# MOLECULAR AND SEROTYPING CHARACTERIZATION OF NON-SHIGA TOXIGENIC *ESCHERICHIA COLI* ASSOCIATED WITH FOOD COLLECTED FROM THE LOCAL MARKET IN FAYOUM GOVERNORATE, EGYPT

**Amr E. M. Mahmoud[1], Ghada O. El-demerdash[2], Mohamed H. H. Roby[3] and Sahar R. Mohamed[2]**

*[1]Biochemistry Department, Faculty of Agriculture, Fayoum University, Fayoum, Egypt.*

*[2]Animal Health Research Institute, Dokki, Giza, Egypt.*

*[3] Food Science and Technology Department, Faculty of Agriculture, Fayoum University, Fayoum, Egypt.*

**ABSTRACT:**

*Escherichia coli* is considered as one of the bacteria that causing diarrhea outbreaks all over the world, and it is responsible for diseases for human and animals as well

A total number of 50 raw milk and 50 raw beef meat samples were collected from the local market in Fayoum Governorate. These samples were subjected for bacteriological, serological and molecular investigations. *E. coli* was isolated from raw milk and raw beef samples with an isolation rate of 58% and 14%, respectively.

Serogrouping of the *E. coli* isolates from the raw milk samples revealed presence of $O_{142}$, $O_{55}$, $O_{111}$, $O_{27}$, and $O_{26}$ with percentage of 20.69%, 20.69%, 17.24%, 17.24%, and 3.45%, respectively. However, the serogrouping of the *E. coli* isolates from raw beef meat samples revealed presence of $O_{111}$, $O_{27}$, $O_{142}$, $O_{55}$, and $O_{127}$ with percentage of 28.56%, 14.28%, 14.28%, 14.28%, and 14.28%, respectively.

Multiplex PCR was applied for the detection of virulence genes including shiga-toxin genes (*stx1* and *stx2*), and the intimin gene (*eae*) which detected in *E. coli*. All the isolates were negative to both *stx1* and *stx2* genes. Meanwhile, the raw milk isolates of $O_{142}$, $O_{55}$, $O_{111}$, and $O_{27}$ were positive to *eae* gene. However, $O_{26}$ isolate was negative to this gene. Also, the raw beef meat isolates of $O_{142}$, $O_{55}$, $O_{111}$, and $O_{27}$ were positive to *eae* gene. But, $O_{127}$ isolate was negative to this gene.

**KEY WORDS:** *E. coli*, Virulence genes, Raw milk and Raw meat.

## INTRODUCTION

Diarrhea is the second cause of death after pneumonia in children aged between 1 to 59 months, with mortality number of 0.509 million per year worldwide. Developing countries had the majority of this mentioned mortality cases of children by diarrhea **(Liu *et al.*, 2016 and WHO, 2017)**. Diarrhea outbreaks are happening all over the world which has the attention as an

important public health problem that caused by *Escherichia coli* bacteria causing diseases for human and animals as well **(Buchholz *et al.*, 2011; Watson *et al.*, 2017 and Valilis *et al.*, 2018).** This Gram-negative bacteria exists as part of the normal flora of animals and humans' gastrointestinal tract and responsible for infection between both of them **(Karmali *et al.*, 2010 and Lim *et al.*, 2010)**. The Majority of its strains are harmless. Unfortunately, there are many virulence factors such as the mobile genetic elements including; bacteriophages, plasmids….etc and pathogenicity islands that may be acquired by these strains resulting in turning them to the pathogenic state **(Kaper *et al.*, 2004).** Moreover, most of *E. coli* carrying hosts are apparently healthy and asymptomatic (**Hussein and Bollinger 2005, and Bogitsh *et al.*, 2018)**. And, ruminants such as sheep, goats and especially cattle, are counted as the main reservoir of *E. coli* bacteria **(Kaper *et al.*, 2004)**. *E. coli* transmission route starts when the bacteria could pass into the food chain via any contaminated food, drinks and water with feces **(Suardana *et al.*, 2017)**. So, *E. coli* transmission may occur via the consumption of any contaminated type of uncooked meat, fruits, vegetables, unpasteurized milk and its products **(Karmali *et al.*, 2010 and Tzschoppe *et al.*, 2012).** Consequently, *E. coli* infection may lead to many food-borne diseases in human including, diarrhea, renal failure, brain failure and hemolytic uremic syndrome which considered as life-threatening disease **(Karmali *et al.*, 2010 and Lim *et al.*, 2010).** There are six pathotypes of this bacterium, enterotoxigenic *E. coli* {ETEC}, enteropathogenic *E. coli* {EPEC}, enteroaggregative *E. coli* {EAEC}, diffusely adherent *E. coli* {DAEC} enterohemorrhagic *E. coli* {EHEC}, and enteroinvasive *E. coli* {EIEC} **(Nataro and Kaper 1998, and Lei *et al.*, 2018).** Also, there are many strains of *E. coli* that produce toxins called "shiga toxins" which cause illness in the vertebrates. These strains are called "shiga-toxin producing" *E. coli* (STEC) or verocytotoxic *E. coli* which are the pathotype group of enterohemorrhagic *E. coli* {EHEC}**(Nguyen and Sperandio 2012; Lacher *et al.*, 2016, and Valilis *et al.*, 2018)**. Also, it is classified by its serotypes which include more than 700 serotypes according to their O and H antigen **(Lacher *et al.*, 2016 and Bai *et al.*, 2018)**. *E. coli* O157:H7 is the major serotype that was associated with human illness world widely. This strain was classified as the most common strain responsible of the *E. coli* outbreaks in the USA, German, Northern Ireland, South Korea, Japan, England, Scotland and many other countries **(Tarr *et al.*, 2005; Money *et al.*, 2010; Buchholz *et al.*, 2011; Dallman *et al.*, 2012; Park *et al.*, 2014; Watahiki *et al.*, 2014; Launders *et al.*, 2016; Saeedi *et al.*, 2017 and Yang *et al.*, 2017).** However, in the last twenty years the non-O157 stains were classified as responsible serotypes for 20 to 50% of the *E. coli* associated illness' outbreaks world widely, these serotypes are including the $O_{26}$, $O_{45}$, $O_{103}$, $O_{111}$, $O_{121}$ and $O_{145}$ **(Wasilenko *et al.*, 2012; Gould *et al.*, 2013;**

