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Prevalence, Virulence and Antibiotic Resistance of *Escherichia coli* Isolated From Fresh, Chilled and Frozen Retail Marketed Beef in Egypt

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Abstract

EXISTANCE of antimicrobial-resistant pathogenic *E. coli* in meats is of great concern due to its harmful effects on human health. Therefore, this study aimed to detect pathogenic *E. coli* in retail beef samples. Herein, seventy-five retail beef samples representing 25 from each fresh, chilled and frozen samples were randomly collected from various butcher shops located in EL-Minya and Beni-Suef governorates. Collected samples were assessed for their microbiological quality using the most probable number technique for enumeration of coliforms, fecal coliforms and *E. coli*. Additionally, *E. coli* isolates were subjected to biochemical, serological and molecular identifications. Antibiotic sensitivity test was conducted by disc diffusion method to evaluate the antibiotic resistance of the isolates. The results showed that the incidence of *E. coli* pathogen in the fresh, chilled and frozen beef samples was 16%, 4% and 4% respectively. The identified serovars in fresh samples were O44: K74 (8%), O111: K58 (4%), and O55:K59 (4%), in chilled was O44:K74 (4%) and in frozen samples was O25:K11 (4%). Genetically, the most prevalent gene was EaeA, followed by ChuA, AdrA, LuxS, and Stx1 with percentages accounted for 100%, 83%, 83%, 83%, 83% and 16%, respectively. All the isolated strains were multi drug resistant (MDR) when examined against different types of antibiotics. It was concluded that the existence of virulent *E. coli* in meat is widely distributed which has a public health significance and need periodical evaluation of the hygienic status of Egyptian meat markets to prevent food poisoning and public health hazards.

Keywords: Beef, Coliform, E. coli, Food safety, MDR, Pathogens, Virulence genes.

Introduction

Meat may become a source of food poisoning organisms due to its perishable nature and richness in nutrients which provides the growth of different microorganisms. Despite of the sterility of muscles of healthy animals, meat could be contaminated by germs from different sources. These sources could be found in the slaughter house including the feet, mud, hides of slaughtered animals, intestinal content, the tools used for dressing, the air and water used to wash the carcasses [1].

Escherichia (E) coli is a common gram negative facultative anaerobic faecal coliform and a member of the Enterobacteriaceae which normally inhabit gut of animals and human but certain strains of *E. coli* have the capacity to acquire pathogenic and virulent genes, even if not all of them are thought to be harmful [2].

The virulence genes of *E. coli* strains are detected by PCR it is a powerful molecular biology technique it is not only highly sensitive and specific also it provides rapid and reliable results [3].

There are some methods which could decrease the carcass biological contamination and limit the occurance of food poisoning such as rapid chilling of adequately spaced carcasses due to surface dehydration during chilling.

Antimicrobial resistance (AMR) is defined as the resistance of microorganisms to an antimicrobial agent this phenomenon enhanced by the misapplication of antimicrobial medicines, the global spread of AMR mainly affects unhealthy and debilitated animals [4]. So, it was found that 61.6% of the *E. coli* isolates from beef samples were multiresistant to antibiotics that mean they

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were resistant to at least three different antibiotics classes [5].

Therefore, this study aimed to detect pathogenic *E. coli* strains in retail beef samples either fresh, chilled or frozen, as well as assess the microbiological quality of these beef samples using the most probable number technique for enumeration of coliforms, fecal coliforms and *E. coli*. Additionally, *E. coli* isolates were subjected to biochemical, serological and molecular identifications. Antibiotic sensitivity test was conducted by disk diffusion method to evaluate the antibiotic resistance of the isolates.

Material and Methods

Chemicals and growth media

All bacterial growth media were purchased from Oxoid (Hampshire, United Kingdom). Antimicrobial discs used for antimicrobial resistance testing were obtained from Sigma (Aldrich).

Sampling collection

A total of 75 random fresh, chilled and frozen beef samples (25 of each) from animals which were slaughtered at regular slaughterhouses as the general Minya slaughterhouse and Bush slaughterhouse in Beni-Suef. The samples were collected from different butcher's shops and hypermarkets at Beni-Suef and El-Minya governorates. All collected samples were undergo the following bacteriological examination after being individually placed in clean, sterile plastic bags identified and transported in an insulated ice box to the department of food safety and technology's laboratory, faculty of veterinary medicine Beni-Suef University under fully aseptic conditions without delay.

