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Original Article *Food Hygiene*

Shigatoxin producing Escherichia coli in camel meat marketed in Behaira governorate, Egypt.

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ABSTRACT

Objective: To detect incidence of Shigatoxin producing Escherichia coli in the examined camel meat samples.

Samples:123 samples

Procedure: All samples were collected from retailed camel carcasses distributed at Albeheira governorate, Egypt. Each individual sample was packaged into a sterile plastic bag then marked and transferred in an insulated icebox to the research laboratory of Food Hygiene and Control Department, Faculty of Veterinary Medicine, Mansoura University, wherein the microbiological examination was done.

Results: Biochemical characteristics suggestive for E. coli colonies (128/156 isolates) were identified as indole +ve, methyl red reaction +ve, Voges Proskauer –ve result, and citrate –ve results. The biochemical tests for the presumptive isolates revealed that only 128 isolates from total of 156 isolates by a percent of 71.87% were biochemically positive E coli and only 92 isolates were confirmed as E. coli based on serological identification and molecular confirmation of E. coli while 46 isolates were confirmed as Shigatoxin producing E. coli containing virulence genes of stx1, stx2, eaeA and hlyA. These results emphasized that retailed camel meat may be a vehicle for EHEC O157:H7 strains which may be of a public health concern, and there is a need to implement protective measures to reduce the levels of their occurrence in meat.

Conclusion and clinical relevance: To our knowledge, this study is one of the fewest studies done to detect the occurrence of shigatoxin producing E. coli in camel carcasses worldwide. Our results clearly determine the incidence of shigatoxin producing E. coli in camels, which may contaminate meat consumed by humans.

Keywords: shigatoxin, camel meat, E. coli, Egypt.

1. INTRODUCTION

Camel meat has exceptional features, as having low fat, good quality protein and cholesterol contents with moderate amounts of polyunsaturated fatty acids. Camels can transmit diseases and infections through consuming their meat, even though the foodborne illness outbreaks associated with the ingestion of camel meat is somewhat low. Camels are considered as reservoirs for ESBL E. coli that has been examined [1].

Meat contamination occur throughout slaughtering procedures originated from hides and gastrointestinal materials of the slaughtered animals, the work environment, the connection of the animal's skin, blood, hair, bile, equipment's, water, limbs, air pollution and worker's hands and clothes [2]. Additionally, abattoir gates are continuously opened without any limitation to go inside and outside from abattoirs can transmit E. coli O157: H7 from the surroundings to abattoir. Carcass splitting and Evisceration occur on the floor in the same place that produce high risks of infection during preparation of carcasses from the intestines, the skin and the ground.

Escherichia coli is a Gram negative, oxidase negative, catalase positive, indole positive and facultative anaerobe belongs to the Enterobacteriaceae family. Pathogenic E. coli are categorized into two main classes; extraintestinal pathogenic and diarrheagenic E. coli that are classified into six groups comprising Enterotoxigenic *E. coli* (ETEC), Entero-invasive E. coli (EIEC), Enteropathogenic E. coli (EPEC), diffusively adherent E. coli (DAEC), Enteroaggregative E. coli (EAEC), and Entero-hemorrhagic E. coli (EHEC)/Shiga toxin-producing E. coli (STEC) [3].

The Shiga toxins produced by numerous serotypes of E. coli may cause anything from uncomplicated diarrhea to hemorrhagic colitis, that can be developed into hemolytic uremic syndrome (HUS) with acute renal failure [4]. Other markers for the progression of haemolytic uremic syndrome (HUS) and haemolytic colitis, are intimin, determined by the eae gene, and enterohaemolysin, determined by the hlyA gene [5]. The non O157 STEC strains (e.g. O111:H4, O117, O26:H11, O103:H2, O121:H7, O44:H8, O11:H8, O127:H6, O124 H28/H) are considered factors for bloody diarrhea, HUS and other intestinal disorders [6].

Shiga-toxin producing Escherichia coli are zoonotic

foodborne microorganisms that have the ability to cause severe human sickness. In 2018 nearly 8 thousand STEC infections were recorded in Europe, with around a percent of 37% were in need of hospitalization and additional medical care and treatment, Actually, in the European Union, STEC comes in the third position after Campylobacter spp. and Salmonella spp on the most pathogenic foodborne microorganisms [4].