**Albonico *et al.*, 2017 and Balamurugan *et al.*, 2017). Chapman *et al.*, (2006),** found that there were more than 50 virulence factors of *E. coli* participated in its pathogenicity. *E. coli* bacteria produce many factors associated with human illness such as Shiga toxins (stx1 and stx2), besides the other virulence factors that responsible for the attachment of the bacteria to the hosts' intestinal epithelial cells. This attaching lesions caused by the intimin protein that encoded by the *eae* gene **(Chapman *et al.*, 2006; Farfan and Torres 2012; and Gharieb *et al.*, 2015)**. So, the main aim of this work is to detect the virulence genes *stx1, stx2 and eae of Escherichia coli* bacteria growing in raw milk and beef meat collected from the local market in Fayoum Governorate. Besides, characterizing the serotypes of the *Escherichia coli* isolates.

## MATERIALS AND METHODS

### Sample collection

A total number of 50 raw milk and 50 raw beef meat samples were collected from the local market in Fayoum Governorate. Samples were collected in sterile marked container then inoculated in Carry and Blair transport medium. The last was kept in ice box for the laboratory bacterial culturing and identification.

### Bacteriological examination

The collected samples were cultured using MacConkey agar. The plates were aerobically incubated up to two days at 37 $^{o}$C. Then the suspected colonies were picked up and tested for Gram's reaction. The positive colonies were identified biochemically by using Vitek2 compact system (bioMérieux, Durham, NC, USA), according to the manufacturer's instructions **(Chatzigeorgiou *et al.*, 2011 and Quinn *et al.*, 2011),** using the Gram-Negative (GN) card which is a complete system for routine identification testing of most clinically significant Gram-Negative organisms. Colonies were transferred to the 0.45 % saline to prepare the organism suspension with a density equivalent to a 0.50 to 0.63 McFarland using a calibrated VITEK® 2 DensiCHEK™ Plus. Then, the last suspension used to fill the test cards for Vitek2 instrument.

### Serological identification

*Escherichia coli* isolates were serologically identified using the rapid diagnostic *E. coli* antisera set (Denka sieken comp. LTD) according to **Edwards and Ewing (1972)**.

### Molecular examination

#### DNA extraction

*Escherichia coli* isolates' DNA extraction was done using the QIAamp DNA Mini kit (Qiagen, Germany, GmbH) with modifications from the manufacturer’s recommendations and according to **(Sambrook *et***

***al.*, 1989).** In brief, a 200 µl of the each sample suspension was added to the proteinase K solution (10 µl), 200 µl of the lysis buffer and incubated at 56˚C for 10 min. Then, 200 µl of 100% ethyl alcohol was added to the lysate. After washing and centrifuging the sample, 100 µl of elution buffer that provided by the kit was used to elute the nucleic acid.

**PCR amplification**

PCR amplification of the *E. coli* isolates' DNA of the virulent genes was carried out using the primers that revealed to (*stx1*, *stx2*, and *eae*) genes as indicated in (Table1). This PCR amplification was applied on 10 random isolates (one of each serotype) of *E. coli*, 5 of each raw milk and beef meat samples for the detection of the virulence genes. The PCR amplification of these primers were utilized in a 25 µl reaction containing 12.5 µl of PCR Master Mix (Takara, Japan), 1 µl of each primer of 20 pmol concentration, 6 µl of DNA template and 4.5 µl of nuclease-free water. The reaction was performed in an (Applied Biosystem Thermal Cycler). Cycling conditions were used as recommended by the manufacturer as follow: primary denaturation: 94˚C/5 min., secondary denaturation: 94˚C/30 sec., annealing: 55˚C/45 sec., extension: 72˚C/45 sec., no. of cycles: 35 and final extension: 72˚C/10 min.

**Analysis of the PCR Products**

1.5 % agarose gel (Applichem, Germany, GmbH) was used to separate the PCR products by electrophoresis in 1x TBE buffer at room temperature using gradients of 5V/cm. For gel analysis, 20 µl of the PCR products was loaded in each gel well. The fragments sizes were determined using a gelpilot 100bp plus DNA Ladders (Qiagen, Germany, GmbH). The gel was photographed by a gel documentation system (Alpha Innotech, Biometra).

**Table (1): Primers used for the detection of virulent genes of *E. coli*, F: Forward and R: Reverse.**

| Target Genes | Primers sequences | Amplified Segment (bp) | Reference |
|---|---|---|---|
| eae | F: ATG CTT AGT GCT GGT TTA GG | 248 | **Bisi-Johnson *et al.*, 2011** |
| | R: GCC TTC ATC ATT TCG CTT TC | | |
| stx1 | F:ACACTGGATGATCTCAGTGG | 614 | **Shetty *et al.*, 2012** |
| | R:CTGAATCCCCCTCCATTATG | | |
| stx2 | F:CCATGACAACGGACAGCAGTT | 779 | **Shetty *et al.*, 2012** |
| | R:CCTGTCAACTGAGCAGCACTTTG | | |

## RESULTS

### The results of bacteriological examination

Out of the 100 raw milk and meat samples collected from the local market in Fayoum Governorate, Egypt, the *E. coli* was isolated as (58%) of the raw milk samples followed by (14%) of the raw beef meat samples as shown in table (2). Also, the biochemical identification of the positive *E. coli* isolates by Vitek2 system is shown in table (3).

