

## Original Research

## Characterization of Shiga Toxin *Escherichia coli* O<sub>157</sub>: H<sub>7</sub> from raw camel milk, Goat Meat and Minced Goat Meat sold at Local Markets in Matrouh Governorate.

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### ABSTRACT

*Escherichia coli* O<sub>157</sub>:H<sub>7</sub> is a major food borne pathogens of concern worldwide so, the present study was carried out for isolation of *E. coli* O<sub>157</sub>:H<sub>7</sub>, molecular characterization,

### INTRODUCTION

*E. coli* O<sub>157</sub>:H<sub>7</sub> serotype is the major source of the current epidemics of diarrhea, hemolytic-uremic syndrome (HUS) and hemorrhagic colitis worldwide (Kwon and Cho, 2015).

Food borne diseases in human comes the third after heart and respiratory diseases (Almeida et al., 2015). Moreover, food born disease outbreaks in third world countries and the Europe reached 91.58 and 38.3 cases per 100000 populations, respectively. (Gould et al., 2013).

detection of virulence genes, antibiogram and phenotypic detection of Extended spectrum beta lactamase (ESBL) producers among isolated strains from raw camel milk goat meat and minced goat meat. A total of 66 samples (44 raw camel milk, 15 goat meat and 7 minced goat meat) were collected from local retail outlets, butcher's shop and super markets in Matrouh Governorate. Samples were subjected to bacterial isolation and identification using cultural, biochemical, PCR, antibiogram and phenotypic detection of ESBL. Out of 66 samples, 7 (10.6%) *E. coli* O<sub>157</sub>:H<sub>7</sub> isolates were recovered. All 7 (100%) tested isolates were positive for *flicH7* gene. 4 (57.14%) isolates were positive for *stx1* but, none of the isolates were positive for *stx2* gene. *E. coli* O<sub>157</sub>:H<sub>7</sub> isolates showed susceptibility to gentamycin (28.6%), chloramphenicol (57.1%), cefepime and cefuroxime (14.3%) while intermediate susceptible to nalidixic acid (71.4%), streptomycin (57.1%), chloramphenicol, ampicillin and cefepime (14.3%), but resistant to cefotaxime and ceftazidime (100%), ampicillin and cefuroxime (85.7%), gentamycin and cefepime (71.4%), streptomycin (42.9%), nalidixic acid and chloramphenicol (28.6%). only 14.3% of isolates were ESBL producers. Consequently, the goat meat is one of the major sources of *E. coli* O<sub>157</sub>:H<sub>7</sub> in Matrouh Governorate. Multiple antibiotic resistant profiles of *E. coli* O<sub>157</sub>:H<sub>7</sub> show a risk for public health and food safety as well as animal production so, proper antibiotics usage in animal production also needs attention.

**Keywords:** *E. coli* O<sub>157</sub>:H<sub>7</sub>, antibiogram, ESBL, cephalosporins, *flicH7*, *stx1*, *stx2*.

Camel's milk is unique in terms of antioxidative factors, antibacterial, antiviral, antifungal, anti-hepatitis, anti-arthritis, treatment for *paratuberculosis*, preventies aging, remedy for autoimmune diseases and cosmetics (Kula, 2016).

Minced meat of goat has high financial impact due to its high nutritive value and the first choice source of animal protein in Matrouh Governorate. The high level of water (99%) in minced meat is appropriate for the growth of different microorganisms (Zafar et al., 2016), spoilage and change in its enzymatic content (Bradeeba and Siva Kumar, 2013), key source of food borne diseases (Elmali and Yaman, 2005).

Meanwhile *Escherichia coli* somatic antigens (O) and H (flagellar) surface antigens form the basis for serological determination of Shiga toxin *E. coli* (STEC) strains. Among STEC serotypes, *E. coli* O<sub>157</sub>:H<sub>7</sub> is the most well known to cause a diverse set of pathologies (Schmidt et al., 1994) and severe illness in human worldwide (Scallan et al., 2011).

