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Antimicrobial Resistance Gene and Molecular Properties of *Escherichia Coli* Isolated from Meat Products in the El-Gharbia Governorate

Eman M. Abdelhady[1], Reda Abdallah[1,2*], Nader Y. Mostafa[1], Ghada A. Kirrella[1], Khaled A Abd El-Razik[3], and Ehab Ali Fouad[4]

[1]*Food Control Department, Faculty of Veterinary Medicine, Kafrelsheikh University, Egypt.*

[2]*Food Safety, Hygiene and Technology department, Faculty of Veterinary Medicine, Badr University in Cairo (BUC), Cairo, Badr City,11829, Egypt.*

[3]*Department of Animal Reproduction, Veterinary Research Institute, National Research Centre, Giza, Egypt.*

[4]*Department of Zoonosis, Veterinary Research Institute, National Research Centre, Giza, Egypt.*

Abstract

THE CURRENT investigation's objective was to identify *Escherichia coli* (*E. coli*), its virulence factors, antibiotic-resistant pattern(s), and antibiotic-resistant genes. A total of sixty samples were collected from diverse supermarkets and grocery stores located in El-Gharbia Governorate, consisting of 20 minced meat samples, 20 sausages, 10 burgers, and 10 luncheon meats. Through culturing and biochemical assays, 45 samples, representing 75%, tested positive for *E. coli* in the meat products. The prevalence was notably higher in minced meat and sausages, both at 100%, while burgers and luncheon meats showed lower rates of 40% and 0%, respectively. To evaluate the antimicrobial susceptibility of the *E. coli* samples, eleven different antimicrobial discs, which are among the most commonly used, were employed. Antibiotics including ceftriaxone, amoxicillin/clavulanic acid, ceftazidime, and erythromycin demonstrated notable sensitivity rates of 73.3%, 66.7%, and 64.4%, respectively. In contrast, there were considerable resistance levels observed for ciprofloxacin, azithromycin, and gentamicin, with resistance rates of 60%, 57.8%, and 51.5%, respectively. A PCR used to identify *E. coli* by looking for the *phoA* (alkaline phosphatase) gene showed that all samples tested positive for the bacteria. In 91.11%, 88.88%, 86.66%, and 24.44% of *E. coli* samples, the *eaeA* (intimin), *tsh* (temperature-sensitive hemagglutinin), *tetA* (tetracycline), and *mcr1*(colistin) genes were discovered, respectively. The aggregate findings suggest that consuming tainted meat products may be a potential means of spreading pathogenic *E. coli*. Concerns have been raised over the potential function of meat products as a reservoir for pathogenic *E. coli* due to the detection of the *tsh* gene mostly or solely in Avian Pathogenic *E. coli* (APEC). When combined, this may present a therapeutic problem or challenges for the consumption of meat products as well as a zoonotic risk to humans.

Keywords: *E. coli*, Meat products, *phoA, eaeA, tsh, tetA, mcr1,* PCR.

Introduction

For most people worldwide, meat and meat products are critical sources of protein, essential amino acids, minerals, vitamins, and other nutrients [1]. Nonetheless, according to Al-Mutairi et al. [1], Rocchetti et al. [2] & Lu et al. [3], they are the perfect medium for the growth and multiplication of microorganisms.

Foodborne infections are recognized as significant risks to public health, primarily due to the substantial expenses related to illness and death. This concern is especially relevant for infections caused by newly identified pathogenic bacteria that exhibit resistance to antibiotics[1] . Although the majority of foodborne pathogens typically result in self-limiting gastroenteritis, there is a possibility of more severe complications, including invasive diseases. One such example is the commensal bacterium present in the human gastrointestinal tract, *E. Coli* [4,5]. While most strains lack virulence, some have acquired traits of toxic or pathogenic virulence that make them

* Corresponding author: Reda Abdallah, E-mail: reda_abdallah@vet.kfs.edu.eg, Tel: +201000639339
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virulent in both people and animals. *E. coli* is a harmful food-borne bacterium that has been linked to several outbreaks caused by contaminated meat products [6]. Numerous virulence factors, such as *phoA, eaeA*, and *tsh*, which are expressed via virulence-associated genes, contribute to *E. coli*'s pathogenicity [7-9].