Isolation and Identification of bacterial pathogens

Preparation of sample homogenate

The samples will be prepared according to the method recommended by [6] as following: -

25 grams of each collected sample was aseptically taken using sterile scissors and forceps and then transferred to a sterile homogenizer flask containing 225 ml of 0.1% sterile buffered peptone water (Oxoid, CM0509) to prepare the first dilution (10^{-1}). The contents of the flask were thoroughly mixed by shaking and one milliliter was transferred into a separate sterile test tube containing 9 ml of 0.1% sterile buffered peptone water (Oxoid, CM0509) and so on until dilution of (10^{-6}) was prepared.

Bacteriological examination techniques

Most probable number of total coliforms (T.C.), fecal coliforms (F.C.) and *Escherichia coli* (*E. coli*) was done in a three-tube series containing inverted Durham's tubes [6]. The MPN of TC was calculated using lauryl sulphate tryptose broth (Oxoid Code: CM0451) at 35^{o}c for 24-48h for the presumptive test and brilliant green bile broth (Oxoid Code: CM0031) at 35^{o}c for 24-48h for the confirmative test. The MPN of F.C. was calculated using E.C. (Oxoid, CM0853) broth tubes containing inverted Durham's tubes at 45^{o}c for 24h. For *E. coli* confirmation (Eijkman test) gas positive tubes from E.C broth were plated onto Eosin Methylene Blue Agar (EMB) agar (Oxoid, CM0069) at 35^{o}c for 24h.The metallic-sheen characteristic colonies were moved to nutrient agar slope tubes, Gram-stained and put through the biochemical tests known as IMViC (Indole, Methyl Red Test, Voges-Proskauer Test, and Citrate Production).

Identification of Escherichia coli

The identification was done by characteristics of the culture and Gram's-stained bacterial films. The typical colonies showing the aspects of *E. coli* were confirmed by oxidase, indole, methyl red, and citrate utilization test according to [6]. Then the cultures which displayed the characteristic biochemical results of *E. coli*, were kept for serological identification. The serological identification was done in the (Animal Health Research Institute), Egypt based on the somatic antigen (O) by slide agglutination method using eight polyvalent and 43 monovalent antisera specific for *E. coli* according to [7]. The *E. coli* antisera sets were from (Denka Seiken Co., Japan).

Using a drop of physiological saline a homogeneous suspension of every isolate was made on a glass slide. After that, a drop of the specified *E. coli* polyvalent antisera was added, and everything was mixed thoroughly. In about one-minute, positive agglutination was clearly visible and could be detected by nacked eye. A partial or delayed agglutination was regarded as negative. For the positive polyvalent one, the same procedure as previously mentioned was used with the monovalent sera.

Antimicrobial sensitivity test:

All biochemically and serologically identified *E. coli* strains were tested against 9 antimicrobial agents to detect their antimicrobial resistance using the disc diffusion method according to the CLSI standards [8]. The test was done on Mueller-Hinton agar (MHA) plates (Oxoid, CM0337B).

The used antibiotic disks were Ampicillin (Amp) 10 µg, Kanamycin (K) 30 µg, Colistin (CT) 25 µg, Sulfamethoxazole-Trimethoprim (Sxt) 25 µg, Tetracycline (Tet) 30 µg, Chloramphenicol (C) 30 µg, Doxycycline (DO) 30 µg, Spiramycin (SP) 100 µg and Gentamicin (Gn) 10 µg.

The diameter of the clear zone of inhibition surrounding each antimicrobial disk was measured using a ruler, the data were categorized as susceptible, intermediate, or resistant after being interpreted using the [8], multi-drug resistant strains were detected.

Molecular identification of virulence and pathotype genes in the identified isolates.