STEC are defined by the production of one or more types of shiga toxin (*stx1* or *stx2* or their variants), beside *eaeA* gene, the plasmid encoding the adherence factor intemin (an outer membrane protein) and *hlyA* gene encoded hemolysin (Bettleheim, 2007).

Sheep act as a reservoir and dissemination of multi drug resistant (MDR) bacteria carrying several antimicrobial resistant genes (ARGs) and extended spectrum  $\beta$ - lactamases (ESBL) as reported by Gozi et al., 2019.

Knowledge of the prevalence of O<sub>157</sub> and non O<sub>157</sub> STEC in various sources is essential to design effective intervention strategies to prevent food borne illness outbreaks in human (Pigatto et al., 2008).

The potential resistant bacteria end up in the environment and near food product of animal origin transferred to causing bacterial antibiotic resistant in human (Aminov, 2009).

*E. coli* was classified as multidrug resistant when presented non susceptibility to  $\geq 1$  antimicrobial in  $\geq 3$  antimicrobial categories. They used  $\beta$ . lactam (ampicillin, amoxicillin clavulonate, ampicillin sulbactam, piperacillin-tazobactam, cefoxitin, cefazolin, cefuroxime, cefepime, Cefotaxime, ceftriaxone, ceftazidime, ertapenem, meropenem, impenem, aztreonam), aminoglycosides (streptomycin, gentamycin, tobramycin, amikacin), fluroquinolones (nalidixic acid, ciprofloxacin), tetracyclines (monocycline, deoxycycline, tetracycline), nitrofurans (nitrofurantoin), sulphonamides (trimethoprim, sulphamethoxazole) and phenicols (chloramphenicol) (Magiorakos et al., 2011).

Previous studies have focused on investigating ESBLs in medical and veterinary clinics; however, few reports have investigated extended spectrum  $\beta$ - lactamases (ESBLs) in foods (Odenthal et al., 2016). Furthermore, these ESBL bacteria can readily be transferred to humans through consumption of contaminated food (Huijbers et al., 2016), these in scare information available on the occurrence of bacterial pathogens contaminating goat's meat and raw camel milk.

The present study was planned to study the Profile of Virulence and Antibiotic Resistance of *E. coli* O<sub>157</sub>:H<sub>7</sub> isolated from goat meat, minced goat meat and raw camel's milk from local markets in Matrouh Governorate and this could be achieved by isolation of *E. coli* O<sub>157</sub>:H<sub>7</sub> on different media, PCR detection of the most virulence genes in isolated *E. coli* O<sub>157</sub>:H<sub>7</sub>, detection of antibacterial susceptibility to different antibiotics

and Characterization of the most important pathogenic isolates phenotypically through detection of ESBL producer isolates.