Antibiotic resistance has recently increased, especially in multi-drug-resistant bacteria (MDR), as a result of the widespread and inappropriate use of antibiotics in human medicine and animal husbandry [10-12]. Human consumption of infected meat products can lead to the rapid dissemination of resistant *E. coli* isolates and their antibiotic resistance [13,14]. Antimicrobial resistance is therefore acknowledged as one of the world's most significant public health issues of the twenty-first century [10]. Foodborne bacteria, especially antibiotic-resistant strains, present significant dangers to consumers. A large portion of foodborne illnesses stem from contaminated meat and meat products, making it essential to monitor the prevalence of *E. coli* pathotypes in these items in Egypt. This involves identifying their virulence factors and levels of antibiotic resistance. The objective of this study was to determine the prevalence of *E. coli* in various meat products through culturing and biochemical assays. Additionally, we employed PCR techniques to detect the *phoA* (alkaline phosphatase) gene, as well as virulence genes such as *eaeA* (intimin) and *tsh* (temperature-sensitive hemagglutinin), along with antibiotic resistance genes including *tetA* (tetracycline) and *mcr1* (colistin) in *E. coli* positive isolates.

Material and Methods

Sampling

A total of twenty samples of minced meat, twenty samples of sausage, ten samples of burgers, and ten samples of luncheon were collected from different supermarkets and grocery stores located in El-Gharbia Governorate, Egypt, during the period from 2023 to 2024.

Bacterial isolation and identification

All samples were rapidly placed in ice and brought to the laboratory for further analysis. The samples that were collected were incubated for one night at 37°C in MacConkey broth. Poured over MacConkey agar, loopfuls of every enriched broth were cultured for eighteen hours at 37°C. Subsequently, the putative lactose fermenter colonies were streaked onto Eosin methylene blue plates and incubated for a full day at 37°C. Gram staining, oxidase, nitrate, urease, catalase, TSI (triple sugar iron agar), coagulase, Indole, methyl red, Voges-Proskaur, citrate (IMVC), and sugar fermentation were used to identify the suspected colonies both visually and biochemically [15].

Antimicrobial susceptibility testing

The conventional disk diffusion method was used to check the antibiotic susceptibility of the purified isolates. To prepare the standardized bacterial suspension inoculums, 0.5 McFarland tubes were matched with sterile normal saline. On Muller-Hinton agar plates, the inoculums were evenly distributed. Afterward, the discs were added, and the plates were incubated for 24 hours at 37^{o}C. There were various classes of antimicrobials used. In accordance with Clinical and Laboratory Standards Institute 2020, the antimicrobial sensitivity test results were interpreted as resistant (R), intermediate (I), and susceptible (S) once the zones of inhibition were observed. Isolates classified as multi-drug-resistant (MDR) were those that showed no sensitivity to more than two distinct antibiotic classes. A total of eleven antimicrobials were utilized, which included amikacin (AK, 30 µg), ampicillin/sulbactam (SAM 10/10 µg), amoxycillin/clavulanic acid (AMC, 20/10 µg), azithromycin (AZM, 15 µg), ceftazidime (CAZ, 30 µg), ciprofloxacin (CIP, 5 µg), gentamicin (GEN, 30 µg), clindamycin (DA, 30 µg), ceftriaxone (CRO, 30 µg), erythromycin (E, 15 µg), and fosfomycin (FF, 30 µg).

DNA extraction

Using the GF-1 Bacterial DNA Extraction Kit (Cat. No. GF-BA-100, Vivantis Technologies, Malaysia), DNA was extracted from bacterial culture in accordance with the manufacturer's instruction.