**Table (2): Prevalence of *E. coli* bacteria isolated from collected samples.**

| No. of examined Samples | *Escherichia coli* | |
|---|---|---|
| | No. of +ve samples | % of +ve samples |
| 50 raw milk samples | 29 | 58 |
| 50 raw beef meat samples | 7 | 14 |

**No.: Number of positive isolates of *E. coli* and %: Percentage in relation to No. of examined samples (50).**

**Table (3): Biochemical details of *Escherichia coli* using Vitek 2 compact system.**

| Table 3: Biochemical details of *Escherichia coli* | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2 | APPA | - | 3 | ADO | - | 4 | PyrA | - | 5 | IARL | - | 7 | dCEL | - | 9 | BGAL | + |
| 10 | H2S | - | 11 | BNAG | - | 12 | AGLTP | - | 13 | dGLU | + | 14 | GGT | - | 15 | OFF | + |
| 17 | BGLU | - | 18 | dMAL | + | 19 | dMAN | + | 20 | dMNE | + | 21 | BXYL | - | 22 | BAlap | - |
| 23 | proA | - | 26 | LIP | - | 27 | PLE | - | 29 | TyrA | - | 31 | URE | - | 32 | dSOR | + |
| 33 | SAC | - | 34 | dTAG | - | 35 | dTER | + | 36 | CIT | - | 37 | MNT | - | 39 | 5KG | + |
| 40 | ILATK | - | 41 | AGLU | - | 42 | SUCT | + | 43 | NAGA | - | 44 | AGAL | - | 45 | PHOS | - |
| 46 | GlyA | - | 47 | ODC | + | 48 | LDC | + | 53 | IHISa | - | 56 | CMT | + | 57 | BGUR | - |
| 58 | O129R | + | 59 | GGAA | - | 61 | IMLTa | - | 62 | ELLM | - | 64 | ILATa | - | | | |

****Vitek2 Gram-negative card well contents according to BioMerieux, manufacturer manual are indicted in *Appendix 1.***

### The results of serotyping of *E. coli* positive isolates

Serogrouping of the 29 *E. coli* isolates from raw milk samples revealed presence of $O_{142}$, $O_{55}$, $O_{111}$, $O_{27}$, and $O_{26}$ with percentage of 20.69%, 20.69%, 17.24%, 17.24%, and 3.45%, respectively. Also, there were 6 isolates untyped as shown in table (4). However, the serogrouping of the 7 *E. coli* isolates from raw beef meat samples revealed presence of $O_{111}$, $O_{27}$, $O_{142}$, $O_{55}$, and $O_{127}$ with percentage of 28.56%, 14.28%, 14.28%, 14.28%, and 14.28%, respectively. Besides, there was one isolate untyped as shown in table (4).

**Table (4): Serotyping of *E. coli* isolates of both raw milk and beef meat samples.**

| *E. coli* serotypes | Raw milk samples | | Raw beef meat samples | |
|---|---|---|---|---|
| | No. of tested strain (29) | % of serotypes | No. of tested strain (7) | % of serotypes |
| $O_{142}$ | 6 | 20.69 | 1 | 14.28 |
| $O_{55}$ | 6 | 20.69 | 1 | 14.28 |
| $O_{111}$ | 5 | 17.24 | 2 | 28.56 |
| $O_{27}$ | 5 | 17.24 | 1 | 14.28 |
| $O_{26}$ | 1 | 03.45 | ---- | ------- |
| $O_{127}$ | ---- | ------- | 1 | 14.28 |
| Untyped | 6 | 20.69 | 1 | 14.28 |

No.: Number of isolates and %: Percentage in relation to No. of tested isolated strains of *E. coli* which is 29 for raw milk samples and 7 for raw meat samples.

**The results of molecular identification of the virulence genes of *E. coli* isolates**

PCR amplification was applied on 10 random isolates (one of each serotype) of *E. coli*, 5 of each raw milk and beef meat samples for the detection of the virulence genes. All the isolates were negative to both the shiga-toxin genes (*stx1*) and (*stx2*) (Figure 1). Meanwhile, the raw milk isolates of $O_{142}$, $O_{55}$, $O_{111}$, and $O_{27}$ were positive to the intimin gene (*eae*). However, $O_{26}$ isolate was negative to *eae* gene of *E. coli* (Figure 2). Also, the raw beef meat isolates of $O_{142}$, $O_{55}$, $O_{111}$, and $O_{27}$ were positive to the *eae* gene. But, $O_{127}$ isolate was negative to *eae* gene of *E. coli* (Figure 2).