Amplification of *E. coli* specific virulence, pathotype and quorum genes: It is performed at (the Biotechnology Unit at the Animal Health Research Institute, Egypt), data on the gene sequences were illustrated in Table (1)

DNA extraction:

DNA extraction from bacterial colonies was performed using the QIAamp DNA Mini kit (Qiagen, Germany, GmbH) Briefly, 200 µl of the sample suspension was incubated with 20µl of proteinase K and 200 µl of lysis buffer at 56^{o}c for 10 min. After incubation, 200 µl of 100% ethanol was added to the lysate. The sample was then washed and centrifuged.

PCR amplification:

Primers as stated in (Table 1) were utilized in a 25-µl reaction then thermocycling parameters were summarized as follows, an initial denaturation cycle at 94^{o}c for 5 minutes then 30 cycles of the subsequent program 94^{o}c for 30 sec, the annealing temperature was 55^{o}c for 45 sec. for each primer. The final extension stage was 72^{o}c for 7 min.

Agarose gel electrophoresis:

The products of PCR were separated by electrophoresis on 1% agarose gel. (Applichem, Germany, GmbH)

Results

The bacteriological evaluation of fresh, chilled and frozen beef samples in table (2) revealed that coliforms (MPN/g) in the examined fresh samples were ranged from 7.4 to > 11×10^2 with mean value 6.869×10^2 ± 60.9, while in chilled samples were ranged between 3.6 and >11×10^2 with mean value 8×10^2 ± 43.9 and in frozen samples were ranged from 3 to >11×10^2 with a mean value 1.80×10^2± 53.5. The fecal coliforms MPN in fresh samples were ranged between <3 and 11×10^2 with mean value 1.984×10^2±22.1, while in chilled samples were ranged from 3 to 11×10^2 with a mean value 5.468×10^2±72.46 and in frozen samples the range was from <3 and 11×10^2 with mean value 63±40 while *E. coli* MPN in fresh samples were ranged between <3 to 35 with mean 4.5±2.1, in chilled <3 to 210 with mean12±2.6 and in frozen <3 to 20 with mean 3±1.

According to the permissible limit of coliforms reported by Egyptian Standard organization for fresh, chilled and frozen beef samples. We found that 14 out of 25 samples of fresh beef were unaccepted for human consumption while in chilled beef all samples were unfit for public consumption and in frozen beef 6 samples unaccepted for human consumption because they were higher than permissible limit of coliforms reported by Egyptian Standard organization for fresh, chilled and frozen beef samples which was (1000, Zero and 100 organisms per gram), respectively.

The results in table (3) finally showed the highest rate of *E. coli* was in fresh samples (16%) followed by chilled and frozen beef samples with (4%) in each one while percentage of isolates in relation to number of suspected *E. coli* isolates respectively 44.44%, 10% and 7.69%. As well as the percentage of *E. coli* isolates biochemically identified in relation to the number of suspected *E. coli* isolates in fresh, chilled and frozen beef samples were (66.66%, 20%, 7.67%) respectively.

This lower percentage of *E. coli* in chilled and frozen beef samples than in fresh beef samples may be due to the low temperature during refrigeration and freezing will affect on the growth of it, because of surface dessication and dehydration.

The results in table (4) illustrated the serotyping of *E. coli* isolates revealed from the examined beef samples. The 6 serotypes were two strains of (O44: k74) (EPEC, EAEC, EHEC) with percent (8%) in relation to examined beef samples and 33.3% in relation to total serotyped *E. coli* isolates, O55: k59 (EPEC, EHEC) (4%) in relation to examined beef samples and 16.66% in relation to total serotyped *E. coli* isolates. and O111: k58 (EPEC, EAEC) (4%) in relation to examined beef samples and 16.66% in relation to total serotyped *E. coli* isolates these serotypes were detected in fresh beef samples. Also, one strain of O44: k74 (EPEC, EAEC, EHEC was isolated from chilled beef samples (4%) in relation to examined beef samples and 16.66% in relation to total serotyped *E. coli* isolates. and one strain O25: k11(EPEC, EAEC) from the frozen beef samples with percentage (4%) in relation to examined beef samples and 16.66% in relation to total serotyped *E. coli* isolates.

Antimicrobial resistance (AMR) tests were performed on all of the *E. coli* strains detected in this study which were performed using the disk diffusion method according to the CLSI standards [8] on Mueller-Hinton agar.