Molecular identification of E. coli, virulence and antibiotic resistance genes using conventional Polymerase Chain Reaction (PCR).

All the *E. coli* bacteriologically isolated and identified were confirmed using PCR targeting *the phoA* gene, and then all *E. coli* isolates were screened for the presence of the following virulence genes: *eaeA*, *tsh,* and antibiotic resistance genes: tetracycline (*tetA)* and colistin (*mcr1*). The used primers with the expected products were listed in Table 1.

PCR reaction was performed using SimpliAmp^{TM} Thermal Cycler (Cat. No. A24811, Applied Biosysytems, USA) in a final volume of 25 µl reaction containing 12.5 µl of 2x MyTaq^{TM} Red Mix Master Mix (Cat. BIO-25043, Meridian Bioscience, UK), 1 µl (10 µM) of each primer, 1 µl of target DNA, and 9.5 µl of DDW. The PCR products were separated by electrophoresis on 1.5% agarose gel, then photographed and analyzed by the InGenius3

gel documentation system (Syngene, UK). The cycling conditions were listed in Table 2.

Results and Discussion

As indicated in Table 3, a total of 75% (45/60) of the 60 samples of meat products obtained from different supermarkets and grocery stores located in El-Gharbia Governorate, were found to be contaminated with *E. coli*. This is in line with the findings of Majueeb et al. [16,17], who identified 72.8% of the *E. coli* isolates. As reported by Anwarullah et al. [18], who identified *E. coli* with an incidence of 14.6%, other researchers isolated *E. coli* from meat products with lower incidence. Sausage and minced meat samples had a higher frequency of *E. coli* isolation from meat products than burger and luncheon samples exhibited.

The increased occurrence of *E. coli* in these specimens could potentially be attributed to improper handling practices, the utilization of unsanitary water contaminated by insects, and insufficient protocols throughout transportation, preservation, and dissemination [19].

The antibiotic susceptibility of the *E. coli* isolates present in each sample is shown in Table 4. Certain antibiotics including ceftriaxone and amoxycillin/clavulanic, ceftazidime, and erythromycin were discovered to have high sensitivities (73.3%, 66.7%, and 64.4%, respectively), whereas gentamicin, azithromycin, and ciprofloxacin were found to have high resistances (60%, 57.8%, and 51.5%, respectively).

High gentamicin resistance (60%) was found in our *E. coli* antibiotic resistance results, this is almost in line with Momtaz et al. [20], who found that 62.29% of patients had strong gentamicin resistance. Azithromycin and ciprofloxacin resistance was also found to be high, at 51.1% and 57.8%, respectively. This is almost in line with the findings of Abdulai Abass et al. [21], who found that ciprofloxacin and azithromycin had high resistance rates of 61.29% and 70.97%, respectively. Farmers' improper use of antibiotics and their disregard for hygiene are to blame for this resistance.

Alkaline phosphatase gene (*phoA*) detection revealed that all isolates tested positive for the *phoA* gene. This finding is consistent with the findings of Chang et al. [22] and Kong et al. [23], who found that all strains of *E. coli* carry the housekeeping gene *phoA*, as shown in Figures 1 and Table 5.

Ninety-one percent of the isolates under investigation tested positive for the *eaeA* gene, which is also known as the intimin gene (*eaeA*). The findings of Nguyen et al. [24], who reported a 9.8% detection rate of the *eaeA* gene, and Mohammadi et al. [25], who stated that all their isolates were *eaeA*-negative, are not consistent with this data. The identification of the intimin gene is primarily associated with the EPEC pathotype [26], and strains that are *eaeA*-positive are thought to be more virulent against humans than strains that are *eaeA*-negative. This suggests that consuming tainted meat products could spread harmful *E. coli*, as shown in Figures 2 and Table 5.