## MATERIALS AND METHODS

**1. Sampling:** A total of 66 samples (44 camel milk, 15 goat meat and 7 minced goat meat) were collected from local retail outlets, butcher's shop and super markets in Matrouh Governorate. 250 ml/g from each sample were delivered to the laboratory of Microbiology, Faculty of Veterinary Medicine, Matrouh University in sterile bags and falcon tubes under refrigeration in an ice container to be subjected to bacteriological examination. **2. Isolation and identification of *E. coli* O<sub>157</sub>:H<sub>7</sub> from collected samples ISO: 16654 (2001):** 25 ml from raw camel milk/25 gram from goat meat and minced goat meat was aseptically transferred into a sterile polyethylene stomacher bag and blended with 225 mL of modified *Escherichia coli* (mEC) broth supplemented with novobiocin and homogenized at 230 rpm for 2 minutes using a stomacher and incubated at 41.5 °C for 6 h and for a further 18-24 h. Then a loopful of the enriched samples was streaked out on the surface of MacConkey's sorbitol agar (SMAC) supplemented with Tellurite - Cefixime Supplement (Himedia) and incubated aerobically at 37°C for 24 h. presumptive colonies were picked up and subjected to morphological and biochemical identification (Quinn et al., 2011). **3. Molecular identification of isolated *E. coli* O<sub>157</sub>:H<sub>7</sub>:** **a. DNA extraction:** DNA Extraction was carried out by boiling method (Sambrook and Russell, 2001) **Table (1):** primer sequences for amplification of virulence genes of *E. coli* O<sub>157</sub>:H<sub>7</sub> **Reference Length of amplified product Primer sequence (5'-3') Gene Target bacteria Fratamico et al., (2000) 625 bp GCGCTGTCGAGTTCTATCGAGC *flicH7* *E. coli* O<sub>157</sub>:H<sub>7</sub> CAACGGTGACTTTATCGCCATTCC Dipineto et al., (2006) 614 bp AACTGGATGATCTCAGTGG *stx* 1 CTGAATCCCCCTCCATTATG 779 bp CCATGACAACGGACAGCAGTT *stx* 2 CCTGTCAACTGAGCAGCACTTTG **Table (2):** Cycling conditions of the primers used for amplification of genes of virulence of *E. coli* O<sub>157</sub>:H<sub>7</sub>: **Target bacteria Gene Initial denaturation Denaturation Annealing Extension No. of cycles Final extention** *E.coli* O<sub>157</sub>:H<sub>7</sub> *flicH7* 94°C 5 min. 94°C 30 sec 57°C 40 sec 72°C 45 sec 35 72°C 10 min. *stx 1* and *stx 2* 94°C 5 min. 94°C 30 sec 58°C 40 sec 72°C 45 sec 35 72°C 10 min. **b. DNA Molecular weight marker:** The ladder was mixed gently by pipetting up and down. Six  $\mu$ l of the required ladder were directly loaded. **c. Agarose gel electrophoresis (Sambrook and Russell, 2001). 4. Antibacterial sensitivity test and screening for ESBL producer among isolated *E. coli* O<sub>157</sub>:H<sub>7</sub> by using cephalosporins.** After preparation of 0.5 McFarland's standard from *E. coli* O<sub>157</sub>:H<sub>7</sub>, the suspension of bacteria was swabbed on the surface of Muller Hinton agar plate then the plate was incubated for about 30 min. The chosen antibiotics discs were applied to adequate spacing so that two discs shouldn't closer than 24 mm from one center to the other**

center and no more than 15 mm from the edges of Petri dish by using sterile fine pointed forceps. The discs were pressed gently to ensure full contact of discs to the medium, and then plates were incubated at 37 °C for 24 h. After incubation, the degree of sensitivity was determined by measuring the easily visible and clear zone of inhibition of growth produced by diffusion of antibiotics from disc into the surrounding medium. The results were interpreted according to **CLSI (2012)** guide lines for the antibiotics. **5. Characterization of ESBLs according to CLSI (2016):** Isolates with reduced susceptibility to CTX and / or CAZ was assessed for the presence of ESBLs, using combination disc test with CTX and CAZ with and without clavulonic acid. An increase in the zone diameter of more than 5mm for either antimicrobial agent tested in combination with clavulonic acid vs. the zone diameter of the agent when tested alone, confirmed the presence of an ESBL producing organisms.

## RESULTS

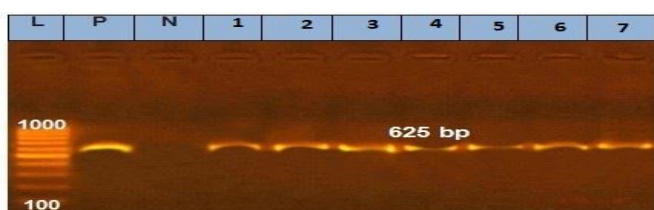
In this study, out of 66 samples (44 raw camel milk, 15 goat meat and 7 minced goat meat samples), 7 *E. coli* O<sub>157</sub>:H<sub>7</sub> (10.6%) isolates were recovered by cultural and biochemical identification. the highest rates of isolation recorded in goat meat 33.33% (5/15), followed by minced goat meat 14.3% (1/7), but the lowest rate of isolation was recorded in camel milk 2.3% (1/44) as shown in **table (3)**.