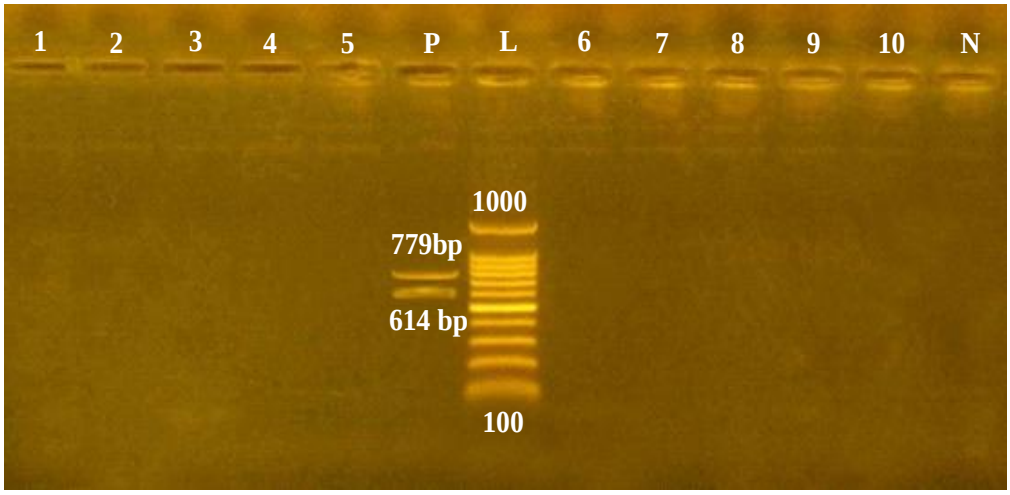


**Fig (1):** Agar gel electrophoresis showed results of multiplex PCR for detection of (*stx1* which amplified at 614bp and *stx2* which amplified at

779bp), L: represents the molecular size marker (100pb plus ladder), N: Negative control, P: Positive control, Lanes from 1:5 represent the raw milk isolates $O_{142}$, $O_{55}$, $O_{111}$, $O_{27}$, and $O_{26}$ which are negative for both *stx1 and stx2* genes, and Lanes from 6:10 represent the raw beef meat isolates $O_{142}$, $O_{55}$, $O_{111}$, $O_{27}$, and $O_{127}$ which are negative for both *stx1 and stx2* genes.

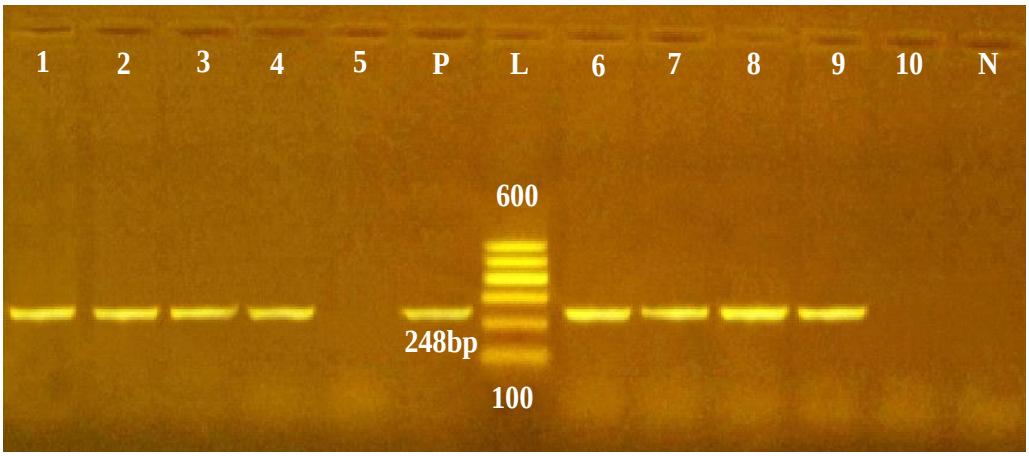


**Fig (2):** Agarose gel electrophoresis showed results of Multiplex PCR for detection of *eae* gene, L: represents the molecular size marker (100pb plus ladder), N: Negative control, P: Positive control of *eae* gene (248bp), Lanes from 1:5 represents the raw milk isolates; Lanes 1:4 are the isolates of $O_{142}$, $O_{55}$, $O_{111}$, and $O_{27}$ which are positive for *eae* gene, Lane 5 is the $O_{26}$ isolate which is negative for the *eae* gene, Lanes from 6:10 represent the raw beef meat isolates; Lanes 6:9 are the isolates of $O_{142}$, $O_{55}$, $O_{111}$, and $O_{27}$ which are positive for *eae* gene, lane 10 is the $O_{127}$ isolate which is negative for the *eae* gene.

## DISCUSSION

*Escherichia coli* considered as one of the bacteria that causing diarrhea outbreaks all over the world, and it is responsible for diseases for human and animals as well **(Buchholz *et al.*, 2011: Watson *et al.*, 2017 and Valilis *et al.*, 2018).** Unfortunately, the developing countries had the majority of mortality cases of children aged from 1 to 59 months caused by diarrhea **(Liu *et al.*, 2016 and WHO, 2017)**. Also, neonatal calf diarrhea is considered as of the most important health problems in livestock causing high economic losses worldwide either directly due to mortality and needs for treatment or indirectly through poor growth **(El-Seedy *et al.*, 2016; Abebaw *et al.*, 2018 and Bokma *et al.*, 2019).**