The temperature-sensitive *E. coli* hemagglutinin, which is encoded by the *tsh* gene, was initially discovered by Provence and Curtiss [25]. Additionally, according to Stathopoulos et al. [27], the *tsh* protein was the first to be recognized as a member of a growing subclass of the IgA protease family of autotransporters found in several pathotypes of *E. coli* as well as *Shigella* spp. 88.88% of the examined isolates in the current investigation proved positive for the *tsh* gene, according to the results of the PCR, as shown in Figures 3 and Table 5.

This is in contrast to Mohamed et al. [28], who found a *tsh* positive frequency of 28%, but it is almost in agreement with Janßen et al. [29] and Saidenberg et al. [30], who found the *tsh* gene in 85.3% and 78.3%, respectively. Our *E. coli* isolates were recovered from meat products; nevertheless, those authors found the *tsh* gene from the APEC (a causative agent of extraintestinal illnesses) isolated from chicken. This could be the result of an underestimation of the *tsh* gene's expression in various animal species or an APEC transmission from chicken to livestok products. This may suggest that humans who consume tainted livestock products run the risk of contracting extraintestinal illnesses.

In the examined isolates, 86.66% of the *tetA* gene—a tetracycline-resistant gene—was detected, as shown in Figure 4 and Table 5. The *tetA* gene was discovered by 88%, 84.2%, 76%, and 88% of the researchers in Daini & Adesemowo [31], Hilbert D.W. [32], Dehkordi et al. [33], and Balasubramaniam et al. [34], respectively. This is almost in agreement with their findings. Although Momtaz et al. [35] and Momtaz et al. [30,36], found the *tetA* gene with 52.63% and 51.63%, respectively, we disagree with their findings.

In the examined isolates, 24.44% of the *mcr1* gene (colistin) was detected, as shown in Figures 5 and Table 5. Joshi et al. [37] and Islam et al. [38,39], who found the *mcr1* gene by 22.8% and 36.4%, respectively, almost agree with this.

The high levels of *E. coli* resistance to antibiotics in the examined samples indicated cross-contamination in local butcher shops, slaughterhouses, storage facilities, and distribution and processing facilities [40]. Their usual medication can be used to treat the low resistance. Before consuming meat, it should be fully cooked to avoid infection in humans.

Conclusion

Meat products are thought to provide an excellent growth medium for several foodborne bacteria. This bacterial species was contaminated during the items' production, distribution, storage, mincing, packaging, and retail sale, which led to its presence in the finished goods. This increases the consumer's health hazards. Thus, it is possible to limit the spread of these dangerous pathogens and produce final products with the highest level of safety by using appropriate handling techniques, thorough hand and surface cleaning, disinfecting the market, practicing good personal hygiene, and following good hygienic practices both during manufacturing and retail sales. PCR is a quick and accurate technique for identifying several foodborne pathogens in samples of meat products. It allows for the quick detection of virulence genes, antibiotic resistance, and bacteria cells, and PCR has been shown to be a reliable identification technique.

Conflict of interest statement

The authors have declared that they have no competing interests with regard to this article's publication.

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Author details:

Eman M. Abdelhady, BVSc, MVSc, Department of Food Control, Faculty of Veterinary Medicine, Kafrelsheikh University, Kafr El Sheikh 33516, Egypt., E-mail: emanelmoselhy2000@gmail.com Tel.: 00201010164752.

Reda Abdallah: BVSc, MVSc, PhD, Department of Food Control, Faculty of Veterinary Medicine, Kafrelsheikh University, Kafr El Sheikh 33516, Egypt. reda_abdallah@vet.kfs.edu.eg mobile: +201000639339; ORCID ID https://orcid.org/0000-0002-020-1441

Nader Y. Mostafa, BVSc, MVSc, PhD, Department of Food Control, Faculty of Veterinary Medicine, Kafrelsheikh University, Kafr El Sheikh 33516, Egypt. Nader@vet.kfs.edu.eg mobile:+2001223260958.

Ghada A. K. Kirrella: BVSc, MVSc, PhD, Department of Food Control, Faculty of Veterinary Medicine, Kafrelsheikh University, Kafr El Sheikh 33516, Egypt. ghada.karala@vet.kfs.ed.eg mobile: +201005582816.