**Table (3):** Prevalence of isolated *E. coli* O<sub>157</sub>:H<sub>7</sub> identified by cultural method and biochemical reactions in different food samples.

Samples		<i>E. coli</i> O <sub>157</sub> :H <sub>7</sub>	
Type	NO.	NO.	%
Camel milk	44	1	2.3
Goat meat	15	5	33.33
Minced goat meat	7	1	14.3
Total	66	7	10.6

% according to number of each sample.

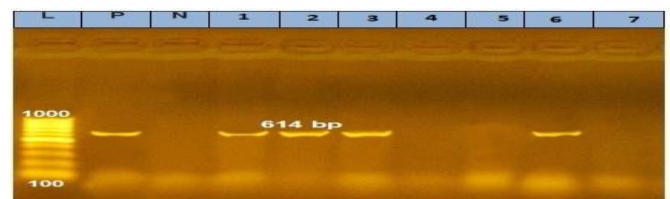
The Seven isolates of biochemically identified *E. coli* O<sub>157</sub>:H<sub>7</sub> were randomly studied for detection of *flicH7* gene using PCR technique. All 7 (100%) tested isolates were positive for *flicH7* gene. The PCR assay yielded amplified products of 625 bp specific for *flicH7* gene as shown in **figure (1)**.



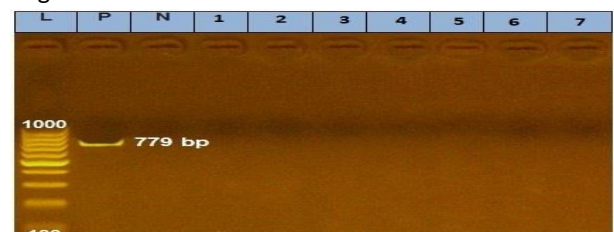
**Figure (1):** Agarose gel electrophoresis (1.5 %) stained with ethidium bromide of the amplified *flicH7* coding gene of the

isolated *E. coli* O<sub>157</sub>:H<sub>7</sub>. Lane (L): DNA molecular weight ladder (100bp ladder). Lane (P): control positive for *flicH7* coding gene, lane (N): negative control for *flicH7* coding gene. Lanes (1, 2, 3, 4, 5, 6 and 7): Positive results for *flicH7* coding gene (specific band at 625bp).

out of seven detected *E. coli* O<sub>157</sub>:H<sub>7</sub> isolates by PCR, only 4 (57.1%) *E. coli* O<sub>157</sub>:H<sub>7</sub> isolates were positive for *stx1* gene (specific band at 614bp) which classified as 2 (50%) isolates from goat meat, one (25%) isolate from raw camel milk and one (25%) isolate from minced goat meat as shown in **figure (2)**, but none of *E. coli* O<sub>157</sub>:H<sub>7</sub> isolates were positive for *stx2* gene (specific band at 779bp) as shown in **figure (3)**.



**Figure (2):** Agarose gel electrophoresis (1.5%) stained with ethidium bromide of the amplified *stx1* gene of the isolated *E. coli* O<sub>157</sub>:H<sub>7</sub>. Lane (L): DNA molecular weight ladder (100bp ladder). Lane (P): control positive for *stx1* gene, lane (N): negative control for *stx1* gene Lane (1 and 6) from goat meat; Lane 2 (raw camel milk) and Lane 3 (minced goat meat): Positive results for *stx1* gene (specific band at 614bp), Lane (4, 5 and 7): negative results for *stx1* gene.



**Figure (3):** Agarose gel electrophoresis (1.5%) and stained with ethidium bromide of the amplified *stx2* gene of the isolated *E. coli* O<sub>157</sub>:H<sub>7</sub>. Lane (L): DNA molecular weight ladder (100bp ladder), Lane (P): control positive for *stx2* gene (specific band at 779bp), lane (N): negative control for *stx2* gene, Lane (1, 2, 3, 4, 5, 6 and 7): negative results for *stx2* gene.