In present study, *E. coli* was isolated from raw milk samples with an isolation rate of 58%. This result was lower than the isolation rate that

described by (**Ombarak *et al.*, (2016)**, who isolated *E. coli* with an incidence of 76.4%. But, this result was higher than the isolation rate that described by **(Metwally and Ali (2015); Bedasa *et al.*, 2018 and Singh *et al.*, 2018),** who isolated *E. coli* with an incidence of 44%, 32% and 17.19% respectively. However, this percentage was almost similar to the rate that obtained by **El Nahas *et al.*, (2015),** who isolated *E. coli* with an incidence of 55%. There were only 7 *E. coli* isolates out of the 50 raw meat samples with an isolation rate of 14%. This percentage was almost similar to the rate that obtained by **Bedasa *et al.*, (2018).** However, this result was higher than the rates described by **Rahimi *et al.*, (2012) and Moawad *et al.*, (2017),** who isolated *E. coli* with an incidence of 8.2% and 11.7%, respectively. This high rate may be explained by that transmission of infection occurs during the milking process by milkers' hands, contaminated equipments and milking machine **Scherrer *et al.*, (2004)**. Also, this may be the same in case of meat rates which is more likely as cause of poor hygienic measures and customs during slaughter, handling, transportation and even during all stages of storage **Rahimi *et al.*, (2010).** Also, contamination level may be varied due to the differences in geographic or national region, processing environments, meat sources and the methodologies which the samples were taken such as; the samples amount, numbers and even the periods of which the samples were tested **Kegode *et al.*, (2008).**

Serogrouping of *E. coli* isolates from the raw milk samples revealed presence of $O_{142}$, $O_{55}$, $O_{111}$, $O_{27}$, and $O_{26}$ with percentage of 20.69%, 20.69%, 17.24%, 17.24%, and 3.45%, respectively. Also, the serogrouping of *E. coli* isolates from raw meat samples revealed presence of $O_{111}$, $O_{27}$, $O_{142}$, $O_{55}$, and $O_{127}$ with percentage of 28.56%, 14.28%, 14.28%, 14.28%, and 14.28%, respectively. The above mentioned results are in agreement with results of **Aisha (2001),** who isolated $O_{26}$, $O_{127}$ and $O_{27}$, **Correa and Marin (2002),** who isolated $O_{26,}$ $O_{55}$, $O_{111,}$ $O_{127}$ and $O_{142}$, **Blanco *et al.*, (2006),** who isolated $O_{55}$, $O_{111,}$ $O_{127}$ and $O_{142}$, **Lin *et al.*, (2011)** who isolated $O_{26}$, $O_{142}$ and $O_{111}$, **Fadel *et al.*, (2017),** who isolated $O_{26}$ ,$O_{27}$,$O_{55}$, $O_{111}$, and $O_{142}$,and **Kalule *et al.*, (2018),** who isolated $O_{26}$, $O_{55,}$ and $O_{111}$,

Molecular characterization of the *E. coli* isolates from both raw milk and beef meat samples through applying different conditions of multiplex PCR for detection of genes encoding virulence factors (*stx1*, *stx2* and *eae*). All the isolates were negative to both the shiga-toxin genes (*stx1*) and (*stx2*) (Figure 1). Meanwhile, the raw milk isolates of $O_{27}$, $O_{55}$, $O_{111}$, and $O_{142}$ were positive to the intimin gene (*eae*). These results agreed with the results of **Blanco *et al.*, (2006)** who found that $O_{55}$, $O_{111}$ and $O_{142}$ are negative for both shiga-toxin genes (*stx1*) and (*stx2*) and positive to the intimin gene (*eae*). However, $O_{26}$ isolate was negative to gene (*eae*) of *E. coli* (Figure 2). This result is in

agreement with the results of **Correa and Marin (2002),** who found that $O_{26}$ is negative for the (eae) gene. Also, the raw meat isolates of $O_{142}$, $O_{55}$, $O_{111}$, and $O_{27}$ were positive to the intimin gene (*eae*). But, $O_{127}$ isolate was negative to gene (*eae*) of *E. coli* (Figure 2). These results agreed with **Blanco *et al.*, (2006)** except for the $O_{127}$ isolate, which was found to be positive for *eae* gene. These differences in expressing some genes for the same serotype may be because the ability of some strains to acquire many virulence factors **(Kaper *et al.*, 2004).** Also, **Correa and Marin (2002)** found that some $O_{55}$ strains are negative for the (*eae*) gene and other $O_{55}$ strains are positive for the same gene. And, **Sanchez *et al.*, (2010)** found that $O_{127}$ was negative to *eae* and *stx1* genes but it was positive to *stx2* gene. And, some serotypes were found to express specific genes and the same serotype are not, like the *E. coli* serotype of $O_{157}$ some isolates expressed both *stx2* and *eae* genes, some expressed only *eae* gene and others were negative to *eae* gene and positive to *stx2* gene.

## CONCLUSION

*E. coli* was found in both raw milk and raw beef meat in Fayoum local market. And, the molecular characterization of its virulence genes indicated that all the isolates are missing the shiga-toxin genes (*stx1* and *stx2*). However, most of the detected *E. coli* serotypes were found to have the *eae* virulence gene, which still needs more attention because the ability of some strains to acquire many virulence factors and may all the isolates of this study have other virulent factors that were not examined in this study. More important, the serotypes of $O_{26}$ and $O_{111}$ which were isolated in this study are classified as a part of the main non-O157 stains that responsible for 20 to 50% of the *E. coli* associated illness' outbreaks world widely. So, strict hygienic measures and intensive care should be applied by the authorities and all the people to overcome this kind of contamination for good health for human and animals and consequently to minimize the economic losses.

## REFERENCES

**Abebaw, R.; Mitku, F. and Fentie, T. (2018):** A review on the importance of calf diarrhea in dairy production system: Ethiopian perspective. *J. Am. Sci.,* 14(10): 71-83.

**Aisha, R. A. (2001):** Comparative studies on diarrhea caused By *E. coli* in farm animals. *J. Egypt. Vet. Med. Assoc.,* 61 (6): 39-49.

**Albonico, F.; Gusmara, C.; Gugliotta, T.; Loiacono, M.; Mortarino, M. and Zecconi, A (2017):** A new integrated approach to analyze bulk tank milk and raw milk filters for the presence of the E. coli serogroups frequently associated with VTEC status. *Res. Vet. Sci.,* 115: 401-406.