Khaled A Abd El-Razik, Department of Animal Reproduction, Veterinary Research Institute, National Research Centre, , El Behouth Street, Cairo 12622, Egypt, Giza, Egypt, khaled707@hotmail.com mobile:+201223670455.

Ehab Ali Fouad, Department of Zoonosis, Veterinary Research Institute, National Research Centre, El Behouth Street, Cairo 12622, Giza, Egypt ORCID ID(0000-0002-2144-0043) ehabfoaud@gmail.com mobile:+201222784463.

Figures and Tables list

TABLE 1. PCR primers used in the study

Gene	Sequence (5´-3´)	Amplicon size (bp)	Reference
phoA	CGATTCTGGAAATGGCAAAAG CGTGATCAGCGGTGACTATGAC	720 bp	Hu et al. (2011)
eaeA	ATGCTTAGTGCTGGTTTAGG GCCTTCATCATTTCGCTTTC	248 bp	Guion et al. (2008)
Tsh	GGTGGTGCACTGGAGTGG AGT CCA GCG TGA TAG TGG	620bp	Dozois et al. (2000)
tetA	GGTTCACTCGAACGACGTCA CTGTCCGACAAGTTGCATGA	577bp	Randall et al. (2004)
mcr1	CGGTCAGTCCGTTTGTTC CTTGGTCGGTCTGTA GGG	309bp	Moosavian and Emam (2019)

TABLE 2. Cycling conditions for the detection of genes in this study

Gene	Initial Denaturation	Denaturation	Annealing	Extension	Final Extension	Cycles
phoA	94°C 5min	94°C 30sec	55°C 30sec	72°C 45sec	72°C 10min	*35*
eaeA	94°C 2min	94°C 20sec	53°C 30sec	72°C 45sec	72°C 10min	35
tsh	94°C 5min	94°C 30sec	55°C 30sec	72°C 45sec	72°C 10min	35
tetA	95˚C 3min	95˚C 20sec	56˚C 30sec	72˚C 45sec	72˚C 7min	35
mcr1	95˚C 3min	95˚C 20sec	56˚C 30sec	72˚C 45sec	72˚C 7min	35

TABLE 3. Prevalence of *E. coli* in different types of meat products:

	Meat samples	
Sample	**Incidence**	**Percentage**
Minced meat	20/20	100%
Sausage	20/20	100%
Burger	4/10	40%
Luncheon	0/10	0%
Total	45/60	**75%**

TABLE 4. Antibiotic sensitivity of *E. coli* isolates

Antibiotics	Sensitive	Resistant
Amikacin (AK, 30 µg)	28[a](62.2%)[b]	17[a](37.8%)[b]
Amoxycillin/clavulanic (AMC, 20/10 µg)	30(66.7%)	15(33.3%)
Ampicillin/sulbactam (SAM 10/10 µg)	27(60%)	18(40%)
Azithromycin (AZM, 15 µg)	19(42.2%)	26(57.8%)
Ceftazidime (CAZ, 30 µg)	30(66.7%)	15(33.3%)
Ciprofloxacin, (CIP, 5 µg)	22(48.9%)	23(51.1%)
Gentamicin (GEN, 30 µg)	18(40%)	27(60%)
Clindamycin (DA, 30 µg)	26(57.8%)	19(42.2%)
Ceftriaxone (CRO, 30 µg)	33(73.3%)	12(26.7%)
Fosfomycin (FF, 30 µg)	28(62.2%)	17(37.8%)
Erythromycin(E,15µg)	29(64.4%)	16(35.6%)

a; number of positive isolates, b; percentage of positive isolates.

TABLE 5. Genetic determinant of *E. coli phoA* gene, virulence factors and antimicrobial resistance genes test.