Diagnosis of pathogenic E. coli necessitates differentiation between non-pathogenic strains that are normal inhabitants of intestinal microflora and pathogenic ones that are causing diseases. Pathogenic types are identified by the prevalence of one or more of the detectable E. coli virulence genes that can be determined by unconventional and conventional molecular methods [7]. Molecular identification is faster and more accurate for the identification of microorganisms compared with the conventional molecular approaches. Quantitative real-time PCR (qRT-PCR) and multiplex polymerase chain reaction (mPCR) are used for the identification of the microbial factors. Additionally, sequencing is an accurate method for species identification [1].

Concerning to human health, antimicrobial resistance results from the misuse of antibiotics in human diseases treatment in the developing countries and livestock production [8]. Illnesses caused by E. coli frequently need antimicrobial management. The higher resistance level of antimicrobials was a sign of their overuse.

Until now, limited studies have been done in the developing countries, comprising Egypt, around the occurrence of Shigatoxin producing Escherichia coli in camel meat. Therefore, the objective of the current study is to detect the incidence of Shigatoxin producing Escherichia coli in camel meat marketed in Behaire governorate, Egypt, and to investigate their public health hazards by detecting some genetic factors as stx1, stx2, hlyA and eae genes in their genetic profile.

2. MATERIALS AND METHODS

2.1. Collection of samples

A total of 123 camel meat samples were collected from retailed camel meat distributed at Albeheira governorate, Egypt. Each individual sample was packaged into a sterile plastic bag then marked and transferred in an insulated icebox to the research laboratory of Food Hygiene and Control Department, Faculty of Vet. Medicine, Mansoura University, wherein the microbiological examination was done.

2.2. Isolation and identification of E. coli strains

Twenty-five grams from every meat sample were mixed with 225 ml of sterile modified tryptone soya broth (Oxoid CM0989) having vancomycin then incubated at 37o C / 18 h. Cultures were inoculated onto sorbitol MacConkey agar enriched with potassium tellurite and cefixime then incubated at 37o C / 24 h. The colonies were detected for the characteristic E. coli O157:H7 typical colonies (colorless, circular and entire edge with brown centers). Presumptive E. coli O157:H7 (sorbitol non fermenting) colonies were cultured onto nutrient agar (Oxoid CM0003s) slopes for further examination. Biochemical examinations used for the diagnosis of E. coli according to MacFaddin [9] comprised indole test, methyl red reaction, Voges Proskauer and citrate utilization. Identification of the enterohemolytic types was done on blood agar plates having 5% of washed sheep erythrocytes. Positive results detected by the presence of a narrow turbid zone of hemolysis within 18–24 h incubation at 37 °C.

2.3. Serotyping of identified Shigatoxin producing Escherichia coli strains

The isolates were identified serologically according to Kok, Worswich and Gowans [10] using E. coli antisera sets **(DENKA SEIKEN Co., Japan)** for identification of the Entero-pathogenic types at the Center of Food Analysis, Faculty of Veterinary Medicine, Benha University, Egypt.

2.4. Detection of E. coli virulence genes by PCR:

For isolation of DNA of E. coli strains, the method reported by Malke [11] using Gene JET Genomic DNA Purification Kit (Fermentas) was performed and used as a template for PCR analyses.

Ninety- two isolates that were identified as positive E. coli strains isolated from camel meat were tested for the incidence of some virulence genes specific for pathotyping of the diarrheagenic E. coli as EHEC-hlyA, eae, stx1 and stx2.

2.5. Electrophoresis of PCR products:

For gel electrophoresis, the following were used: agarose powder, ethidium bromide, Tris, boric acid EDTA (TBE) 10x (Tris base (1 M) 121g, Boric acid (1M) 51.3 gm, EDTA 2H2O 3.72 g, Water to one liter) DNA ladder (molecular marker): 100 bp (Fermentas, lot No: 00052518). DNA amplified products "PCR master Mix "(Fermentis) which contains: PCR buffer, 2.5mM MgCl2, 200 mM each the four deoxy-nucleoside triphosphates d.ATP. d.CTP. dGTP and dTTP, 2.5 U of Taq DNA polymerase. Positive strains were analyzed by application of PCR for identification of the particular genes of E. coli was basically achieved by using primers (Pharmacia Biotech) as shown in the following table (1).