**Bai, X.; Mernelius, S.; Jernberg, C.; Einemo, I. M.; Monecke, S.; Ehricht, R.; Lofgren, S. and Matussek, A. (2018):** Shiga toxin-producing *Escherichia coli* infection in Jönköping county, Sweden: occurrence and molecular

characteristics in correlation with clinical symptoms and duration of stx shedding. *Front. Cell. Infect. Microbiol.,* 8:125. doi: 10.3389/fcimb.2018.00125**.**

**Balamurugan, S.; Ahmed, R.; Gao, A. and Strange, P. (2017):** Comparison of the fate of the top six non-O157 shiga-toxin producing *Escherichia coli* (STEC) and *E. coli* O157:H7 during the manufacture of dry fermented sausages. *Int. J. Food Microbiol.*, 259: 14-21.

**Bedasa, S.; Shiferaw, D.; Abraha, A. and Moges, T. (2018):** Occurrence and antimicrobial susceptibility profile of *Escherichia coli* O157:H7 from food of animal origin in Bishoftu town, Central Ethiopia. *Int. J. Food Contam.,* 5 (2): 1-8.

**Bisi-Johnson, M. A.; Obi, C. L.; Vasaikar, S. D.; Baba, K. A. and Hattori, T. (2011):** Molecular basis of virulence in clinical isolates of *Escherichia coli* and *Salmonella* species from a tertiary hospital in the Eastern Cape, South Africa. *Gut Pathogens*, 3 (9): 1-8.

**Blanco, M.; Blanco,J. Dahbi, G.; Mora,A.; Alonso, M. P.; Varela, G.; Gadea,M. P.; Schelotto,F.; Gonza, E. A. and Blanco, J. (2006):** Typing of intimin (eae) genes from enteropathogenic Escherichia coli (EPEC) isolated from children with diarrhoea in Montevideo, Uruguay: identification of two novel intimin variants (mB and jR/b2B). *J. Med. Microbiol.,* 55: 1165-1174.

**Bogitsh, B. J.; Carter, C. E. and Oeltmann, T. N. (2018):** *Human parasitology*, 5th ed.; Elsevier, London, United Kingdom.

**Bokma, J.; Boone, R.; Deprez, P. and Pardon, B. (2019):** Risk factors for antimicrobial use in veal calves and the association with mortality. *J. Dairy Sci.,* 102: 607-618.

**Buchholz, U.; Bernard, H.; Werber, D.; Böhmer, M.; Remschmidt, C.; Wilking, H.; Deleré, Y.; Heiden, M.; Adlhoch, M.; Dreesman, J. and Ehlers, J. (2011):** German outbreak of Escherichia coli O104:H4 associated with sprouts. *N. Engl. J. Med.*, 365: 1763-1770.

**Chapman, T. A.; Wu, X. Y.; Barchia, I.; Bettelheim, K. A.; Driesen, S.; Trott, D.; Wilson, M. and Chin, J. J. (2006):** Comparison of virulence gene profiles of *Escherichia coli* strains isolated from healthy and diarrheic swine. Appl. Environ. Microbiol., 72: 4782-4795.

**Chatzigeorgiou, K. S.; Sergentanis, T. N.; Tsiodras, S.; Hamodrakas, S. J. and Bagos, P.G., (2011):** Phoenix 100 versus Vitek 2 in the identification of gram-positive and gram-negative bacteria: a comprehensive meta-analysis. *J. clin. microbiol.*,49: 3284-3329.

**Correa, M. G. P. and Marin, J. M. (2002):** O-serogroups, eae gene and EAF plasmid in *Escherichia coli* isolates from cases of bovine mastitis in Brazil. *Vet. Microbiol.,* 85: 125-132.

**Dallman, T.; Smith, G. P.; O'brien, B.; Chattaway, M. A.; Finlay, D., Grant, K. A. and Jenkins, C. (2012):** Characterization of a verocytotoxin - producing enteroaggregative *Escherichia coli* serogroup O111:H21 strain

associated with a household outbreak in Northern Ireland. *J. Clin. Microbiol*., 50: 4116-4119.

**Edwards, P. R. and Ewing, W. H. (1972):** Identification of *Enterobacteriacae*, 3rd ed., Burgess Pub. Co., MN, USA.

**El Nahas, A. W.; Mohamed, H. A.; El Barbary, H. A. and Mohamed, H. S. (2015):** Incidence of E. coli in raw milk and its products. *Benha Vet. Med. J.*, 29 (1): 112-117.

**El-Seedy, F. R.; Abed, A. H.; Yanni, H. A. and Abd El-Rahman, S. A. A. (2016):** Prevalence of *E. coli* and *Salmonella* in neonatal calves with diarrhea. *J. Basic and Applied Sci. Cell. Mol. Bio.* 62: 21–28. .

**Fadel, H. M.; Afifi, R. and Al-Qabili, D. M. (2017):** Characterization and zoonotic impact of Shiga toxin producing *Escherichia coli* in some wild bird species. *Vet. World,* 10 (9): 1118-1128.

**Farfan, M. J. and Torres, A. G., (2012):** Molecular mechanisms that mediate colonization of Shiga toxin-producing *Escherichia coli* strains. *Infect. Immun.,* 80: 903-913.

**Gharieb, R. M.; Fawzi, E. M.; Attia, N. E. and Bayoumi, Y. H. (2015):** Calf diarrhea in Sharkia province, Egypt: diagnosis; prevalence, virulence profiles and zoonotic potential of the causative bacterial agents. *Int. J. Agric. Sci. Vet. Med.,* 3 (2): 71-87.