GENE	Meat samples (45)	
	Incidence	**Percentage**
phoA	45	100%
eaeA	41	91.11%
tsh	40	88.88%
tetA	39	86.66%
mcr1	11	24.44%

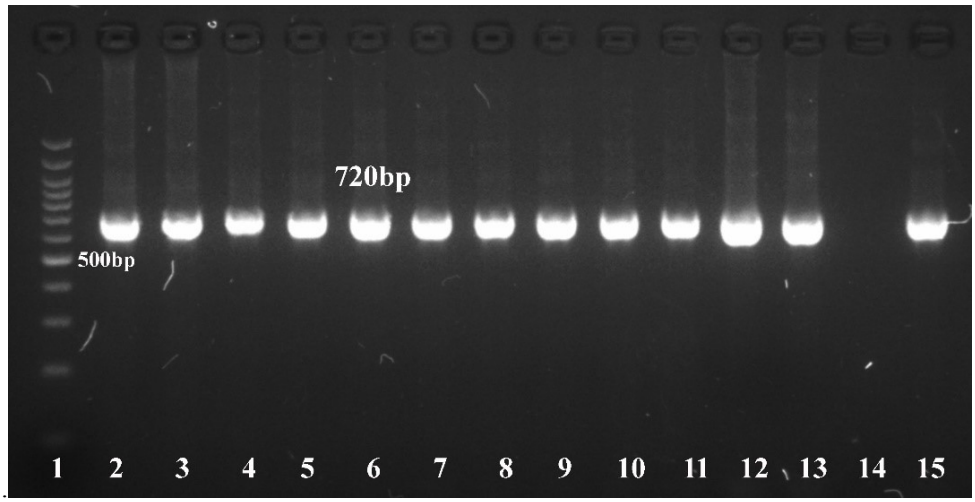

Fig. 1. Agarose gel electrophoresis of *E. coli* PCR product. Lane1: 100bp DNA ladder, Lane 2-13: Amplified *phoA* gene (720 bp), Lane 14 negative control and Lane 15 positive control.

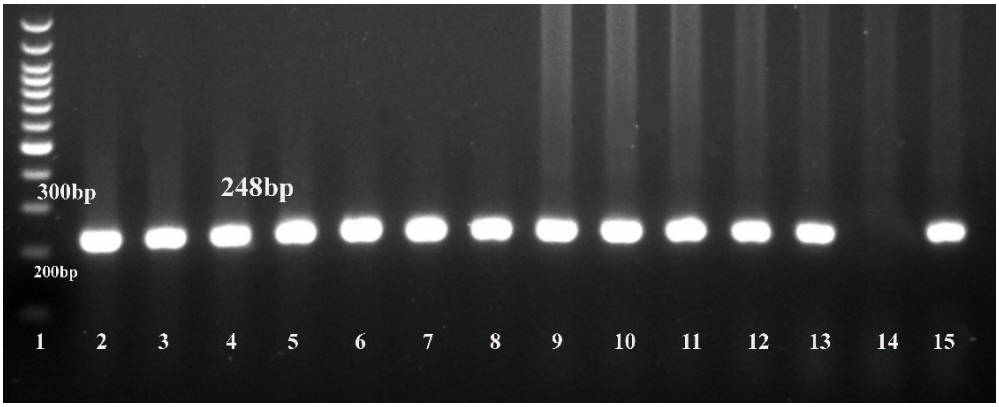

Fig. 2. Agarose gel electrophoresis of *E. coli* PCR product. Lane1: 100bp DNA ladder, Lane 2-13: Amplified *eaeA* gene (248 bp) Lane 14 negative control and Lane 15 positive control.