The current data in table (5) showed all the isolated strains of *E. coli* were (MDR), O111, O55 (fresh beef) and O44 (chilled) were intermediate susceptible to Gn (Gentamicin, 10 µg) but were resistant to K (Kanamycin, 30 µg), Amp (Ampicillin,10 µg), SP (Spiramycin,100 µg), DO (Doxycycline,30 µg), Sxt (Sulfamethoxazole-Trimethoprim, 25µg), Tetracycline (Tet) 30µg, CT (Colistin, 25 µg), C (Chloramphenicol, 30 µg). The

previous mentioned serotypes with resistance percentage 0% for gentamycin and 100% for another antibiotics (Fig. 1).

The other serotypes are (MDR) with resistant % (100) for eight antibiotics which were used in the present study.

Table (6) represent the virulence genes which were detected in the 6 isolates which were analyzed using the conventional PCR in the serologically identified strains (Fig. 2). The most prevalent gene was (EaeA) gene which followed by chuA (pathotyping gene), adrA (biofilm gene), LuxS and stx1 with percent (100%, 83%, 83%, 83%, 83%, 16%), respectively.

Discussion

The mean value of coliforms and *E. coli* in fresh samples were lower than the critical limit reported by [9] for fresh meat which is 1000cfu/g for coliforms and 500cfu/g *E. coli*, Also the mean value of coliforms in the present study lower than reported by [10] from different locations in Ethiopia, with mean values (3.36×10^4, 3.73×10^4, 5.80×10^4). Therefore, high prevelance of microbial contamination of raw beef in [10] study might be related to improper handling of animals during slaughter, unhygienic dressing, evisceration, transportation and marketing. Also, high temperature of his study area would have contributed in increasing the microbial contamination.

The mean value of fecal coliforms in fresh samples higher than study stated by [11] which its range (1.83-4.73). but lower than stated by [12] where fecal coliforms mean was $2\times10^2 \pm 10^2$.

The mean value of coliforms and *E. coli* in chilled beef exceeded critical limit reported by [9] for chilled which is zero for both coliform and *E. coli* per gram. But mean of coliforms and *E. coli* lower than reported by [13] with values ($4.2x10^3 \pm 0.03\times10^3$) ($0.3 \pm 0.3$) CFU/g, respectively.

The fecal coliforms in chilled samples were ranged from 3 to 11×10^2 with a mean value $5.468\times10^2\pm72.46$ which was higher than study reported by [14] which its value ranged from 108.07 to 210.

Coliforms and *E. coli* mean values in frozen beef exceeded critical limit reported by [15] for frozen meat which is 100/g for coliforms and zero/g for *E. coli*. But lower than the results mentioned by [16] with mean value of coliforms in frozen beef ($7.1x10^3 \pm 0.02\times10^3$).

There is a significant difference between the means of coliform and *E. coli* in different samples at $p < 0.05$.

The prevalence of *E. coli* in fresh beef samples in the present study (16%) disagreement with that reported by [16] with prevalence (4%). However, the results were near to those reported by [17].

E. coli prevalence in frozen samples was lower than that reported by [18], [19], [20]. who isolated *E. coli* with percentage (5.71%, 11.1% and 30%), respectively. However, low figure has been reported in Egypt by [21] who isolated *E. coli* from samples of imported frozen meat with percentage 2.86% and 1.42% and also in alignment with that reported by [13] where prevalence 4% in chilled and 2% in frozen sample.

The overall variation in the previously mentioned prevalence could be attributed to many causes including variations in hygiene, breed, geographic origin, sampling and isolation techniques, fecal and hide contact with the carcass, methods of meat transportation to the butcher house, rumen content removal techniques, abattoir conditions, study design, temperature, and antimicrobial substance treatment during the process (disinfectants).

Through this investigation beef samples from supermarkets and meat shops with low *E. coli* percentage than fresh beef. Because the way meat is sold in traditional markets is more open than in supermarkets and customers can select meat by easily touching or holding it. In addition to temperature difference.