**Gould, L. H.; Mody, R. K.; Ong, K. L.; Clogher, P.; Cronquist, A. B.; Garman, K. N.; Lathrop, S.; Medus, C.; Spina, N. L.; Webb, T. H.; White, P. L.; Wymore, K.; Gierke, R. E.; Mahon, B. E.; Griffin, F.T. ( 2013):** Increased recognition of non-O157 shiga toxin–producing *Escherichia coli* infections in the United States during 2000-2010: epidemiologic features and comparison with E. coli O157 infections. *Foodborne Pathog. Dis.,* 10; 453-460.

**Hussein, H. S. and Bollinger, L. M. (2005):** Prevalence of Shiga toxin-producing *Escherichia coli* in beef cattle. *J. Food Prot.,* 68: 2224-2241.

**Kalule, J. B.; Keddy, K. H. and Nicol, M. P. (2018):** Characterisation of STEC and other diarrheic *E. coli* isolated on CHROM agar™ STEC at a tertiary referral hospital, Cape Town. *BMC Microbiol.,* 18 (55): 1-8.

**Kaper, J. B.; Nataro, J. P. and Mobley, H. L. (2004):** Pathogenic *Escherichia coli*. *Nat. Rev. Microbiol.*, 2: 123-140.

**Karmali, M. A.; Gannon, V. and Sargeant, J. M. (2010):** Verocytotoxin-producing *Escherichia coli* (VTEC), *Vet. Microbiol.,* 140 (3-4): 360-370.

**Kegode, R. B.; Doetkott, D. K.; Khaitsa, M. L. and Wesley, I. V. (2008):** Occurrence of Campylobacter species, Salmonella species and generic *Escherichia coli* in meat products from retail outlets in the Fargo metropolitan area. *J. Food Safety*, 28: 111-125.

**Lacher, D. W.; Gangiredla, J.; Patel, I.; Elkins, C. A. and Feng, P. C.H. (2016):** Use of the *Escherichia coli* identification microarray for characterizing the health risks of Shiga toxin-producing *E. coli* isolated from foods. *J. Food Prot.*, 79: 1656-1662.

**Launders, N.; Locking, M. E.; Hanson, M.; Willshaw, G.; Charlett, A.; Salmon, R.; Cowden, J. and Adak, G. K. (2016)**: A large Great Britainwide outbreak of STEC O157 phage type 8 linked to handling of raw leeks and potatoes. *Epidemiol. Infect.*, 144: 171-181.

**Lei, L.; Rehman, M. U.; Huang, S.; Zhang, L.; Wang, L.; Mehmood, K.; Zhang, H.; Tong, X.; Wang, M. and Li, J. (2018):** Antimicrobial resistance and prevalence of diarrheagenic *Escherichia coli* (DEC), in diarrheic yaks of Tibetan Plateau, China. *Acta Trop.*, 182: 111-114.

**Lim, J. Y.; Yoon, J. W. and Hovde, C. J. (2010):** A brief overview of *Escherichia coli* O157:H7 and its plasmid O157. *J. Microbiol. Biotechnol.*, 20 (1): 5-14.

**Lin, A.; Nguyen, L.; Lee, T.; Clotilde, L. M.; Kase, J. A.; Son, I.; Carter, J. M. and Lauzon, C. R. (2011):** Rapid *O* serogrouping of the ten most clinically relevant STECs by Luminexmicrobead-based suspension array. *J Microbiol. Methods*, 87 (1): 105-110.

**Liu, L.; Oza, S.; Hogan, D.; Chu, Y.; Perin, J.; Zhu, j.; Lawn, j. E.; Cousens, S.; Mathers, C. and Black, R. E. (2016):** Global, regional, and national causes of under-5 mortality in 2000-15: an updated systematic analysis with implications for the Sustainable Development Goals. *Lancet*, 388: 3027-3035.

**Metwally, A. M. M. and Ali, F. H. M. (2015):** *Escherichia coli* $O_{157}$ in dairy products from retailers and small dairy shops. *J. Food and Dairy Sci., Mansoura Univ.*, 6 (5): 349-355.

**Moawad, A. A.; Hotzel, H.; Awad, O.; Tomaso, H.; Neubauer, H.; Hafez, H. M. and El-Adawy, H. (2017):** Occurrence of Salmonella entericaand Escherichia coli in raw chicken and beef meat in northern Egypt and dissemination of their antibiotic resistance markers. *Gut Path.,* 9 (57): 1-13.

**Money, P.; Kelly, A. F.; Gould, S. W. J.; Denholm-Price, J.; Threlfall, E. J. and Fielder, M. D. (2010):** Cattle, weather and water: mapping *Escherichia coli* O157:H7 infections in humans in England and Scotland. *Environ. Microbiol.*, 12: 2633-2644.

**Nataro, J. P. and Kaper, J. B. (1998):** Diarrheagenic *Escherichia coli*. *Clin. Microbiol. Rev.*, 11: 132-201.

**Nguyen, Y. and Sperandio, V. (2012):** Enterohemorrhagic E. coli (EHEC) pathogenesis. *Front. Cell. Infect. Microbiol.*, 2:90. doi: 10.3389/fcimb.2012.00090**.**

**Ombarak, R. A.; Hinenoya, A.; Awasthi, S. P.; Iguchi, A.; Shima, A.; Elbagory, R. M. and Yamasaki, S. (2016)**: Prevalence and pathogenic potential of Escherichia coli isolates from raw milk and raw milk cheese in Egypt. *Int. J. Food Microbiol.*, 221: 69-76.