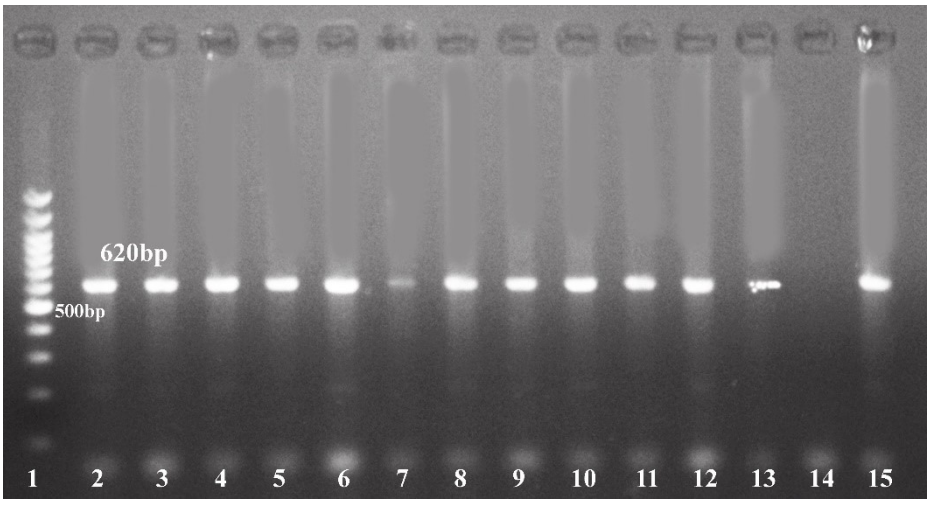

Fig. 3. Agarose gel electrophoresis of *E. coli* PCR product. Lane1: 100bp DNA ladder, Lane 2-13: Amplified *tsh* gene (620 bp), Lane 14 negative control and Lane 15 positive control.

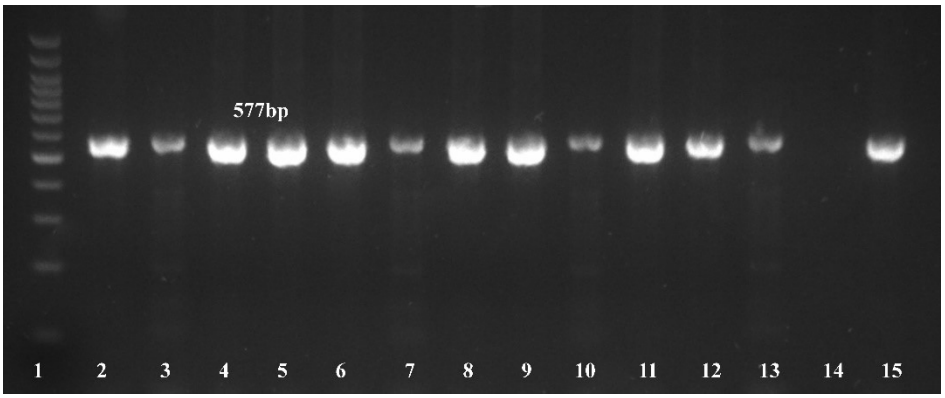

Fig. 4. Agarose gel electrophoresis of *E. coli* PCR product. Lane1: 100bp DNA ladder, Lane 2-13: Amplified *tetA* gene (577 bp), Lane 14 negative control and Lane 15 positive control.

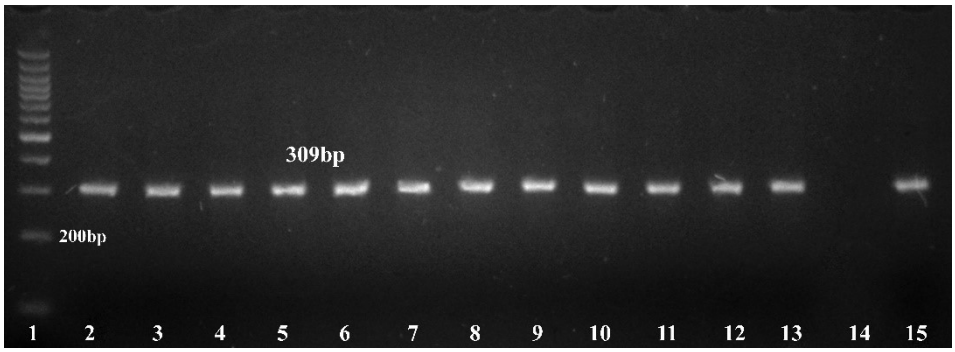

Fig. 5. Agarose gel electrophoresis of *E. coli* PCR product. Lane1: 100bp DNA ladder, Lane 2-13: Amplified mcr_1 gene (309 bp), Lane 14 negative control and Lane 15 positive control.

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جين مقاومة المضادات الحيوية والخصائص الجزيئية لبكتيريا الإشريكية القولونية المعزولة من منتجات اللحوم في محافظة الغربية

ايمان محمد عبد الهادي[1] ، رضا عبد الله [1،2] ، نادر يحيي مصطفي[1] ، غادة عبد العاطي قرله[1]، خالد عبد الرازق[3] وايهاب علي فؤاد[2]

[1] قسم مراقبة الاغذية - كلية الطب البيطري- جامعة كفر الشيخ - مصر.

[2] قسم الامراض المشتركة - معهد البحوث البيطرية، المركز القومي للبحوث، الجيزة، مصر.

[3] قسم التكاثر الحيواني، معهد البحوث البيطرية، المركز القومي للبحوث، الجيزة، مصر.

الملخص

كان الهدف من التحقيق الحالي هو تحديد الإشريكية القولونية، وعوامل ضراوتها، وأنماط مقاومة المضادات الحيوية، والجينات المقاومة للمضادات الحيوية. تم جمع ستين عينة من مواقع مختلفة داخل محافظة الغربية، تتكون من 20 قطعة من اللحم المفروم، و20 سجق، و10 برجر، و10 لانشون . من خلال استخدام زراعة العينات والاختبارات الكيميائية الحيوية، تم العثور على 75% عينة إيجابية من الإشريكية القولونية في منتجات اللحوم؛ وكانت النسبة أعلى في اللحم المفروم والسجق (100%)، وأقل في لحم البرجر والانشون (0% و40% على التوالي). تم استخدام أحد عشر قرصًا مضادًا للميكروبات - الأكثر استخدامًا - لتقييم حساسية عينات الإشريكية القولونية للمضادات الميكروبية. تم العثور على أن المضادات الحيوية الفردية مثل سيفترياكسون وأموكسيسيلين/كلافولانيك، وسيفتازيديم، وإريثروميسين تتمتع بحساسية عالية (73.3%، 66.7%، و64.4% على التوالي). ومع ذلك، كانت هناك مقاومات كبيرة للسيبروفلوكساسين، والأزيثرومايسين، والجنتاميسين (60%، 57.8%، و51.5% على التوالي). أظهر اختبار تفاعل البوليميراز المتسلسل (PCR) المستخدم لتحديد بكتيريا الإشريكية القولونية من خلال البحث عن جين *phoA* أن جميع العينات التي تم اختبارها كانت إيجابية للبكتيريا. تم اكتشاف جينات *eae(A) وtsh وtetA(A) وmcr1* في 91.11% و88.88% و86.66% و24.44% من عينات الإشريكية القولونية، على التوالي. تشير النتائج الإجمالية إلى أن استهلاك منتجات اللحوم الملوثة قد يكون وسيلة محتملة لنشر الإشريكية القولونية الممرضة. تم إثارة المخاوف بشأن الوظيفة المحتملة لمنتجات اللحوم كمصدر لجراثيم الإشريكية القولونية الممرضة، وذلك بسبب اكتشاف جين tsh في الغالب أو بشكل حصري في الإشريكية القولونية الممرضة للطيور. (APEC). عند الجمع بينهما، قد يمثل ذلك مشكلة علاجية أو تحديات لاستهلاك منتجات اللحوم بالإضافة إلى خطر حيواني المنشأ على البشر..

الكلمات الدالة: الإشريكية القولونية، منتجات اللحوم، *phoA، eae(A)، tsh، tetA(A)، mcr1*، تفاعل البوليميراز المتسلسل (PCR).