The serological identified isolates in fresh beef samples were closely same to [17] who detected O44 and O111 isolates from fresh samples and differ than that reported by [22] who isolated one strain (O18) from fresh. Serotypes in chilled and frozen differ than detected by [13] who isolated O55, O125 from chilled and O114 from frozen samples.

The percentage of serological identified isolates in relation to number of suspected *E. coli* isolates in fresh, chilled and frozen samples (44.44%, 10% and 7.69%), respectively.

"Multi Drug Resistant" (MDR) refers to strains that show resistance to three or more antimicrobial agent types [23]. The current data in table (5) showed all the isolated strains of *E. coli* were (MDR). O111, O55 (fresh beef) and O44 (chilled) were intermediate susceptible to Gn (Gentamicin, 10 µg) but resistant to other antibiotics.

In addition to O25 (frozen) and O44 (fresh) were resistant to all antibiotic K (Kanamycin, 30 µg), Amp (Ampicillin,10 µg), SP (Spiramycin,100 µg), DO (Doxycycline,30 µg), Sxt (Sulfamethoxazole-Trimethoprim, 25 µg), Tetracycline (Tet) 30µg, CT (Colistin, 25 µg), C (Chloramphenicol, 30 µg) with inhibition zone diameter respectively 6 mm, 2 mm, 11 mm, 3 mm, 4 mm, 0 mm, 1 mm, 2 mm, and 2 mm. but the inhibition zone diameter of intermediate susceptible serotypes (O111, O55) and O44 chilled to gentamycin is 14 mm and without inhibition zone to other antibiotics.

The results of antimicrobial resistance (AMR) were similar to that mentioned by [24]. due to the highest prevalence of resistance was recorded for tetracycline, ampicillin, trimethoprim-sulfamethoxazole and colistin. On the other hand, these results were disagreed with that reported by [16] due to *E. coli* was sensitive to ciprofloxacin, suphamethoxazole/trimethoprim and gentamicin.

Incidence of *E. coli* virulence genes in serologically confirmed *E. coli* strains from different beef samples showed that eaeA was the most prevalently detected gene in the present study similar to the results reported by [25]. who detected it in 11 isolates. EaeA gene is responsible for intimin protein production which help *E. coli* in attachment of to the intestinal cells it produces intestinal lesions characterized by cytoskeletal changes such as polymerized F-actin accumulation [26].

The Stx1 is known as the most important toxin. The clinical significance of Stx1 producing haemorrhagic colitis and haemolytic-uremic syndrome due to injuries to endothelial cells, eukaryotic ribosome and hindrance of protein synthesizing [27]. This gene was detected in one strain (o44: k74) in chilled beef in this study similar that reported by [28]. But the low prevalence of stx1in the present study 16% disagree with that reported by [29]. which its incidence (21%).

ChuA gene can encode a heme-binding protein is important for outer membrane proteins production involved in the utilization of heme and its protein [30]. ChuA gene present in all previous mentioned serotypes in this study except in O111: k58 but was detected in O111: K58 by [31].

Quorum sensing (QS) marker amplified LuxS gene was presented in all mentioned serotypes except o44: k74 in chilled but it is reported in o44: k74 serotype in fresh samples these result similar to that mentioned by [31].

The adrA gene is a responsible for biofilm formation which provides resistance against drugs and immune system. Numerous genes linked to the production of biofilm have been discovered [32]. AdrA present in all serotypes in the present study except o25: k11 this result has got close to that reported by [33]. who detected adrA in O55, O111, O86, O91, O103, O125, O128, O44 and O16.

Conclusion

The prevalence of *E. coli* in fresh, chilled and frozen beef indicates unhygienic production and processing of these foods. The display temperature of different types of retail marketed beef might affect on growth of microorganism. The incidence of multi-drug resistant and virulent *E. coli* in meat may pose a serious public health threat on human health.

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Declaration of Conflict of Interest

The authors declare that there is no conflict of interest.

TABLE 1. Primer sequences of target genes, length of the amplified product, and annealing temperatures.

Gene	Primer Sequence 5'-3'	Amplified product	Annealing temperature (°C) / Time (sec)	Reference
Stx1	ACACTGGATGATCTCAGTGG CTGAATCCCCCTCCATTATG	614 bp	58˚C 40 sec.	[34]
chuA	GACGAACCAACG GTCAGGAT TGCCGCCAGTACCAAAGACA	279 bp	55˚C 30 sec.	[35]
eaeA	ATGCTTAGTGCTGGTTTAGG GCCTTCATCATTTCGCTTTC	248 bp	51˚C 30 sec.	[36]
adrA	ATGTTCCCAAAAATAATGAA TCATGCCGCCACTTCGGTGC	1113 bp	50˚C 40 sec.	[37]
LuxS	ATGCCGTTGTTAGATAGCTTCA GATGTGCAGTTCCTGCAACTTC	513 bp	55˚C 40 sec.	[38]

TABLE 2. Most probable numbers (MPN) of coliforms, fecal coliforms, and *E. coli* in fresh, chilled and frozen beef samples. Data are represented by means ± standard errors (SE) of 25 samples (n=25).

Parameters	Fresh beef			Chilled beef			Frozen beef		
	No.	%	Mean ±SE	No.	%	Mean ± SE	No.	%	Mean ± SE
Coliforms MPN	25	100	$6.87\times10^2 \pm 60.9^{b}$	25	100	$8.0\times10^2 \pm 43.9^{b}$	20	80	$1.80\times10^2 \pm 53.5^{a}$
Fecal coliforms MPN	23	92	$1.98\times10^2 \pm 22.1^{b}$	25	100	$5.46\times10^2 \pm 72.46^{a}$	18	72	$6.3\times10 \pm 40^{b}$
***E. coli* MPN**	9	36	4.5 ± 2.1^{a}	10	40	$1.2\times10 \pm 2.6^{a}$	13	52	3 ± 1^{a}

Where, No.= Number of positive samples, %= Percentage of positive samples

Different small letter (a, b, …) superscripts within row indicates significance difference between means at $p < 0.05$

TABLE 3. Results of biochemical and serological identifications of suspected *E. coli* isolates.

Beef sample types	No. of suspected *E. coli* isolates	Biochemically confirmed *E. coli*-IMVC* pattern (+/+/-/-)		Serologically typed *E. coli* isolates		
		No.	%*	No	%	%*
Fresh beef	9	6	66.66	4	16	44.44
Chilled beef	10	2	20	1	4	10
Frozen beef	13	1	7.69	1	4	7.69

*IMVC = Indole, Methyl Red, Voges Proskauer, Citrate Utilization

% = percentage of isolates in relation to number of examined samples

%* = percentage of isolates in relation to number of suspected E. coli isolates.

TABLE 4. Serotypes of *E. coli* strains isolated from fresh, chilled, and frozen beef samples (n=25).

Samples	Serotype:	No.	%*	%**
Fresh beef	O44: k74	2	8%	33.3
	O55: k59	1	4%	16.66
	O11: k58	1	4%	16.66
Chilled beef	O44: k74	1	4%	16.66
Frozen beef	O25: k11	1	4%	16.66

%* = percentage of serotypes in relation to examined beef samples in each type.

%**= percentage of serotypes in relation to total serotyped E. coli isolates.

TABLE 5. Antibiotic sensitivity profile of isolated *E. coli* strains from different beef samples.

Origin (serotype n)	Number of resistant serotypes (resistant %)							
	Gn	K	Amp	SP	DO	Sxt	Tet	C
Fresh beef (4)	2 (50)	4 (100)	4 (100)	4 (100)	4 (100)	4 (100)	4 (100)	4 (100)
O55: K59 (1)	0 (0)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)
O44: K74 (2)	2 (100)	2 (100)	2 (100)	2 (100)	2 (100)	2 (100)	2 (100)	2 (100)
O111: K58 (1)	0 (0)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)
Frozen beef (1) O25: K11	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)
Chilled beef (1) O44: K74	0 (0)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)

Gn (Gentamicin, 10 µg), K (Kanamycin, 30 µg), Amp (Ampicillin,10 µg), SP (Spiramycin, 100 µg), DO (Doxycycline,30 µg), Sxt (Sulfamethoxazole-Trimethoprim, 25 µg), Tetracycline (Tet) 30 µg, CT (Colistin, 25µg), C (Chloramphenicol, 30 µg).

TABLE 6. Incidence of *E. coli* virulence genes in serologically confirmed *E. coli* strains from different beef samples (n=6).

Isolate No.	Sample types	*E. coli* serotype	*chuA*	*Stx1*	*LuxS*	*eaeA*	*adrA*
1	Fresh beef	O55: K59	+	-	+	+	+
2	Fresh beef	O44: K74	+	-	+	+	+
3	Fresh beef	O44: K74	+	-	+	+	+
4	Fresh beef	O111: K58	-	-	+	+	+
5	Frozen beef	O25: K11	+	-	+	+	-
6	Chilled beef	O44: K74	+	+	-	+	+

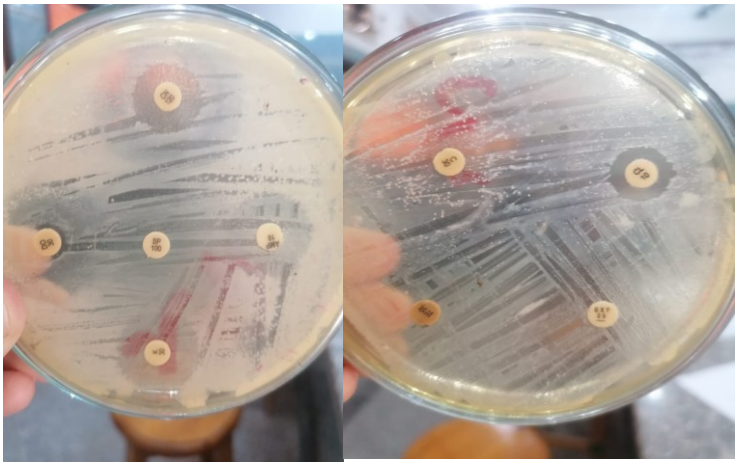

Fig 1. Photo samples of different degrees of antibiotic resistance/sensitivity of serologically identified *E. coli* strains using disc diffusion assay on Muller Hinton agar.

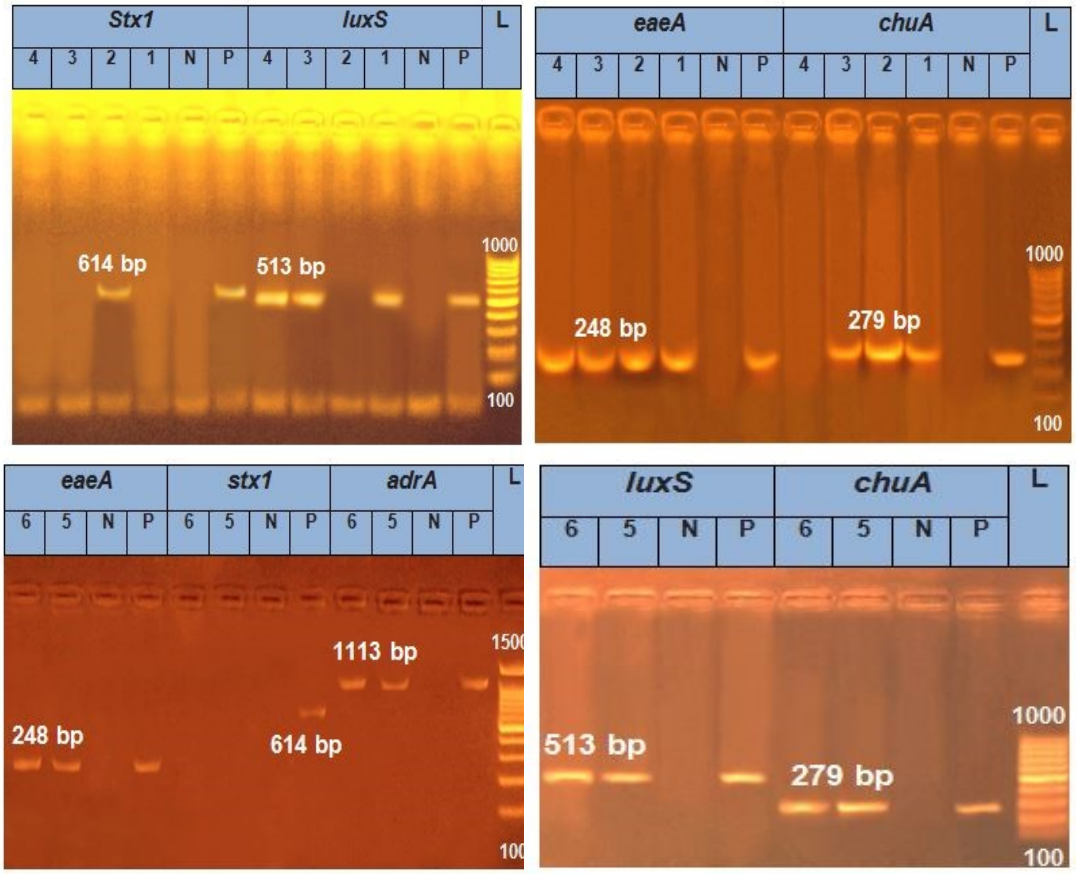

Fig. 2. Gel electrophoresis photos of molecularly identified virulence genes of serologically typed *E. coli* strains: *chuA, stx1, LuxS, eaeA* and *adrA* genes are detected at 279, 614, 513, 248 and 1113 bp respectively. P= control positive, N= control negative.

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ضراوة ومقاومة المضادات الحيوية لمعزولات بكتريا الايشريشيا كولاي من اللحوم المباعة بالتجزئة (الطازجة، المبردة، المجمدة)

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الملخص

ان وجود مسببات الامراض المقاومة لمضادات الميكروبات الايكولاي في اللحوم يشكل مصدر قلق كبير بسبب آثارها الضارة على صحة الإنسان. ولذلك، هدفت هذه الدراسة إلى الكشف عن سلالات الإشريكية القولونية المسببة للأمراض في عينات لحوم البقر بالتجزئة في الأسواق المصرية. هنا، تم جمع خمسة وسبعين عينة من لحوم البقر بالتجزئة تمثل 25 عينة من كل من العينات الطازجة والمبردة والمجمدة بشكل عشوائي من محلات الجزارة المختلفة الموجودة في محافظتي المنيا وبني سويف. تم تقييم العينات المجمعة للتأكد من جودتها الميكروبيولوجية باستخدام تقنية العدد الأكثر احتمالا لتعداد القولونيات والقولونيات البرازية والقولونيات. بالإضافة إلى ذلك، تم إخضاع عزلات الإشريكية القولونية للتشخيصات البيوكيميائية والمصلية والجزيئية. تم إجراء اختبار الحساسية للمضادات الحيوية بطريقة الانتشار القرصي لتقييم مقاومة العزلات للمضادات الحيوية. أظهرت النتائج أن نسبة الإصابة الإشريكية القولونية في عينات لحوم الأبقار الطازجة والمبردة والمجمدة كانت 16%، 4% و4% على التوالي الطازجة بنسبه تتراوح علي التوالي (4%،4%،8%)(المعزولات من العينات O44,o111,o55الطازجة (المعزولة من العينات المجمدة بنسبه 4% O25من العينات المبرده بنسبه O44%4 وراثيا تم اكتشاف الجينات الأكثر انتشار في المعزولات بنسبه تتراوح على توالي (16%،83%،83%،100%)، EaeA chuA,adrA,lux,stx1 جميع السلالات المعزولة مقاومة للأدوية المتعددة عند فحصها ضد أنواع مختلفة من المضادات الحيوية. وقد خلص إلى أن وجود بكتيريا الإشريكية القولونية الخبيثة في اللحوم منتشر على نطاق واسع وهو أمر له أهمية على الصحة العامة ويحتاج إلى تقييم دوري للوضع الصحي لأسواق اللحوم المصرية لمنع التسمم الغذائي ومخاطر الصحة العامة.

الكلمات الدالة: لحم البقر، القولونيات، الإشريكية القولونية، الأنماط المصلية وجينات الضراوة للإشريكية القولونية في اللحوم مقاومه للمضادات الحيوية المتعددة.