**Park, J. H.; Oh, S. S.; Oh, K. H.; Shin, J.; Jang, E. J.; Jun, B. Y.; Youn, S. K. and Cho, S. H. (2014)**: Diarrheal outbreak caused by atypical enteropathogenic *Escherichia coli* O157:H45 in South Korea. *Foodborne Pathog. Dis.*, 11: 775-781.

**Quinn, P. J.; Markey, B. K.; Leonard, F. C.; Hartigan, P.; Fanning, S. and Fitzpatric, E. S. (2011):** Veterinary microbiology and microbial diseases.2nd edition. Willy-Blackwell publisher, IN, USA.

**Rahimi, E.; Ameri, M. and Kazemeini, H. R. (2010):** Prevalence and antimicrobial resistance of Campylobacter species isolated from raw camel, beef, lamb and goat meat in Iran. *Foodborne Path. Dis.,* 7; 443-447.

**Rahimi, E.; Kazemeini, H. R. and Salajegheh, M. (2012):** *Escherichia coli* O157:H7/NM prevalence in raw beef, camel, sheep, goat, and water buffalo meat in Fars and Khuzestan provinces, Iran. *Vet. Res. Forum*, 3 (1): 13-17.

**Saeedi, P.; Yazdanparast, M.; Behzadi, E.; Salmanian, A.; Mousavi, S.; Nazarian, S. and Amani, J. (2017):** A review on strategies for decreasing *E. coli* O157:H7 risk in animals. *Microb. Pathog.,* 103: 186-195.

**Sambrook, J.; Fritscgh, E. F. and Mentiates (1989):** Molecular coloning. A laboratory manual, Cold spring Harbor Laboratotry press, NY. USA.

**Sanchez, S.; Martınez, R.; Garcıa, A.; Vidal, D.; Blanco, J.; Blanco, M.; Blanco, J. E.; Mora, A.; Herrera-Leon, S.; Echeita, A.; Alonso, J. M. and Rey, J. (2010):** Detection and characterisation of O157:H7 and non-O157 Shiga toxin-producing *Escherichia coli* in wild boars. *Vet. Microbiol.,* 143: 420-423.

**Scherrer, D.; Coti, S.; Muehlberr, J. E.; Zweife, C. and Stephan, R. (2004):** Phenotypic and genotypic characteristics of *S. aureus* isolates from raw bulk-tank milk samples. *Vet. Microbiol*., 101:101-107.

**Shetty, V. A.; Kumar, S. H.; Shetty, A. K.; Karunasagar, I. and Karunasagar, I. (2012):** Prevalence and Characterization of Diarrheagenic Escherichia coli Isolated from Adults and Children in Mangalore, India. *J. Lab. Phys.* 4 (1): 24-29.

**Singh, A.; Chhabra, D.; Sikrodia, R.; Shukla, S.; Sharda, R. and Audarya, S. (2018):** Isolation of *E. coli* from bovine mastitis and their antibiotic sensitivity pattern. *Int. J. Curr. Microbiol. App. Sci.,* 7 (10): 11-18.

**Suardana, I. W.; Widiasih, D. A.; Nugroho, W. S.; Wibowo, M. H. and Suyasa, I. N. (2017):** Frequency and risk-factors analysis of Escherichia coli O157:H7 in Bali-cattle. *Acta Tropica*, (172): 223-228.

**Tarr, P. I.; Gordon, C. A. and Chandler, W. L. (2005):** Shiga-toxin-producing *Escherichia coli* and haemolytic uraemic syndrome. *Lancet,* 365: 1073-1086.

**Tzschoppe, M.; Martin, A. and Beutin, L. (2012):** A rapid procedure for the detection and isolation of enterohaemorrhagic *Escherichia coli* (EHEC) serogroup O26, O103, O111, O118, O121, O145 and O157 strains and the aggregative EHEC O104:H4 strain from ready-to-eat vegetables. *Int. J. Food Microbiol.*, 152; 19-30.

**Valilis, E.; Ramsey, A.; Sidiq, S. and DuPont, H. L. (2018):** Non-O157 Shiga toxin-producing Escherichia coli-A poorly appreciated enteric pathogen: Systematic review. *Int. J. Infect. Dis.,* 76: 82-87.

**Wasilenko, J. L.; Fratamico, P. M.; Narang, N.; Tillman, G. E.; Ladely, S.; Simmons, M. and Cray, W.C. (2012):** Influence of primer sequences and DNA extraction method on detection of non-O157 Shiga toxin-producing *Escherichia coli* in ground beef by real time PCR targeting the eae, stx, and serogroup-specific genes. *J. Food Prot.*, 75: 1939-1950.

**Watahiki, M.; Isobe, J.; Kimata, K.; Shima, T.; Kanatani, J.; Shimizu, M.; Nagata, A.; Kawakami, K.; Yamada, M.; Izumiya, H.; Iyoda, S.; Morita-Ishihara, T.; Mitobe, J.; Terajima, J.; Ohnishi, M. and Sata, T. (2014):** Characterization of enterohemorrhagic *Escherichia coli* O111 and O157 strains isolated from outbreak patients in Japan. *J. Clin. Microbiol.*, 52: 2757-2763.

**Watson, V. E.; Jacob, M. E.; Flowers, J. R.; Strong, S. J.; DebRoy, C. and Gookin, J. L. (2017):** Association of atypical enteropathogenic *Escherichia coli* with diarrhea and related mortality in kittens. *J. Clin. Microbiol.*, 55: 2719-2735**.**

**Widiasih, D. A.; