act on producing clinically observed signs and lesions (5). Over the course of the majority of the last few decades, there has been an increase in the utilization of antimicrobials in the poultry production industry. Antibiotics are utilized in the therapeutic setting for the purpose of treatment. Antibiotics are utilized as stimulants for development and disease prevention at the same time. A significant contributor to the development of bacterial resistance in chicken production is the excessive use of antibiotics in poultry production. This is the primary cause of the problem. Disease-causing bacteria are found in significant numbers in the intestines of animals. These bacteria are capable of causing illness. Due to the fact that these bacteria have the capability of being transmitted to individuals through feces or the food chain, they have the potential to eventually become a source of public health problems (6,7,8).

In contrast to other animals, such as pigs, cattle, and others, it has been discovered that poultry is the principal reservoir of bacteria that produce Extended-Spectrum β-Lactamases (ESBLs), which are categorised as enteric bacteria. The fact that chicken is a reservoir of bacteria that create ESBL presents a challenge for those who work in the meat industry, as well as for farmers and workers who sell meat. An increase in the colonization of β-lactamase-producing bacteria of the intestines was discovered in people who work near poultry (9,10,11).

Bacterial resistance is becoming more complex and serious year by year, especially after the emergence of carbapenemase, which can degrade carbapenems (imipenem, ertapenem, panipenem, doripenem, biapenem, meropenem) and other β-lactam antibiotics, making disease prevention and control more challenging (12,13).

β-lactam antibiotics are a category of antimicrobials that are capable of targeting a wide range of microorganisms. Antibiotics are compounds that possess a β-lactam ring inside their molecular structure, being the defining characteristic of antibiotics. The term "antibiotics" refers to these chemical substances. In order to treat illnesses that are brought on by Gram-positive and Gram-negative bacteria, antimicrobial drugs are regularly supplied and have been utilized all over the world. This is done for the aim of treating illnesses. These

medications have been applied in the treatment of a wide array of diseases and conditions. A lot of research have shown that these compounds are quite successful in the treatment of infections. This has been proved by the findings of these investigations (14,15). The development of β-lactamase, a hereditary enzyme component that leads to the resistance of Gram-negative bacteria to β-lactam antimicrobial medications, is quite common among pathogenic Gram-negative bacteria (16,17).

β-lactamases represent a wide group of enzymes capable of degrading β-lactam antibiotics by opening the β-lactam ring and converting the antibiotic from an active form to an inactive form that does not affect the bacterial cell (18). Among the most important classifications used in classifying β-lactamase enzymes are: the functional classification and the molecular classification. The functional classification depends on the basic substance on which the enzyme works and the inhibitors that inhibit it (19), and consists of the following groups: Group1: includes cephalosporinase enzymes, or what is known as AmpC. These enzymes work on cephalosporin antibiotics as substrate and are not inhibited by EDTA, clavulanic acid, or tazobactam. Examples of these enzymes are ACT, AmpC, ADC-33 (20,21). Group 2: includes the largest group of β-lactamase enzymes, which are serine enzymes, and contains a number of subgroups (2a, 2b, 2bc, 2br). The basic substances of this group range between penicillins, cephalosporins, monobactam, and carbencillins, and they are not inhibited by EDTA but by tazobactam and clavulanic acid. Examples of which include Extended-Spectrum β-Lactamase enzymes (ESBLs), TEM, OXA, CepA, CAZ, and CTX-M (22). Group 3: includes metallo-β-lactamases, which are enzymes that use zinc ions in the active site and work to decompose carbapenems. Most of these enzymes are not inhibited by tazobactam or clavulanic acid, but rather by EDTA. This group can be divided into two subgroups: 3a and 3b, as 3a represents metalloenzymes that hydrolyze many substrates and 3b represents the metalloenzymes that degrades carbapenems only, examples of which include IMP, SIM, NDM, and VIM (23).

According to the molecular classification, also known as the Ambler classification, β-lactamases are classified based on the similarity in amino acid sequences. This classification system is also known as the Ambler classification. This categorization is comprised of four categories: The letters A, B, C, and D. Included in this are the categories that are listed above. Serine enzymes are classified as belonging to classes A and D in accordance with this categorization. Serine enzymes are classified as belonging to classes A and D because they hydrolyze their substrates by creating an acyl-enzyme through an active site serine. This classification is established using the classification system. Metalloenzymes that make use of zinc ions in their active site in order to improve β-lactam hydrolysis are classed as belonging to class B, whereas AmpC enzymes are classified as belonging to class C (24).

The current study aimed to isolate bacterial species causing respiratory infections in poultry farms in Mosul city, detect to their antimicrobial resistance patterns and evaluate the prevalence of (ESBL)- and carbapenemase-encoding genes in them.

Materials and methods

Sample collection

A total of forty-five swabs were collected from the internal organs (air sacs, heart, lung, and liver) of broiler chickens that were exhibiting indications of respiratory illnesses. Between the months of March and June of 2023, samples were gathered from broiler chicken farms in Mosul city in collaboration with the Dutch laboratory known as the (Animal Health Diagnostic Center). The microbiological processing of the samples was carried out as quickly as feasible after they were transferred to the laboratory.

Isolation and Identification

Following the inoculation of the swabs onto MacConkey agar plates, the plates were then placed in an incubator at a temperature of 37 degrees Celsius for a period of 37°C for (24-48)hours (25). The pure bacterial isolates were preserved on the nutrient agar medium in the refrigerator, with monthly checking and re-culturing .

Bacterial isolates were primarily identified by observing the cultural characteristics of the colonies on MacConkey agar. Thin smears of pure colonies were prepared and stained with Gram stain to observe the shape

and reaction of the cells (26). Identification of the of the isolates was conducted using Analytic Profile Index (API 20E) strips (Biomerieux Co., France).

Antimicrobial Susceptibility Testing

Disc diffusion was the method that was utilized in this test, and it was carried out in a manner that was in accordance with the criteria that were established by the Clinical Laboratory Standards Institute(27). Fresh bacterial suspensions were prepared in the nutrient broth to achieve a suspension turbidity equivalent to 0.5 tube of the McFarland standards (1.5 x108 CFU/ml) (25). After using sterile cotton swabs to inoculate Mueller-Hinton agar plates with the bacterial suspensions, the plates were allowed for (15) minutes to allow for absorption to take place. Following this, antimicrobial discs manufactured by Bioanalyse Company in Turkey were handed out. After 16–18 hours of incubation at 35 °C, the plates were removed. Following the classification of the isolates into susceptible, moderately susceptible, and resistant categories, the diameter of the inhibitory zone was measured, which included the diameter of the disk. This classification was based on the guidelines of the CLSI. Additionally, MDR, XDR, and PDR isolates were found to be present (28).

Phenotypic Detection of ESBLs

In line with the directions that were provided by the CLSI, bacterial suspensions that were comparable to the 0.5 McFarland standard were prepared, and then they were inoculated onto Mueller-Hinton agar. After placing discs containing cefotaxime, ceftazidime, and aztreonam on the surface of the agar, the mixture was then incubated at a temperature of (35) °C for a period of time ranging from 18 to 16 hours.After the incubation period had passed, the diameter of the inhibitory zones was measured, and the results were compared to the CLSI table that had been developed specifically for this particular test(26).

Phenotypic Detection of Carbapenemases

A suspension of the fresh bacterial culture that had a turbidity that was comparable to that of a 0.5 McFarland standard tube was utilized in order to inoculate a Mueller-Hinton Agar plate. After that, the suspension required a large amount of diluting (1:10). One disk of meropenem was placed without EDTA, while the other disc contained 10 µl of EDTA at a concentration of 0.5 M. Both discs were introduced into the instrument. Following the completion of the incubation period, a positive result for the detection test was obtained in the case of the disc that contained EDTA. This was accomplished by observing an increase in the diameter of the inhibition zone that was equal to or more than 7 mm (29,30).

Molecular Study

The six isolates that were found to be the most resistant were chosen for the molecular analysis. The Geneaid kit from Taiwan was utilized for the extraction of DNA, and the procedure was carried out in accordance with the manufacturer's instructions (www.geneaid.com). In preparation for further research, the isolated DNA was stored at a temperature of -20 °C.

A confirmatory molecular identification for the six isolates was conducted using 16s rRNA primers using (GoTaq G2Green master Mix Compatibility).. The presence of carbapenemases and ESBLs genes was also investigated using PCR technique. Molecular detection was carried out using thermocycler (SensoQuest GmbH, Germany). The reaction mixture (final volume 25 µl) was composed of (12.5 µl) master mix (Promega Co.), (2 µl) DNA template, (2 µl) of each of the forward and reverse primer, and (6.5 µl) distilled water (free of ions and Nuclease enzymes). Table (1) shows the reaction conditions, amplification product size, and primer sequences used in the study. The outcomes of the polymerase chain reaction (PCR) were assessed through the utilization of agarose gel electrophoresis, which consisted of 2% agarose in 1x (TAE) buffering. Visualization and confirmation of the gene bands were both made possible by the employment of a UV transilluminator.

Table 1: Primer and PCR conditions used in the study

Genes	Primer sequences	Product size (bp)	PCR conditions				Reference
			cycles	**Denaturation**	Annealing	**Extension**	
16S rRNA	27 F-AGAGTTTGATCMTGGCTCAG 1552 R-AAGGAGGTGATCCARCCGCA	1495	30	95°C 30 sec	55°C 30 sec	72°C 90 sec	(31)
bla$_{NDM}$	F-GGGCAGTCGCTTCCAAGGT R-GTAGTGCTCAGTGTCGGCAT	475	35	94°C 60 sec	55°C 45 sec	72°C 90 sec	(32)
bla$_{OXA48}$	F-TTGGTGGCATCGATTATCGG R-GAGCACTTCTTTTGTGATGGC	744	30	95°C 60 sec	55°C 60 sec	72°C 60 sec	(33)
bla$_{OXA23}$	F-GATCGGATTGGAGAACCAGA R-ATTTCTGACCGCATTTCCA	501	30	95°C 60 sec	55°C 60 sec	72°C 60 sec	(34)
bla$_{NMC-A}$	F-TGCGGTCGATTGGAGATAAA R-CGATTCTTGAAGCTTCTGCG	399	35	94°C 30 sec	46°C 45 sec	72°C 60 sec	(35)
bla$_{GES}$	F-GCTTCATTCACGCACTATT R-CGATGCTAGAAACCGCTC	323	35	94°C 30 sec	46°C 45 sec	72°C 60 sec	(35)
bla $_{VIM}$	F-GATGGTGTTTGGTCGCATA R-CGAATGCGCAGCACCAG	390	36	94°C 30 sec	52°C 4 0sec	72°C 50 sec	(36)
bla *OXA58*	F-CGATCAGAATGTTCAAGCGC R-ACGATTCTCCCCTCTGCGC	529	30	95°C 60 sec	55°C 60 sec	72°C 60 sec	(37)
bla KPC	F- TGTCACTGTATCGCCGTC R-CTCAGTGCTCTACAGAAAACC	882	30	95°C 60 sec	55°C 60 sec	72°C 60 sec	(38)
bla SIM	F-TACAAGGGATTCGGCATCG R-TAATGGCCTGTTCCCATGTG	570	36	94°C 30 sec	52°C 40 sec	72°C 50 sec	(36)
bla SPM	F-AAAATCTGGGTACGCAAACG R-ACATTATCCGCTGGAACAGG	271	36	94°C 30 sec	52°C 40 sec	72°C 50 sec	(36)

bla IMP	F-GGAATAGAGTGGCTTAAYTCTC R-CCAAACYACTASGTTATCT	188	36	94°C 30 sec	52°C 40 sec	72°C 50 sec	(36)
blaTEM	F: CATTTCCGTGTCGCCCTTATTC R: CGTTCATCCATAGTTGCCTGAC	800	35	95°C 30 sec	54°C 30 sec	72°C 30 sec	(39)
bla SHV	F:CGCCTGTGTATTATCTCCCTGTTAGCC R- TTGCCAGTGCTCGATCAGCG	843	35	95°C 30 sec	58°C 30 sec	72°C 30 sec	(39)
blaCTX-M	F: CGCTTTGCGATGTGCAG R: ACCGCGATATCGTTGGT	550	35	95°C 30 sec	52°C 30 sec	72°C 30 sec	(39)

Results

Fifty-five Gram-negative isolates were recovered from the samples collected in the study, more than one species were recovered from some samples. The most isolated species was *Escherichia coli* (56.36%), followed by *Klebsiella pneumoniae* (14.54%), *Pseudomonas aeruginosa* (12.7%),.*Proteus mirabilis* (10.9%), while *Proteus vulgaris* was the least isolated species (5.5%) as shown in Table 2.

Table 2: Bacterial species isolated in the study.

Species	**N (%)**
Escherichia coli	31(56.36%)
Klebsiella pneumoniae	8(14.54%)
Pseudomonas aeruginosa	7(12.7 %)
Proteus mirabilis	6(10 .9 %)
Proteus vulgaris	3 (5.5%)
Total	55 100%

The isolates showed absolute resistance to amoxicillin - clavulanic acid, cefotaxime, amoxicillin, and nalidixic acid, while their resistance rates to tetracycline, ciprofloxacin , and ampicillin were 96.4%, 94.6 %, and 90.9% respectively. They were also resistant to trimethoprim- sulfamethoxazole, ceftazidime, gentamicin and cefoxitin at rates of 87.2%, 80%, 58.2% and 52.7%, respectively. Aztreonam, imipenem, and meropenem showed the least resistance rates with a percentage of 27.3%, 14.5%, and 12.7 %, respectively (Table 3).

Table 3: Susceptibility testing results of bacterial isolates to the antimicrobials under study.

Antimicrobia Agent	**symbol**	**Concentration µg /disc**	**susceptible**		**Intermediate**		**resistant**	
			n	**%**	**n**	**%**	**n**	**%**
Amoxicillin-clavulanic acid	AMC	30	-	-	-	-	55	100
Ceftazidime	CAZ	30	5	9.1	6	10.9	44	80
Cefotaxime	CTX	30	-	-	-	-	55	100
Norfloxacin	NOR	30	7	12.7	4	7.3	44	80
Meropenem	MEM	10	30	54.6	18	32.7	7	12.7
Cefoxitin	FOX	30	4	7.3	22	40	29	52.7
Nitrofurantoin	F	300	2	3.64	13	23.64	40	72.73

Trimethoprim-Sulfamethoxazole	SXT	25	4	7.3	3	5.5	48	87.2
Aztreonam	ATM	30	22	40	18	32.7	15	27.3
Imipenem	IPM	10	41	74.6	6	10.9	8	14.5
Gentamicin	CN	10	17	30.9	6	10.9	32	58.2
Amoxicillin	AX	10	-	-	-	-	55	100
Ampicillin	AM	10	2	3.6	3	5.5	50	90.9
Ciprofloxacin	CIP	10	2	3.6	1	1.8	52	94.6
Nalidixic acid	NA	10	-	-	-	-	55	100
Kanamycin	K	30	5	9.1	16	29.1	34	61.8
Chloramphenicol	C	30	12	21.8	2	3.6	41	74.6
Tetracycline	TE	30	1	1.8	1	1.8	53	96.4

The bacterial species in the study varied in their resistance to antibiotics. All bacterial species showed absolute resistance to amoxicillin, amoxicillin - clavulanic acid, cefotaxime, and nalidixic acid, while the isolates of *Proteus mirabilis*, *Proteus vulgaris* and *Pseudomonas aeruginosa* showed absolute resistance to ceftazidime. *Pseudomonas aeruginosa* isolates also showed absolute sensitivity to meropenem and imipenem, while *Klebsiella pneumoniae*, *Proteus mirabilis* and *Escherichia coli* isolates were resistant to meropenem at rates of 50% ,33.3 %,3.22%, respectively (Table 4).

Table 4: Susceptibility testing results according to bacterial species.

Species	**Antibiotic**																	
	AMC		**CAZ**		**CTX**		**NOR**		**MEM**		**FOX**		**F**		**SXT**		**ATM**	
	n	**%R**	**n**	**%R**	**n**	**%R**	**n**	**%R**	**n**	**%R**	**n**	**%R**	**n**	**%R**	**n**	**%R**	**n**	**%R**
Escherichia coli (31)	31	100	21	67.7	31	100	27	87.1	1	3.22	15	48.3	17	54.8	25	80.6	6	19.3
Klebsiella pneumonia (8)	8	100	7	87.5	8	100	7	87.5	4	50	4	50	7	87.5	7	87.5	3	37.5
seudomonas aeruginosa (7)	7	100	7	100	7	100	4	57.14	-	-	7	100	7	100	7	100	1	14.28
Proteus mirabilis (6)	6	100	6	100	6	100	4	66.6	2	33.3	3	50	6	100	6	100	3	50
Proteus vulgarus (3)	3	100	3	100	3	100	2	66.6	-	-	-	-	3	100	3	100	2	66.6
	55		44		55		44		7		29		40		48		15	

ISOLATES	IMP		CN		AX		AM		CIP		NA		K		C		TE	
	n	%R	n	%R	n	%R	n	%R	n	%R	n	%R	n	%R	n	%R	n	%R
Escherichia coli (31)	2	6.45	16	51.6	31	100	30	96.7	31	100	31	100	17	54.8	24	77.41	30	96.77
Klebsiella pneumonia (8)	2	25	5	62.5	8	100	8	100	8	100	8	100	5	62.5	6	75	8	100
seudomonas	-	-	7	100	7	100	7	100	5	71.42	7	100	6	85.71	6	86	6	85.71

aeruginosa (7)																		
Proteus mirabilis (6)	3	50	3	50	6	100	4	66.6	5	83.3	6	100	4	66.6	3	50	6	100
Proteus vulgarus (3)	1	33.3	1	33.3	3	100	1	33.3	3	100	3	100	-	-	2	67	3	100
	8	-	32		55		50		52		55		34		41		53	

The most six resistant isolates were selected for further investigation. Their identification was molecularly confirmed (three isolates were *Klebsiella pneumoniae* and the other three were *Proteus mirabilis*) figure 1.

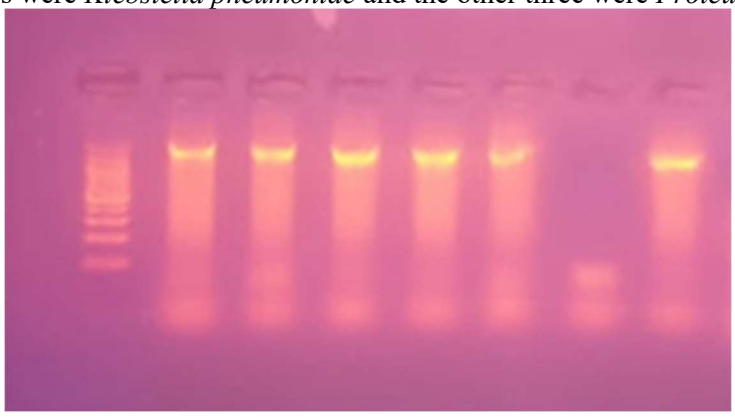

Figure 1: Molecular confirmation of isolates based on 16S rRNA sequence.

Phenotypic and molecular detection of ESBLs and carbapenemase showed that all six isolates were ESBLs producers, as the detection rates of SHV and TEM reached 100% for each, while that of CTX-M was 66.6 % (Tables 5,6) Phenotypic detection of carbapenemase enzymes gave a positive result for five isolates out of six (83.3%). Molecular detection of these enzymes showed the presence of only two enzymes, namely VIM and NDM, at rates of 83.3% and 33.3% respectively(figure2). The rest of carbapenemase genes were negatively detected (Tables 5 and 6) .

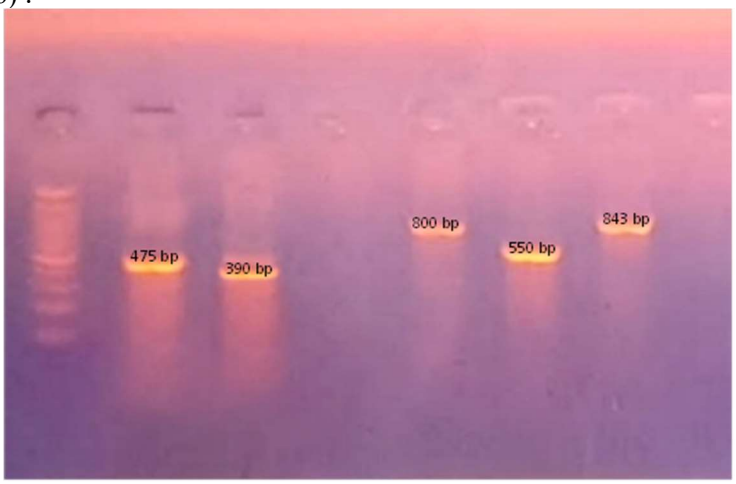

Figure 2 : Molecular detection of carbapenemase and ESBL genes in *Klebsiella pneumoniae* 2 *bla* VIM 390bp, *bla*NDM 475bp, *bla SHV843bp, blaTEM800bp, blaCTX-M550bp*

Table 5: Phenotypic detection of carbapenemase and ESBLs enzymes.

Isolate	carbapenemase	ESBLs
Klebsiella pneumoniae 1	+	+
Klebsiella pneumoniae 2	+	+
Klebsiella pneumoniae 3	+	+
Proteus mirabilis 1	+	+
Proteus mirabilis 2	+	+
Proteus mirabilis 3	-	+
6	5 (83.3%)	6 (100%)

Table 6: Distribution of carbapenemase and ESBLgenes among bacterial isolates.

Isolates	Genes													
	Carbapenemase											ESBL		
	NDM	*OXA48*	*OXA23*	*NAM-C*	*GES*	VIM	*OXA58*	KPC	SIM	SPM	IMP	TEM	*SHV*	*CTX-M*
Klebsiella pneumoniae 1	-	-	-	-	-	+	-		-	-	-	+	+	-
Klebsiella pneumoniae 2	+	-	-	-	-	+	-	-	-	-	-	+	+	+
Klebsiella pneumoniae 3	-	-	-	-	-	+	-	-	-	-	-	+	+	+
Proteus mirabilis 1	-	-	-	-	-	+	-	-	-	-	-	+	+	+
Proteus mirabilis 2	+	-	-	-	-	+	-	-	-	-	-	+	+	+
Proteus mirabilis 3	-	-	-	-	-	-	-	-	-	-	-	+	+	-
	2	0	0	0	0	5	0	0	0	0	0	6	6	**4**
	33.3%					**83.3%**						**100%**	**100%**	**66.6%**

Discussion

The respiratory system is the primary entry point for a variety of infections that are responsible for chronic problems in hens. These infections tend to be transmitted through the respiratory system. As an additional point of interest, respiratory issues are the leading cause of losses in the poultry sector (40). The chicken business is currently facing a big crisis that is producing significant economic losses all over the world. This problem is caused by respiratory illnesses, which are affecting poultry population. It has been discovered that the bacterial respiratory illnesses that are the most dangerous to hens are those that are caused by Enterobacteriaceae,

Mycoplasma gallisepticum, Staphylococcus, *Bordetella avium*, and *Pasteurella multocida* (41).

Our result is consistent with the finding of Veeraselvam (42), who recorded the isolation of *Escherichia coli*, *Pseudomonas aeruginosa*, Klebsiella species, and Salmonella species, at rates of 34.42%, 6.82%, 4.15%, and 16%, respectively. Additionally, the study of Berag (43) which was conducted in Sudan, also reported the isolation of *E. coli*, Pseudomonas species, Klebsiella species, Proteus species, and Morganella species at rates of 17.92%, 10.4%, 4.72%, 2.83%, and 11.3%, respectively. Rashid et al (44) from Iraq indicated the isolation of *Escherichia coli* from poultry infected with respiratory infections at a rate of 82.4%. Another study conducted in Egypt pointed to the isolation of P. aeruginosa at rate of 42.5% of infected poultry and the rate of isolation of this bacteria from infected liver and lungs of recently dead poultry was 22.2%. (45). On the other hand, researchers from China indicated the following isolation rates: *Escherichia coli*, 53.04%, *Proteus mirabilis* 7.92% and *Klebsiella pneumoniae* 4.67% (46). This variation isolation rates may be a reflection of the geographical region and the nature of local isolates in each location, which determines the dominant species in each.