As shown in **table (4)**, the antibiotic resistance pattern revealed that *E. coli* O<sub>157</sub>:H<sub>7</sub> isolates showed susceptibility (28.6%) and (57.1%) to gentamicin and chloramphenicol, respectively and 14.3% of isolates showed susceptibility to cefepime and cefuroxime while (71.4%), (57.1%) of isolates showed intermediate susceptibility to nalidixic acid and streptomycin, respectively and only (14.3%) of isolates showed intermediate susceptibility to chloramphenicol, ampicillin and cefepime.

**Table (4):** Results of antibiotic sensitivity of isolated *E. coli*O<sub>157</sub>:H<sub>7</sub>

Antibiotics	<i>E. coli</i> O <sub>157</sub> :H <sub>7</sub>					
	7					
	S		I		R	
	NO.	%	NO.	%	NO.	%
Gentamycin	2	28.6	0	0	5	71.4
Nalidixic acid	0	0	5	71.4	2	28.6
Streptomycin	0	0	4	57.1	3	42.9
Chloramphenicol	4	57.1	1	14.3	2	28.6
Ampicillin	0	0	1	14.3	6	85.7
Cefotaxime	0	0	0	0	7	100
Ceftazidime	0	0	0	0	7	100
Cefepime	1	14.3	1	14.3	5	71.4
Cefuroxime	1	14.3	0	0	6	85.7

%: According to total number of isolates (7 isolates).

#### DISCUSSION

R: resistant. S: susceptible. I: intermediate susceptible

Out of 7 isolates of *E. coli* O<sub>157</sub>:H<sub>7</sub> showed resistance to third generation cephalosporins (Cefotaxime and/ or ceftazidime), 6 (85.7%) isolates were non ESBL producer when tested by double disk synergy test and combination disk method, but only 1 (14.3%) isolate was ESBL producer by double disk synergy and combination disk method as shown in **table (5)** and **Figure (4)**.

**Table (5):** ESBL producing bacteria among isolated *E. coli* O<sub>157</sub>:H<sub>7</sub>

	ESBL producer		Non ESBL producer	
	NO.	%	NO.	%
<i>E. coli</i> O <sub>157</sub> :H <sub>7</sub>	1	14.3	6	85.7

%: According to number of isolates.

**Figure (4):** Combination disk test of *E. coli* O<sub>157</sub>:H<sub>7</sub>

Food borne pathogens are major threat to food safety, especially in developing countries, where hygienic and sanitation facilities are often poor. *E. coli* O<sub>157</sub>:H<sub>7</sub> is one of the most common causes of outbreaks of food borne diseases in Egypt (**Ahmed and Shimamoto, 2014**) and all over the world (**Alice et al., 2010**).

As shown in **table (3)**, the obtained results were similar to that recorded by **Zdragas et al. (2009)** who isolated *E. coli* O<sub>157</sub>:H<sub>7</sub> at a percentage of 12.9% from milk samples, but lower than that reported by **Garbaj et al. (2016)** who isolated *E. coli* O<sub>157</sub>:H<sub>7</sub> at a percentage of 11% from she camel's milk and higher than that reported by **Hessain et al. (2015)**, **Karmi (2019)** and **Dadi et al. (2020)** who isolated *E. coli* O<sub>157</sub>:H<sub>7</sub> at percentage of 2.97% from raw meat and meat products, 2.5% from raw and processed meat and 0% from milk samples, respectively. These variations may be due using different samples as well as collection of samples in different season.

This study detected presence of *flicH<sub>7</sub>* coding gene of the isolated *E. coli* O<sub>157</sub>:H<sub>7</sub> from different food samples at a percentage of 100%. these results were higher than that recorded by **Abong'o and Momba (2009)** who found that PCR for amplification of *flic* coding gene of *E. coli* O<sub>157</sub>:H<sub>7</sub> at a

percentage of 2.8% and **Al-Ajmi et al. (2020)** who reported that 33.3% of *E. coli* O<sub>157</sub>:H<sub>7</sub> isolates possessed *flicH*<sub>7</sub> gene while none of the isolates had *stx1*. These difference may be attributed to using different technique in DNA extraction.

The recovered results showed that presence of *stx1* gene in *E. coli* O<sub>157</sub>:H<sub>7</sub> isolated from different food samples at a percentage of 57.1%. Those results were similar to that recorded by **Onmaz et al. (2020)** who found that 50% *E. coli* O<sub>157</sub> isolates possess *stx1* gene.

On the other hand, those results were higher than that reported by **Kiranmayi and Krishnaiah (2010)** who found that PCR for amplification of *stx1* gene of *E. coli* O<sub>157</sub>:H<sub>7</sub> at a percentage of 44.4%, but lower than the result obtained by **Shahzad et al. (2013)** who identified *stx1* gene at a percentage of 73% by using PCR. These variations may be attributed to difference in time of gene expression.

The obtained results revealed that absence of *stx2* gene in all isolated *E. coli* O<sub>157</sub>:H<sub>7</sub> from different food samples. These results were similar to that recorded by **Kargar and Homayoon (2015)** who found that 14.29% of *E. coli* O<sub>157</sub>:H<sub>7</sub> isolates carried *stx1* and *eaeA* genes and none of the isolates had *stx2* and *hly* genes, but disagree with that reported by **Tahamtan et al (2010) and Wang et al. (2014)** who found *stx2* gene in *E. coli* O<sub>157</sub>:H<sub>7</sub> at a percentage of 34.93% and 40%, respectively. These differences may be due to differences in conditions of PCR technique.

As shown in **table (4)**, 100% of isolates showed resistance to cefotaxime and ceftazidime, 85.7% of isolates showed resistance to ampicillin and cefuroxime, 71.4% of isolates showed resistance to gentamicin and cefepime, 42.9% of isolates showed resistance to streptomycin, while (28.6%) of isolates showed resistance to nalidixic acid and chloramphenicol. These results agree with that reported by **Egbule et al. (2016)** who found that *E. coli* O<sub>157</sub> exhibited 100% resistance to ceftazidime, but lower than that obtained by **Welde et al. (2020)** who reported that 55.6% of *E. coli* O<sub>157</sub>:H<sub>7</sub> isolates showed susceptibility to gentamycin, but showed resistance to ampicillin and streptomycin at a percentage of 100% and 77.8%, respectively, but higher than that recovered by **Bedasa et al. (2018)** who found that 71.4% of *E. coli* O<sub>157</sub>:H<sub>7</sub> isolates were resistant to ampicillin and only 14.3% *E. coli* O<sub>157</sub>:H<sub>7</sub> isolates showed intermediate susceptibility to streptomycin and **Bisi Johnson et al. (2018)** who found that 80% of *E. coli* O<sub>157</sub>:H<sub>7</sub> showed resistance to Cefotaxime. this higher resistance may be due to frequent using of the same antibiotic in treatment which lead to increase of resistance of bacteria.

As shown in **table (5) and Figure (4)**, these results were lower than that reported by **Bisi Johnson et al. (2018)** who found that of the 64 isolates resistant to third generation

cephalosporins, 58 (90.6%) were ESBL positive phenotypically. These variations may be attributed to using different sample type.

## CONCLUSION

The findings obtained in this study revealed that goat meat is considered the major sources of *Escherichia coli* O<sub>157</sub>:H<sub>7</sub> in Matrouh Governorate. The occurrence of *Escherichia coli* O<sub>157</sub>:H<sub>7</sub> and its multiple antibiotic resistant profiles shows a risk for public health and food safety as well as animal production.

## CONFLICT OF INTEREST

The authors declare no conflicts of interest related to this report.

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