Table 1: Primers for PCR

Primer	Oligonucleotide sequence (5' → 3')	Product size (bp)	References
stx1 (F)	5' ACACTGGATGATCTCAGTGG '3		
stx1 (R)	5' CTGAATCCCCCTCCATTATG '3	614	
stx2 (F)	5' CCATGACAACGGACAGCAGTT '3		[29]
stx2 (R)	5' CCTGTCAACTGAGCAGCACTTTG '3	779	
eaeA (F)	5' GTGGCGAATACTGGCGAGACT '3		[30]
eaeA (R)	5' CCCCATTCTTTTTCACCGTCG '3	890	
hylA (F)	5' ACGATGTGGTTTATTCTGGA '3		[31]
hylA (R)	5' CTTCACGTGACCATACATAT '3	165	

2.6. Antibiotic Resistance and MAR index of Isolated E. coli:

Antimicrobial susceptibility was recognized by the single diffusion method according to Mary and Usha [12] for the isolated E.coli strains. Sensitivity discs with variable concentrations were used to determine the susceptibility of the isolated strains (Oxoid Limited, Basingstoke, Hampshire, UK). Fourteen antibiotics were used at the following drug concentrations: erythromycin (15 µg), ampicillin (10 µg), penicillin (10 IU), amikacin (30 µg), cefotaxime (30 µg), sulphamethoxazole (25 µg), tetracycline (30 µg), ipipenem (10 µg), ceftriaxone (30 µg), cephalothin (30 µg), levofloxacin (5 µg), ciprofloxacin (5 µg), clindamycin (10 µg), and gentamicin (10 µg). The tested strains were categorized into susceptible, intermediate and resistant. Multiple Antibiotic Resistance (MAR) index for each strain was determined according to the formulation set by Singh, Yadav, Singh and Bharti [13] as follow: MAR index= No. of resistance (Isolates classified as intermediate were considered sensitive for MAR index) / Total No. of tested antibiotics.

3. RESULTS

3.1 Identification of E. coli strains:

In this study, Morphological characters of Presumptive E coli cultures (300 isolates) on sorbitol MacConkey agar supplemented with cefixime and potassium tellurite media base were appeared as circular, colorless with brown centers. Suggestive E. coli isolates (156 isolates) were subjected to biochemical tests, (128 isolates) were identified as indole +ve, methyl red reaction +ve, Voges Proskauer –ve and citrate –ve results. The biochemical tests for the presumptive isolates revealed that only 128 isolates by percentage of 71.87% were biochemically positive E coli and only 92 isolates were verified as E. coli based on serological identification and molecular confirmation of E. coli while 46 isolates were confirmed as Shigatoxin producing E. coli containing virulence genes of stx1, stx2, eaeA and hlyA.

3.2 Serotyping of identified Shigatoxin producing Escherichia coli strains:

Identification of E. coli strains requires the differentiation of nonpathogenic members from the normal flora. Serotypic markers sometimes are very close with the specific categories of E. coli [14]. The identified serovars of enteropathogenic E. coli strains in this research were serotyped into 10 different serotypes included, O111:H4(n=12), O117:H4(n=12), O26:H11(n=5), O103:H2(n=7), O121:H7(n=14), O44:H18(n=7), O11:H8(n=12), O127:H6(n=7), O124 (n=6) and O157:H7 (n=10) as observed in (table2).

Table 2: Seropathotypes of isolated E. coli strains from the examined camel meat samples.

Pathotype	Serotype	No. of *E. coli* contaminated samples	Target genes			
			Stx 1	Stx 2	eae A	hyl A
EHEC	O157:H7	10 (10.86 %)	+	+	+	+
	O111:H4	12 (13.04 %)	+	+	+	+
	O117:H4	12 (13.04 %)	+	-	-	-
	O26:H11	5 (5.43%)	+	+	-	+
	O103:H2	7 (7.60 %)	+	+	-	+
	Total	46 (50%)				
EPEC	O121:H7	14 (15.21 %)	+	-	-	-
	O44:H18	7 (7.60 %)	-	+	-	-
	Total	21 (22.81%)				
ETEC	O11:H8	12 (13.04 %)	-	+	-	+
	O127:H6	7 (7.60 %)	-	+	-	-
	Total	19 (20.64%)				
EIEC	O124	6 (6.52%)	-	-	-	-
Total		92 (100%)				

3.3 Detection of E. coli virulence genes by PCR:

From the observed results, Interestingly, four strains of the verified 10 E. coli strains in this study having both stx1and stx2 genes, 2 strains having stx1 gene only and 3 strains containing stx2 gene only. Two of these 10 E. coli strains were also positive for eae gene which encodes intimin, an important binding protein of pathogenic STEC as E. coli O157:H7 and O111:H4 which is more virulent than

other strains not carrying this gene and is considered more toxigenic and hazardous to consumer health. However, only 5 strains harbored hylA gene.