Table (4) shows the percentage of resistance of Gram-negative bacteria to antimicrobials according to the bacterial type, where *Escherichia coli* isolates showed absolute resistance to amoxicillin - clavulanic acid, cefotaxime, and nalidixic acid and showed high resistance to ciprofloxacin, tetracycline, amoxicillin, and ampicillin. In contrast, they showed low resistance to meropenem, and imipenem. This is in consistence with a previous study conducted in Iraq which indicated that *Escherichia coli* had absolute resistance to tetracycline, trimethoprim-sulfamethoxazole, chloramphenicol and absolute sensitivity to imipenem (44).

Pseudomonas aeruginosa isolates showed absolute resistance to most antibiotics except for tetracycline and chloramphenicol, and absolute sensitivity to meropenem and imipenem. In a previous study in Egypt, these bacteria isolated from sick and dead poultry showed absolute resistance to tetracycline, trimethoprim-sulfamethoxazole, and ampicillin. They showed resistance to amoxicillin - clavulanic acid and cefotaxime at a rate of 92%, 88%, respectively (45).

Our result showed that *Klebsiella pneumoniae* isolates were resistant to many antibiotics and highly resistant to ceftazidime, norfloxacin at a rate of 87.5% for each, as well as meropenem, imipenem at a rate of 25%, 50%, respectively. In a recent study by alchalaby et al (47) conducted in Mosul, *Klebsiella pneumoniae* was absolutely resistant to amoxicillin, trimethoprim-sulfamethoxazole and sensitive to imipenem.

As for *Proteus mirabilis*, *Proteus vulgaris* they showed absolute resistance to amoxicillin - clavulanic acid, cefotaxime, and nalidixic acid, tetracycline, trimethoprim-sulfamethoxazole, while *Proteus vulgaris* showed absolute sensitivity to meropenem, kanamycin, cefoxitin, while the *Proteus mirabilis* showed resistance to meropenem, and imipenem. In Egypt a recent study indicated the absolute resistance of Proteus mirabilis to amoxicillin and trimethoprim-sulfamethoxazole, while it was resistant to ceftazidime and cefoxitin with 57.1% for each, and with a rate of 8.6% for each of meropenem and imipenem (48).

There are three basic processes that can be ascribed to the resistance of Gram-negative bacteria to β-lactam antibiotics. These drugs include penicillins, carbapenems, and cephalosporins. Porin-mediated resistance, which decreases the uptake of antibiotics, efflux pumps, which pump the antibiotic outside the cells, and enzyme-mediated resistance, which is mediated through the acquisition of β-lactamase genes, are the mechanisms that are included in this category. In most cases, the presence of an overexpression of β-lactamases that have low affinity for antibiotics is often accompanied by a decrease in the absorption of antibiotics or an increase in the efflux of medicines. These resistance determinants can have an effect on the kinetics of their diffusion, depending on the properties of the resistance determinants(49).

The results of the susceptibility test for the antimicrobials selected for the study showed that all isolates were multiple drug-resistant (MDR), in addition to the identification of 53 (96.36%) isolates as extensively drug-resistant (XDR), and 2 (3.63%) isolates as pan drug-resistant (PDR). In the event that these definitions for MDR, XDR, and PDR were used all over the world, it would be possible to compare data and would lead to a better understanding of the problem of bacteria that are resistant to interventions that involve strong antibiotics(50).

The study of ALGammal et al (48) conducted in Egypt revealed that most of the *Proteus mirabilis* isolated from poultry were XDR and these isolates contain extended-spectrum β-lactamases, where the presence rate

was 100% for both CTX-M and TEM, and the presence rate of carbapenemase represented by the NDM gene was 5.7%. Also, many studies have indicated the presence of NDM in *Proteus mirabilis* isolated from poultry (51 ,52). This is consistent with our results, which showed a detection rate of 100% for both SHV and TEM, while CTX-M was detected in 66.6% of the isolates and NDM in 33.3% of them.

In a study conducted in Iraq, researchers indicated the occurrence of CTX-M and SHV enzymes in *Klebsiella pneumoniae* isolated from poultry and the absence of TEM (47). Furthermore, it was recorded that NDM was present at a rate of 42% in *Klebsiella pneumoniae* isolated from poultry in Egypt (53).

Plastids are responsible for maintaining the prevalence of ESBL genes in poultry because of the ease with which these genes can be transferred from commensal bacteria to pathogens in poultry (54). Plasmids are transmitted to consumers through food chain and contact with animals. The transmission of ESBLs genes in animals and humans leads to failure and difficulty of treatment in both cases in addition to economic losses (55).

Human activities have been linked to the development of ESBL in the environment, particularly in aquatic habitats. This is especially true in environments where animals are present. This can occur either as a result of the transmission of determinants that contain genes that are connected with direct human activities or as a result of the release of antimicrobials into the environment as a result of indirect human activities. Both of these scenarios are possible. There is a possibility of both of these outcomes(56,57). The prevalence of ESBL varied around the world. For instance, a high rate was recorded in Germany (85.8%) , while in Lebanon the rate was 28% and in Finland less rate was recorded (14%), and so on (58,59,60).

The most common gene found in isolates producing carbapenemase, isolated from food-producing animals, was NDM gene (61). These enzymes have the ability to degrade all β-lactam antibiotics and spread rapidly. Thus, they become a serious threat to public health (62). Ali et al (63) indicated that NDM gene was prevalent with ESBL in poultry in Egypt, while VIM and KPC were not detected. In Iraq, Kanaan et al (64) noted that NDM was prevalent in bacteria causing contamination of meat and egg in poultry, whereas VIM gene was absent. Our results are consistent with the mentioned studies regarding the NDM gene. Concerning the VIM gene, the current study found that the prevalence of this gene was higher in comparison to other studies, and this may attributed to the local and dominant isolates associated with the geographical region and epidemiology of resistant strains.

Increasingly, bacteria that are resistant to carbapenems are being discovered in animals that are used for food production, which poses a significant risk to the general population's health. In spite of the fact that carbapenems have not been granted a license to be used on animals, this problem is getting more and more widespread. The rapid expansion of NDM-producing bacteria among animals that are employed for food production has been accelerated as a result of the horizontal transmission of NDM genes on mobile genetic elements. These elements include insertion sequences, transposons, and the plasmids that are connected with them. This has occurred as a consequence of the utilization of mobile genetic elements for the purpose of transmitting genes associated with NDM. Because of this, there has been a rise in the number of animals that are used in the process of producing food. This is a consequence of the fact that. Additionally, as a consequence of this, the rate at which these germs are transmitted from one individual to another has increased. As a result of the fact that, this is a consequence(65).

There was a larger incidence of bacteria that produce carbapenemase among farm laborers (67%) compared to veterinarians (33%), according to the findings of studies. Based on this information, it appears that the virus is spread by direct contact between humans and broilers on the farm. This is due to the fact that in spite of the fact that they live on the farm, farm workers are in constant and direct contact with animals. (53) There have been a number of studies that have investigated the genetic similarities that exist between carbapenemase-producing bacteria that come from human origins and those that originate from animal origins(66, 67) showing that the plasmid carrying NDM genes isolated from humans have 75 to 90% similarity to that recorded from poultry sources (68).

Conclusion

The findings of this study revealed that Gram-negative bacilli, which were isolated from broiler farms located in the city of Mosul, exhibited the presence of genes that encoded carbapenemase and extended-spectrum β-lactamase (ESBLs). These genes were shown to be present in the animals. As a consequence of the increased utilization of carbapenems in the metropolitan area, the findings of this study show that instances of such strains have emerged and spread throughout the broiler farms in the city. As a consequence of this, these farms will eventually constitute a new source for the propagation of bacteria that are capable of producing carbapenemase and ESBL within the future. The study highly recommends taking the necessary measures to control and monitor the carbapenems and the new generations of cephalosporin usage, as well as introducing antimicrobials from other categories in poultry treatment to prevent an increased dissemination of these enzymes from broiler farms to people and environment.

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Conflict of interest

There is no conflict of interest.

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Article highlights

The study detected carbapenemase and extended-spectrum β-lactamase (ESBLs) genes in Gram-negative bacilli isolated from broiler farms in Mosul city. This finding indicates the emergence and the spread of such strains in the city's broiler farms, rendering them a new source for the dissemination of carbapenemase- and ESBL-producing bacteria in the future with the growing use of carbapenems in the city