Molecular Detection of Antibiotic Resistance Genes in *E.Coli* Isolated From Poultry Meat

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Abstract

This study was conducted to determine the presence of *Escherichia coli* in fresh poultry meat. Two hundred and thirty samples of poultry meat and liver were randomly collected from different section of a poultry slaughterhouse in Ismailia Governorate. The isolation, identification, serological classification and antibiogram of *E.coli* strains using the disk diffusion technique were done. The detection of alkaline phosphatase (*pho*A) and the resistance genes of Streptomycin, Tetracycline and Quinolones (*Aad*a1, *tet*A and *qnr*S) respectively by polymerase chain reaction (PCR) for 9 isolates of different serotypes. *E.coli* was isolated with a percentage of 69.5 %. A total of 25 *E. coli* isolates were obtained, of which, O1 (five strains), O18 and O146 (four strains each), O27, O78, O86A, O125, O151 and O159 (two each). *E.coli* strains showed 100% resistance against erythromycin, penicillin and tetracycline, 92% resistance against Trimethoprim / Sulfamethoxazole, 84% resistance against Streptomycin, 80% resistance against Amoxicillin /Clavulnic acid, 72% resistance Enrofloxacin and no resistance for Gentamicin at all. The detection of *pho*A gene in *E.coli* by PCR showed 100% of strains carried the *pho*A gene sequence and all *E.coli* strains were positive with *qnr*S, *Aad*a1 *and* *Tet*A except for one strain, which were negative for *qnr*S. It could be concluded that the increasing antibiotic resistance observed in different serogroups of isolates from poultry meat may lead to the resistance to other pathogenic bacteria, beside the failure of treatment strategies for human beings.

Introduction

Poultry meat is a good source of animal protein, appealing to consumers very easily due to its sensorial attributes. Contamination can occur at several points throughout the processing operation, the most incriminating factors being the temperature abuse and improper handling or preparation ***(Ayres, 1995).*** Chicken products are suspected to be sources of foodborne pathogens and/or antimicrobial-resistant bacteria for humans ***(Asai et al., 2014).***

Multi resistant bacteria are frequently found in poultry ***(Dierikx et al., 2013).*** Their presence can be caused by selection pressure on bacteria due to the indiscriminate use of antimicrobials in aviculture

as feed additives or as therapy ***(Marshall and Levy, 2011).***

As a commensal bacterium in warm-blooded food animals, *E. coli* could be transmitted from the intestine to other carcass parts during slaughtering and processing. *E.coli* contamination in animal-derived meat products, especially chicken, is common and has been reported in many countries ***(Sheikh et al. 2012).*** *E. coli* presence in food materials is considered to be an indicator for the presence of other pathogenic bacteria in the respective food items ***(Shar et al., 2010).***

The prevalence of antimicrobial resistance among food-borne pathogens increased during recent decades ***(Akbar and Anal, 2014a).*** Antimicrobial resistance in *E. coli* is of particular concern because it is the most common Gram-negative pathogen in humans, the most common cause of urinary tract infections, a common cause of both community and hospital-acquired bacteriemia ***(Salvadori et al., 2004)*** as well as a cause of diarrhea ***(Kaper et al., 2004)***. In addition, resistant *E. coli* strains have the ability to transfer antibiotic resistance determinants not only to other strains of *E. coli*, but also to other bacteria within the gastrointestinal tract and to acquire resistance from other organisms ***(Österblad M et al., 2000).***

The specific objective of this study was planned as the following: isolation and identification of *E.coli* from poultry meat in poultry slaughterhouse, serotyping of isolated *E. coli*, detection of antibiotic resistance pattern of *E.coli* by using of the disc diffusion technique and detection of alkaline phosphatase (*pho*A) and antibiotic resistance genes; *qnr*S, *Aad*a1 and *Tet*A (A) by polymerase chain reaction (PCR).

Material and Methods

Collection of samples

Two hundred and thirty samples of poultry meat and liver were randomly collected from different section of a poultry slaughterhouse in the Ismailia city (80 before handling by workers and 150 after handling by workers) from November 2016 till April 2017. All samples were collected in sterile plastic bags and transported to the microbiology and clinical pathology laboratory in Ismailia on icebox for microbiological examination.

Bacteriological screening of clinical specimens

Collected Samples were firstly cauterized by using a hot spatula (surface sterilization) then the cauterized parts were removed by using sterilized scalpel and forceps, then inoculated in buffer peptone water and incubated at 37°C for 18±2 hrs under aerobic condition ***(ICMSF,1978)***. A loopful from each sample was separately streaked into MacConkey's agar medium and incubated at 37°C for 24hr for primary isolation. The suspected colonies were examined

for their colonial morphology, microscopical examination and biochemical characters according to ***Quinn et al. (2002).***

Serotyping of E. coli

Twenty five *E. coli* isolates were serotyped based on their somatic (O) antigens at an Animal health research institute, Dokki, Egypt.

***Antibiotic sensitivity test of E.coli isolates*ggg** to **Koneman *et al*. (1999)** and **Quinn *et al*. (2002).** Subcultures from each *E .coli* serovars were prepared and the tested for sensitivity to eight antibiotics Amoxicillin-Clavulnic acid, Enrofloxacin, Erythromycin, Gentamicin, Penicillin, Streptomycin, Trimethoprim-Sulphamethoxazole and Tetracycline.

Genotypic characterization of E. coli (polymerase chain reaction PCR):

A. Extraction of DNA according to QIAamp DNA mini kit instructions Catalogue no.**51304 (Sambrook et al., 1989)**

B. Preparation of PCR Master Mix according to **Emerald Amp GT PCR mastermix (Takara)** Code No. **RR310A** kit**:**

Emerald Amp GT PCR mastermix (2x premix) **12.5 *µl***
PCR grade water **4.5 *µl***
Forward primer (20 pmol) **1 *µl***
Reverse primer (20 pmol) **1 *µl***
Template DNA **6 *µl***

Table (1): Oligonucleotide primers sequences

Primer	Sequence	Amplified product	Reference
*pho*A	**F** CGATTCTGGAAATGGCAAAAG	720 bp	**Hu et al. (2011)**
	R CGTGATCAGCGGTGACTATGAC		
*qnr*S	**F** ACGACATTCGTCAACTGCAA	417 bp	**Robicsek et al. (2006)**
	R TAAATTGGCACCCTGTAGGC		
*aad*A1	**F** TATCAGAGGTAGTTGGCGTCAT	484 bp	**Randall et al. (2004)**
	RGTTCCATAGCGTTAAGGTTTCATT		
*tet*A(A)	**F** GGTTCACTCGAACGACGTCA	576 bp	
	R CTGTCCGACAAGTTGCATGA		

A.

B. Cycling conditions of the primers during cPCR

Temperature and time conditions of the primers during PCR are shown in the table according to specific authors and Emerald Amp GT PCR mastermix (Takara) kit

Table (2): Cycling conditions of the different primers used during the study

Gene	Primary denaturation	Secondary denaturation	Annealing	Extension	No. of cycles	Final extension
qnrs	94˚C 5 min.	94˚C 30 sec.	55˚C 45 sec.	72˚C 45 sec.	35	72˚C 10 min.
aada1	94˚C 5 min.	94˚C 30 sec.	54˚C 45 sec.	72˚C 45 sec.	35	72˚C 10 min.
tetA(A)	94˚C 5 min.	94˚C 30 sec.	50˚C 45 sec	72˚C 45 sec	35	72˚C 10 min.
phoA	94˚C 5 min.	94˚C 30 sec.	55˚C 45 sec.	72˚C 45 sec.	35	72˚C 10 min.

D. DNA Molecular weight marker

The Gel Pilot 100 bp ladder was mixed gently by pipetting up and down. 6 µl of the required ladder were directly loaded.