The association of the four virulent genes (eae gene along with both stx1and stx2 and hylA gene) was present in 2 strains of STEC serotypes (O157:H7 and O111:H4). PCR is a powerful method to identify the targeted DNA of various types of pathogens in different samples from food. It gives extremely accurate, specific and reliable results to differentiate between pathogenic E. coli isolates and normal intestinal flora.

Using multiplex PCR for categorizing the isolated *E. coli* strains, we could identify ten isolates of O157:H7 strains, depending upon amplification of *eae*A, *hylA*, and *sx1 and sx2* genes **(Figure, 1)**.

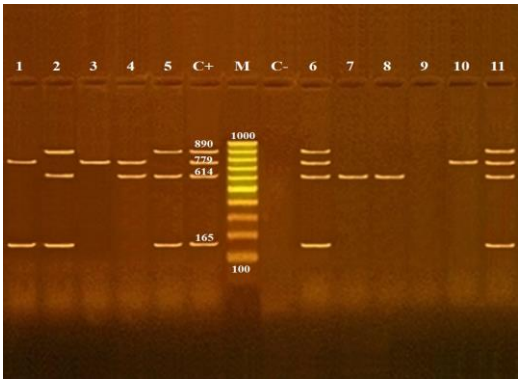

Figure 1. Photograph (1): Agarose gel electrophoresis of multiplex PCR

Agarose gel electrophoresis of multiplex PCR of *stx1* (614 bp), *stx2* (779 bp), *eaeA* (890 bp) and *hlyA* (165 bp) virulence genes for characterization of *Enteropathogenic E. coli.* Lane M: 100 bp ladder as molecular size DNA marker. Lane C+: Control positive *E. coli* for *stx1*, *stx2*, *eaeA* and *hlyA* genes. Lane C-: Control negative, Lanes 7 (O117) & 8 (O121): Positive *E. coli* for *stx1* gene, Lanes 3 (O44) & 10 (O127): Positive *E. coli* containing *stx2* gene. Lane 1 (O11): Positive *E. coli* for *stx2* and *hlyA* genes, Lanes 2 (O26) & 5 (O103): Positive *E. coli* for *stx1*, *stx2* and *hlyA* genes, Lanes 6 (O111) & 11 (O157): Positive *E. coli* for *stx1*, *stx2*, *eaeA* and *hlyA* genes, Lane 9 (O124): Negative *E. coli* for *eaeA, hlyA* genes, *stx1* and *stx2*.

3.4 Electrophoresis of PCR products

Agarose gel electrophoresis of multiplex PCR of *stx1* (614 bp), *stx2* (779 bp), *eaeA* (890 bp) and *hlyA* (165 bp) virulence genes for characterization of *Enteropathogenic E. coli.* Lane M: 100 bp ladder as molecular size DNA marker. Lane C+: Control positive *E. coli* for *stx1*, *stx2*, *eaeA* and *hlyA* genes. Lane C-: Control negative, Lanes 7 (O117) & 8 (O121): Positive *E. coli* for *stx1* gene, Lanes 3 (O44) & 10 (O127): Positive *E. coli* containing *stx2* gene. Lane 1 (O11): Positive *E. coli* for *stx2* and *hlyA* genes, Lanes 2 (O26) & 5 (O103): Positive *E. coli* for *stx1*, *stx2* and *hlyA* genes, Lanes 6 (O111) & 11 (O157): Positive *E. coli* for *stx1*, *stx2*, *eaeA* and *hlyA* genes, Lane 9 (O124): Negative *E. coli* for *eaeA, hlyA* genes, *stx1* and *stx2*.

3.5 Antibiotic Resistance and MAR index of Isolated E. coli

An antibiogram sensitivity test was performed on ninety-two *E. coli* strains and revealed that *E. coli* was highly sensitive to Ceftriaxone (97.8%) and Ipipenem (95.7%). Intermediate resistance was exhibited against cefotaxim by percentage of (7.7%). However, a higher resistance pattern varied among the other tested drugs; the highest resistance (100%) was recorded against Clindamycin and Penicillin followed by Erythromycin (94.5%) (Table3).

Table3. Antimicrobial susceptibility of E. coli:

Antibiotic	Resistant		Intermediate		Sensitive	
	NO	%	NO	%	NO	%
Clindamycin (CL)	92	100	0	0	0	0
Penicillin (P	92	100	0	0	0	0
Erythromycin (E)	87	94.5	5	5.5	0	0
Tetracycline (T)	73	79.3	6	6.5	13	14.1
Cephalocin (CN)	64	69.5	9	9.8	19	20.6
Ampicillin (AM)	54	58.6	4	4.1	34	37.3
Sulphamethoxazol (SXT)	43	46.7	8	8.8	41	44.5
Amikacin (AK)	36	39.1	9	9.8	47	51.1
Cefotaxim (CF)	27	29.3	7	7.7	58	63
Ciprofloxacin (CP)	18	19.6	0	0	74	80.4
Gentamicin (G)	12	13	4	4.3	76	82.7
Levofloxacin (L)	5	5.4	7	7.6	80	87
Ipipenem (IPM)	2	2.2	2	2.2	88	95.7
Ceftriaxone (C)	2	2.2	0	0	90	97.8

CL: Clindamycin P: Penicillin E: Erythromycin T: tetracycline CN: Cephalothin AM: Ampicillin SXT: Sulphamethoxazol AK: Amikacin CF: Cefotaxime CP: Ciprofloxacin G: Gentamicin L: Levofloxacin IPM: Ipipenem C: Ceftriaxone

The MAR index average of E. coli strains was 0.468 (table4) that reveals multiple resistant patterns in E. coli populations. The higher MAR index (more than 0.2) in this research is not surprising as E. coli has 100% resistance to tested antibiotics as clindamycin, penicillin.

Table 4. Classification of E. coli isolates (n = 92) based on their antibiotic resistance profiles against the 14 antibiotic agents tested and their multiple antibiotic resistance (MAR) index.

Antimicrobial resistance phenotype[1]	Number and (%) of isolates	Serotypes	MAR index [2*]
CL, P, E, T, CN, AM, SXT, AK, CF, CP, G, L	5 (5.4%)	O157: H7 (1) O26: H11 (1) O11 : H8 (3)	0.857
CL, P, E, T, CN, AM, SXT, AK, CF, CP, G	7 (7.6%)	O157: H7 (3) O127: H6 (1) O121 : H7 (3)	0.786

CL, P, E, T, CN, AM, SXT, AK, CF, CP	6 (6.5%)	O103: H2 (2) O44: H18 (2) O11 : H8 (2)	0.714
CL, P, E, T, CN, AM, SXT, AK, CF	9 (9.8 %)	O11: H8 (2) O121: H7 (3) O111: H4 (3) O26 : H11 (1)	0.643
CL, P, E, T, CN, AM, SXT, AK	9 (9.8%)	O44: H18 (2) O127: H6 (1) O117: H4 (2) O111 : H4 (4)	0.571
CL, P, E, T, CN, AM, SXT	7 (7.6%)	O103: H2 (3) O121: H7 (2) O117 : H4 (2)	0.500
CL, P, E, T, CN, AM	11 (12%)	O157: H7(2) O124 (1) O121: H7 (3) O111: H4 (3) O26 : H11 (2)	0.428
CL, P, E, T, CN	10 (10.9%)	O103: H2 (2) O44: H18 (3) O121: H7 (2) O117 : H4 (3)	0.357
CL, P, E, T	9 (9.8%)	O127: H6 (3) O124 (1) O121: H7 (1) O117: H4 (3) O26 : H11 (1)	0.286
CL, P, E	14 (15.2%)	O157: H7 (4) O127: H6 (2) O124(3) O11: H8 (3) O111 : H4 (2)	0.214
CL, P	5 (5.4%)	O124 (1) O11: H8 (2) O117 : H4 (2)	0.143
Average = 0.468			

4. DISCUSSION

4.1 Identification of E. coli strains

The achieved results are in line with those obtained by Abdalla, Suliman, Ahmed and Bakhiet [15] who examined a total of 384 swab samples (rump, brisket, neck and shoulder) from cattle carcasses and found *E. coli* in a percentage of (8.86%). Lower occurrence of Enteropathogenic *E. coli* organisms as percentage of (0.0%, 13.33%, 13.33%, and 33.33%**)** of camel meat, spleen, liver and kidney respectively, were previously isolated from Qaliubiya governorate by A, I and M [16]. Other study in Khuzestan and Fars provinces, Iran, was conducted by Rahimi, Khamesipour, Yazdi and Momtaz [17] who found that the highest occurrence of *E. coli* O157 was found in beef meat samples by (8.2%), (5.3%) in water buffalo, (4.8%) in sheep, (2.0%) in camel, and (1.7%) in goat. Differences in the incidence of shiga toxin *E. coli* in these studies resulted from variances in research procedures as sample collection, animal history and sampling site (e.g., origin, cleanliness, season, and age).

4.2 Serotyping and molecular identification of Shigatoxin producing Escherichia coli strains

Higher prevalence of *E. coli* O157:H7 strains in camel meat samples by (10.86%) in this study may be attributed to the irritable contamination of carcass with *E. coli* O_{157}*:* H_7 during the slaughtering process, In this context Elder, Keen, Siragusa, Barkocy-Gallagher, Koohmaraie and Laegreid [18] who claimed that when the contents of the animal's intestine are allowed to come into contact with the carcass, the *E. coli* bacteria are eventually mixed into the meat. Thorough cooking is required to prevent *E. coli* O_{157}*:* H_7 poisoning when the meat is eaten by the consumer.

Another study was investigated by Faten, Amani, Mervat and Gaafar [19] who isolated *E. coli* strains from camel offal samples with an incidence of 10% in lung samples, 10% in liver samples, 0 % in heart samples and the serotypes of *E. coli* were O26: K60(B6), O111: K58(B9) and O119: K69(B19). However, El-Shamy [20] detected the incidence of *Escherichia coli* isolated from 25 liver samples from cattle as 40%, 7(28%) Enteropathogenic, 2 (8%) Enterohaemorrhagic and 1 sample (4%) Enterotoxigenic that also identified serologically as O26, O127, O111, O143 and O128.

Serotyping of *E. coli* is a significant method to create the correct analysis and epidemiological examinations for foodborne outbreaks. New *E. coli* pathotypes often emigrate from the intestine are recognized as class of *E. coli*. This serotyping only could not be a reliable method to classify *E. coli* strains but also to identify the specific virulence factors [21].

Our results ensure that there is a probability for isolation of pathogenic *E. coli* from food samples. This decides the difficulty to determine if the strain of *E. coli* detected from animal or food samples is pathogenic or not, and also demonstrates that the decision could not be created absolutely on the existence or the number of virulence factors. The serotyping is an important complementary method to investigate the cause of outbreaks from non O157 *E. coli* strains [22].

4.3 E. coli virulence genes

The most common virulence genetic profile in camel isolates was eae/stx1/ ehlyA. Consequently, they are considered pathogenic to humans; besides, their capability to cause illness and outbreaks is usually accompanied with stx2-producing strains [23]. Our results revealed that O111 had stx1, stx2, eaeA and hylA, O26, O103 had stx1, stx2 and hylA, O44, O127 possessed only stx2, O124 did not possess any virulence genes and this is in line with A, Hassan Hassan, Heikal and Barhoma [24] who found that E. coli O26, O111 had stx1, stx2 and eaeA, O124 was negative for all genes, O103 was positive for stx1 and stx2 genes and and Shawish Shawish [25] who found that E. coli O26 and O111 had Stx1, Stx2 and eae genes, but our results were different from A, Hassan Hassan, Heikal and Barhoma [24] as he found that O44 had only stx1 and O127 had stx1and stx2 genes.

4.4 Antibiotic Resistance and MAR index

The higher resistance to tetracyclines in this work by a percentage of (79.3%) is in line with the study of Daini and Adesemowo [26] who detected 88% and 54% resistance in STEC strains against tetracyclines and gentamicin besides [27], also stated that the resistance percentage to tetracycline in *E. coli* isolated from man and animals is 94.4%. It is exciting that isolates from camels and cattle exhibited resistance to ciprofloxacin and nalidixic acid that are fluoroquinolones and quinolones, respectively. The most resisted antibiotics are ampicillin, tetracycline trimethoprim and gentamycin, respectively also detected [28].

The incidence and multidrug resistance of shigatoxin producing *E. coli* in this study indicate an unacceptable level of hygiene and sanitation practices in meat management and misuse of antimicrobial agents.

Conclusions and Future Prospective

This work provides great information on microbiological safety and quality of camel carcass at slaughterhouses in Egypt. Moreover, it can be used as a standard for other studies and to improve practical guidelines. To our knowledge, our study is one of the fewest studies detecting the incidence of shigatoxin producing *E. coli* in camel carcasses globally. The Shigatoxin producing *E. coli* bacterial load in fresh camel meat was very high which can be related to unhygienic conditions at the slaughterhouse. Multidrug resistance to furthermost tested antimicrobial agents was also detected. This work clarified that municipal slaughterhouses in Egypt do not have the essential sanitary and hygienic principles. Camel meat could be an important source of food-borne pathogens to humans so, good teaching of meat supervisors has to be implemented in camel slaughterhouses to produce meat with high keeping quality properties to keep the consumer safe and decrease the public health risk to the minimum levels. We should balance the use of antimicrobial agents in medicine and agriculture. Additionally, constant antimicrobial susceptibility investigation is necessary. More studies are recommended to confirm the causes of carcass contamination.

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Declarations

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Conflict of interest

The authors declare that there is no conflict of interests.

Availability of data and material

Not applicable

Code availability

Not applicable

Authors' contribution:

Conceived and designed the study: HZ and YA. Conducted the experiments and analyzed the results: KS, HZ, ME and YA. Drafted the manuscript: HZ and YA.

All authors reviewed the manuscript. All authors read and approved the final manuscript.

Consent to participate

Not applicable

Consent for publication

All authors agree to publish the findings of the current research.

5. REFERENCES

1 Fadlelmula, A., Al-Hamam, N.A., and Al-Dughaym, A.M.: 'A potential camel reservoir for extended-spectrum β-lactamase-producing Escherichia coli causing human infection in Saudi Arabia', Tropical Animal Health and Production, 2015, 48, (2), pp. 427-433

2 Koffi-Nevr, R., Koussemon, M., and Coulibaly, S.O.: 'Bacteriological Quality of Beef Offered for Retail Sale in Cote d'ivoire', American Journal of Food Technology, 2011, 6, (9), pp. 835-842

3 Monaghan, Á., Byrne, B., Fanning, S., Sweeney, T., McDowell, D., and Bolton, D.J.: 'Serotypes and virulotypes of non-O157 shiga-toxin producing Escherichia coli (STEC) on bovine hides and carcasses', Food Microbiology, 2012, 32, (2), pp. 223-229

4 'Joint EFSA/ECDC technical report: Shiga toxin/verotoxin-producing Escherichia coli in humans, food and animals in the EU/EEA, with special reference to the German outbreak strain STEC O104', EFSA Supporting Publications, 2011, 8, (6)

5 Karmali, M.A., Gannon, V., and Sargeant, J.M.: 'Verocytotoxin-producing Escherichia coli (VTEC)', Veterinary Microbiology, 2010, 140, (3-4), pp. 360-370

6 Son, I., Binet, R., Maounounen-Laasri, A., Lin, A., Hammack, T.S., and Kase, J.A.: 'Detection of five Shiga toxin-producing Escherichia coli genes with multiplex PCR', Food Microbiology, 2014, 40, pp. 31-40

7 Stromberg, Z.R., Van Goor, A., Redweik, G.A.J., Wymore Brand, M.J., Wannemuehler, M.J., and Mellata, M.: 'Pathogenic and non-pathogenic Escherichia coli colonization and host inflammatory response in a defined microbiota mouse model', Dis Model Mech, 2018, 11, (11), pp. dmm 035063

8 Pornsukarom, S., van Vliet, A.H.M., and Thakur, S.: 'Whole genome sequencing analysis of multiple Salmonella serovars provides insights into phylogenetic relatedness, antimicrobial resistance, and virulence markers across humans, food animals and agriculture environmental sources', BMC Genomics, 2018, 19, (1), pp. 801-801

9 MacFaddin, J.F.: 'Biochemical tests for identification of medical bacteria, williams and wilkins', Philadelphia, PA, 2000, 113

10 Kok, T., Worswich, D., and Gowans, E.: 'Some serological techniques for microbial and viral infections', Practical Medical Microbiology (Collee, J.; Fraser, A.; Marmion, B. and Simmons, A., eds.), 14th ed., Edinburgh, Churchill Livingstone, UK, 1996, pp. 179-204

11 Malke, H.: 'T. Maniatis, E. F. Fritsch and J. Sambrook, Molecular Cloning: A Laboratory Manual, X + 545 S., 61 Abb., 28 Tab. Cold Spring Harbor, N. Y. 1982. Cold Spring Harbor Laboratory', Zeitschrift für allgemeine Mikrobiologie, 1984, 24, (1), pp. 32-32

12 Mary, C., and Usha, M.S.: 'Incidences of multi-drug resistance Escherichia coli isolates in Panipuri sold in Bangalore', International Food Research Journal, 2013, 20, (2), pp. 1007

13 Singh, S., Yadav, A.S., Singh, S.M., and Bharti, P.: 'Prevalence of Salmonella in chicken eggs collected from poultry farms and marketing channels and their antimicrobial resistance', Food Research International, 2010, 43, (8), pp. 2027-2030

14 Nataro, J.P., and Kaper, J.B.: 'Diarrheagenic Escherichia coli', Clin Microbiol Rev, 1998, 11, (1), pp. 142-201

15 Abdalla, M.A., Suliman, S.E., Ahmed, D.E., and Bakhiet, A.O.: 'Estimation of bacterial contamination of indigenous bovine carcasses in Khartoum (Sudan)', African Journal of Microbiology Research, 2009, 3, (12), pp. 882-886

16 A, H., I, H., and M, B.: 'Traceability of enteropathogenic E. coli in cattle and camel carcasses', Benha Veterinary Medical Journal, 2016, 31, (1), pp. 50-55

17 Rahimi, E., Khamesipour, F., Yazdi, F., and Momtaz, H.: 'Isolation and Characterization of Enterohaemorragic Escherichia coli O157:H7 and EHEC O157:NM from Raw Bovine, Camel, Water Buffalo, Caprine and Ovine Milk in Iran', Kafkas Universitesi Veteriner Fakultesi Dergisi, 2012

18 Elder, R.O., Keen, J.E., Siragusa, G.R., Barkocy-Gallagher, G.A., Koohmaraie, M., and Laegreid, W.W.: 'Correlation of enterohemorrhagic Escherichia coli O157 prevalence in feces, hides, and carcasses of beef cattle during processing', Proceedings of the National Academy of Sciences, 2000, 97, (7), pp. 2999-3003

19 Faten, S.H., Amani, M.S., Mervat, S.H., and Gaafar, M.H.: 'Enterobacteriaceae in edible offal', J. Ben Vet Med, 2013, 25, pp. 77-87

20 El-Shamy, R.H.M.: 'Quality assurance of internal edible offal produced from food animals abattoirs in Alexandria', 2011

21 Barlow, R.S., Hirst, R.G., Norton, R.E., Ashhurst-Smith, C., and Bettelheim, K.A.: 'A novel serotype of enteropathogenic Escherichia coli (EPEC) as a major pathogen in an outbreak of infantile diarrhoea', Journal of Medical Microbiology, 1999, 48, (12), pp. 1123-1125

22 Fantelli, K.: 'Prevalence and characteristics of Shigatoxin-producing Escherichia coli and Listeria monocytogenes strains isolated from minced meat in Switzerland', International Journal of Food Microbiology, 2001, 70, (1-2), pp. 63-69

23 'The Community Summary Report on Food-Borne Outbreaks in The European Union in 2007', EFSA Journal, 2009, 7, (5)

24 Hassan, M.A., Heikal, G.I., and Barhoma, R.M.: 'Traceability of enteropathogenic E. coli in cattle and camel carcasses', 2016

25 Shawish, R.R.M.: 'Prevalence of shiga toxin-producing Escherichia coli in some beef products', 2015

26 Daini, O.A., and Adesemowo, A.: 'Antimicrobial susceptibility patterns and R-plasmids of clinical strains of Escherichia coli', Aust J Basic Appl Sci, 2008, 2, pp. 397-400

27 Odugbemia, T.: 'Multidrug resistance in E. coli 0157 strains and the public health implication', Science, 2007, 3, (3), pp. 22-33

28 Elmonir, W., Shalaan, S., Tahoun, A., Mahmoud, S.F., Remela, E.M.A., Eissa, R., El-Sharkawy, H., Shukry, M., and Zahran, R.N.: 'Prevalence, antimicrobial resistance, and genotyping of Shiga toxin-producing Escherichia coli in foods of cattle origin, diarrheic cattle, and diarrheic humans in Egypt', Gut Pathog, 2021, 13, (1), pp. 8-8

29 Dhanashree, B., and Mallya, P.S.: 'Detection of shiga-toxigenic Escherichia coli (STEC) in diarrhoeagenic stool & meat samples in Mangalore, India', Indian Journal of Medical Research, 2008, 128, (3), pp. 271

30 Mazaheri, S., Salmanzadeh Ahrabi, S., and Aslani, M.M.: 'Shiga toxin-producing Escherichia coli isolated from lettuce samples in tehran, iran', Jundishapur J Microbiol, 2014, 7, (11), pp. e12346-e12346

31 Fratamico, P.M., Sackitey, S.K., Wiedmann, M., and Deng, M.Y.: 'Detection of Escherichia coli O157:H7 by multiplex PCR', J Clin Microbiol, 1995, 33, (8), pp. 2188-2191