E. Agarose gel electrophoresis according to **(Sambrook *et al.*, 1989)**

Results

The prevalence of E.coli in poultry meat samples:

The bacteriological examination of 230 poultry meat samples revealed in 160 samples (69.5 %) positive reaction to *E.coli* (40 samples before handling by workers and 120 samples after handling by workers with a percentage of 50% and 80%, respectively

Serotyping of E.coli isolated from poultry meat samples

Serological identification of *E.coli* isolates recovered from the number of 25 poultry meat samples taken randomly from the pooled samples is revealed as the following O1(20%), O18(16%), O27(8%), O78(8%), O86A(8%), O125(8%) , O146(16%) , O151 (8%) and O159(8%).

Results of Antibiotic sensitivity test (Results of agar disc diffusion technique)

The result of antibiogram of different *E.coli* serovars isolated from poultry meat revealed that, 100% of all serovars were resistant to Erythromycin, Penicillin and Tetracycline and 100% susceptible to Gentamicin. O1 serotype is intermediately susceptible to Amoxicillin/ Clavulnic acid and Enrofloxacin; O27 intermediately susceptible to Trimethoprim/ Sulfamethoxozole, O78 and O125 were intermediately susceptible to Streptomycin and O151 serotype was intermediately susceptible to Enrofluxacin as shown in table (4).

Polymerase chain reaction (PCR) for E.coli isolates

Detection of alkaline phosphatase gene (phoA):

PCR used for identification of *E.coli* by detection of *pho*A gene which revealed that all of the samples were *E.coli* positive for alkaline phosphatase gene as shown in figure (1)

*Detection of antibiotic resistance genes; qnr*S, *Aad*a1 *and Tet*A (A) *in E.coli isolates*

Antibiotic resistance genes distribution in *E.coli* serogroups isolated from various poultry meat samples revealed that; all *E.coli* strains examined for presence of *qnr*S, *aad*a1 *and tet*A were positive except for one *E.coli* strain, which were negative for *qnr*S, as shown in table(5) figures(2,3 &4).

Table (3): Prevalence of *E.coli* serotypes from different poultry meat samples:

Serotypes		NO.	%	Before handling by workers		After handling by workers	
monovalent	polyvalent			NO.	%	NO.	%
O1	1	5	20	2	8	3	12
O18	3	4	16	2	8	2	8
O27	4	2	8	_	_	2	8
O78	4	2	8	2	8	_	_
O86A	1	2	8	_	_	2	8
O125	2	2	8	1	4	1	4
O146	2	4	16	_	_	4	16
O151	3	2	8	_	_	2	8
O159	4	2	8	2	8	_	_
Total		25	100%	9	36%	16	64%

Table (4): The results of antimicrobial sensitivity test for the examined *E.coli* strains (n=25)

Chemotherapeutic agent	S		I		R	
	No. of isolates	%	No. of isolates	%	No. of isolates	%
Amoxicillin/ clavulanic acid	0	0	5 (O1)	20%	20	80%
Enrofloxacin	0	0	7 (O1,151)	28%	18	72%
Erythromycin	0	0	0	0	25	100%
Gentamicin	25	100%	0	0	0	0
Penicillin	0	0	0	0	25	100%
Streptomycin	0	0	4(O78,125)	16%	21	84%
Tetracycline	0	0	0	0	25	100%
Trimethoprim/ sulfamethoxazole	0	o	2 (O27)	8%	23	92%

S=Sensitive **I**=Intermediate **R**=Resistant

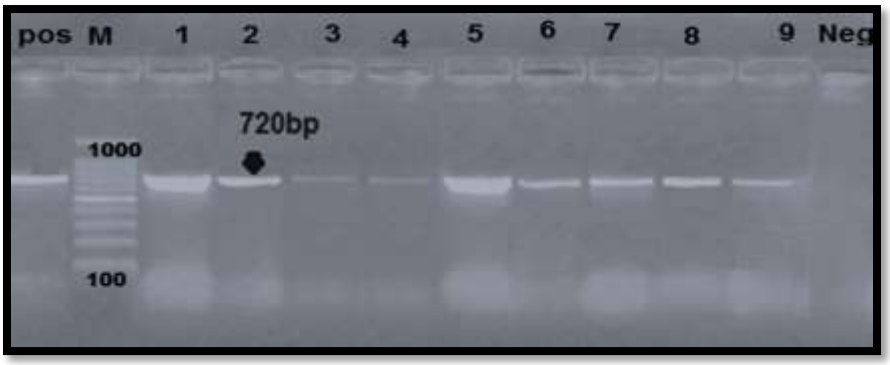

Figure (1) Agarose gel electrophoresis of amplified *pho*A gene PCR product (720bp): from randomly selected *E. coli* (9 isolates). M: molecular size marker (100bp); Positive amplifications were presented as following; lane 1, 2, 3, 4, 5, 6, 7, 8 and Lane 9. Pos: positive control sample. Neg: negative control sample.

Table (5) the distribution of antibiotic resistance genes in *E.coli* serogroups isolated from various poultry meat samples

Sample		Results		
		***qnr*S**	***Aada*1**	***Tet*A(A)**
1	**O1**	+	+	+
2	**O18**	+	+	+
3	**O27**	+	+	+
4	**O78**	+	+	+
5	**O86A**	+	+	+
6	**O125**	+	+	+
7	**O146**	+	+	+
8	**O151**	-	+	+
9	**O159**	+	+	+

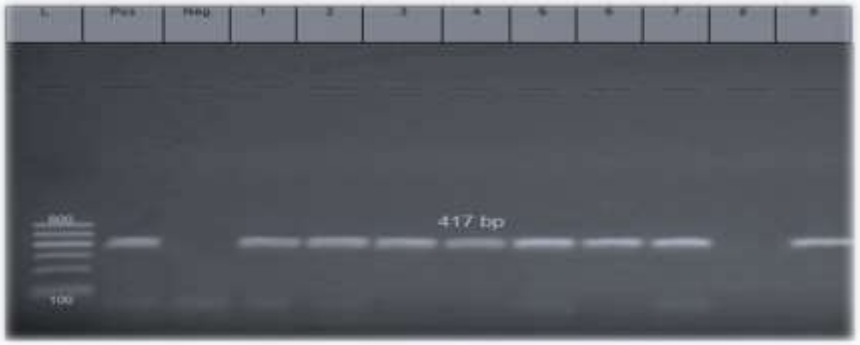

Figure (2) Agarose gel electrophoresis of amplified *qnr*S gene PCR product (417bp): M = molecular weight marker (100bp) = lanes 01to 07 and lane 9 were and lane 8 was negative. Neg = control negative reaction. Pos = control positive reaction.

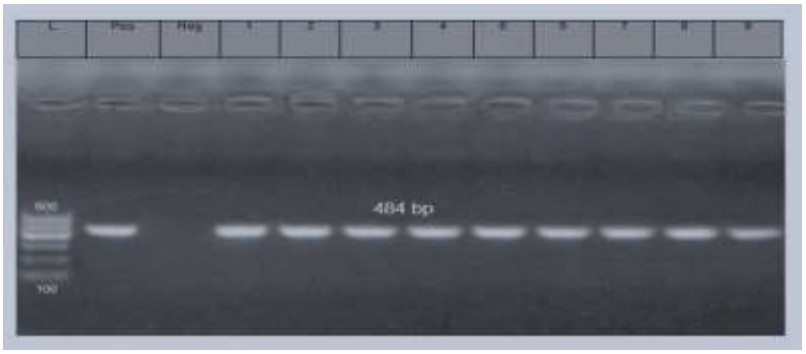

Figure (3) Agarose gel electrophoresis of amplified *Aad*a1 gene PCR product (484bp): L = molecular weight marker (100bp) = 01-0 9 *E.coli* samples positive resistance reaction to *Aad*a1 gene. Neg = control negative reaction. Pos = control positive reaction.

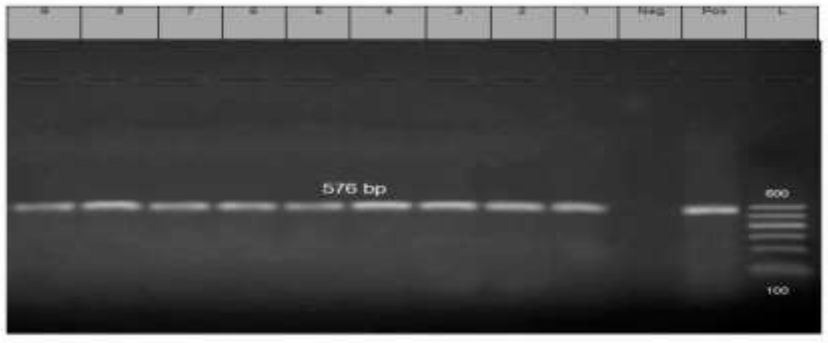

Figure (4) Agarose gel electrophoresis of amplified *Tet*A (A) gene PCR product (576): L = molecular weight marker (100bp) = 01-0 9 *E.coli* samples positive resistance reaction to *Tet*A (A) gene. Neg = control negative reaction. Pos = control positive reaction.

Discussion

In this study, *E.coli* was isolated with a percentage 69.5%. These results nearly similar to ***Bonyadian et al. (2011)*** who isolated *E. coli* from poultry carcasses with a percentage of 57.3%. Also ***Samaha et al. (2012)*** isolated *E.coli* with an incidence of 68% of chicken meat. Furthermore ,***Nosair et al. (2015)*** reported the rate of isolation of *E. coli* in frozen chicken carcasses, chilled chicken carcasses and freshly slaughtered chicken carcasses were 72% (18 isolates), 76% (19 isolates) and 80% (40 isolates), respectively.

With regard to serotyping, the predominance of *E. coli* serotype O1 (5 isolates), followed by O18, O146 (4 isolates, each), O27, O78, O86A, O125, O151 and O159 (2 isolates, each). As *E. coli* O125, O86 and O119 were previously isolated from chicken meat in Egypt by ***Saad et al. (2012).*** Also, **El-Jakee et al. (2012)** identified *E. coli* O27, O78 and O86 from chicken; while ***Nabawy et al. (2016)*** recorded the predominance of *E. coli* serotype O125 (7 isolates), followed by O142, O124, untypable (3 isolates, each), O126, O78 (2 isolates, each), O25, O127, O91, O86, O119 (one isolate, each) isolated from poultry slaughterhouse.

The result of antibiogram of different *E.coli* serovars isolated from poultry meat revealed that, 100% of all serovars were resistant to erythromycin, penicillin and tetracycline and 100% susceptible to gentamicin. O1 serotype is intermediately susceptible to amoxicillin/ clavulnic acid and enrofloxacin; O27 intermediately susceptible to trimethoprim/ sulfamethoxozole, O78 and O125 were intermediately susceptible to streptomycin and O151 serotype was intermediately susceptible to enrofluxacin. These results agreed with ***Momtaz et al. (2012)*** who found out of 57 *E. coli* isolates tested, all were resistant to one or more antimicrobial agent. Resistance to tetracycline was the most common finding (91.2%), followed by resistance to sulfamethoxazole (45.6%), chloramphenicol and trimethoprim (29.8%). All *E. coli* isolates were susceptible to streptomycin, cephalothin, gentamicin and ampicillin. ***Dayse et al., (2010)*** stated that resistance of *E.coli* isolated to amoxicillin was 5%, which opposite with the obtained result (80%). Enrofloxacin resisted the *E.coli* growth by 72% which was higher than the result reported by ***Kmet et al., (2013)*** which was (24%). Gentamicin was highly sensitive of growth of *E.coli* isolates (100%) which agreed with ***Kmet et al., (2013)*** and less agreed with ***Veldman et al.(2013)*** who reported sensitivity by 21.5%.

In the present study, all the *Escherichia coli* isolates showed positivity for the alkaline phosphatase gene (*pho*A), with an incidence of 100%. These results

were totally come in agreement with the finding of ***Abdulgayeid et al. (2015).***

Regarding the presence of antibiotic resistance genes, the results were all positive for tetracycline (*tet*A), streptomycin (*Aad*a1) and quinolones (*qnr*S) except one isolate was negative to quinolones. These results are similar to those of ***Dan et al. (2015)*** reported that all of the *E. coli* isolates tested positive to at least one antibiotic. The isolates resistant to tetracycline tested positive for *tet*A gene (66.6%). These results are matched with recordes of ***Abdulgayeid et al. (2015)*** who detected presence of *tetA* gene by 100% and higher than ***Lollai et al. (2005)*** who demonstrated that two strains out of three were harboring the *tet*A gene. ***Momtaz et al. (2012)*** found that, genes *qnr*A gene was identified in 36.8% of isolates which were lower than obtained results. According to ***Nabawy et al. (2016)*** the predominant distribution of the resistance genes in phenotypic resistant *E. coli* of *tet*A gene was (68%).

Conclusion

From the previously mentioned results, it is concluded that the higher incidence of *Escherichia coli* contamination was recorded in various poultry meat samples from different section of a poultry slaughterhouse in the Ismailia city (69.5%). These results may indicate the increased contamination or poor hygiene in poultry slaughter house. On the other hand, the increasing alarm of antibiotic resistance observed in different serogroups of *Escherichia coli* isolates from poultry meat and the burden of antibiotic resistance genes which can transfer resistance to other pathogenic bacteria, beside the failure of treatment strategies for human beings. So, it should be performed the hygienic measures and Biosafety of the slaughterhouse Also, it must advise to detect *E. coli* by the rapid test PCR for confirmation.

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الملخص العربي

دراسات عن الجينات المقاومة المضادات الحيوية للايشريشياكولاى المعزولة من لحوم الدواجن

أجريت هذه الدراسة لعزل ميكروب الايشريشياكولاى من لحوم الدواجن الطازجة حيث تم جمع مائتين وثلاثين عينة من لحوم الدواجن والكبد عشوائيا من اقسام مختلفة من مسلخ للدواجن في محافظة الإسماعيلية. حيث تم عزله ، والتصنيف المصلي ، واختبار الحساسية للمضاد الحيوي لسلالات الايشريشياكولاى باستخدام تقنيةاقراص المضادات الحيوية. وايضا الكشف عن الفوسفاتيز القلوية (*pho*A) والجينات المقاومة للمضادات الحيوية. الستربتومايسين، التتراسيكلين والكينولونات (*qnr*S وtetA،Aada1) بواسطة تفاعل البوليميراز المتسلسل (PCR) حيث تم عزل الايشريشياكولاى بنسبة 69.5٪. ونتيجة التصنيف المصلى ل 25 عزلة من الايشيرشياكولاى: O1 (خمسة سلالات)، O18 و O146 (أربع سلالات لكل منهما)، O27، O78، O86A، O125، O151 و O159 اثنين لكل منهما وقد أظهرت السلالات مقاومة 100٪ ضد الاريثروميسين والبنسلين والتتراسيكلين ومقاومة 92٪ ضد تراي ميثوبريم / سلفاميثوكسازول ومقاومة 84٪ ضد الستربتومايسين ومقاومة 80٪ ضد أموكسيسيلين / حمض كلافولنيك ومقاومة إنروفلوكساسين 72٪ ولا مقاومة للجنتاميسين على الإطلاق. بالكشف عن جينات *pho*A في الايشرشياكولاى بواسطة PCR100٪ من السلالات حملت تسلسل الجين *pho*Aوجميع سلالات الايشرشياكولاى كانت إيجابية مع *qnr*S، *Aada*1 و *tet*A باستثناء سلالة ايشرشياكولاى واحدة، والتي كانت سلبية لـ*qnr*S. ويمكن استنتاج أن مقاومة المضادات الحيوية المتزايدة التي لوحظت في مجموعات مختلفة من عزلات الايشرشاكولاى من لحم الدواجن قد تؤدي إلى مقاومة البكتيريا المسببة للأمراض الأخرى، إلى جانب فشل استراتيجيات العلاج للبشر.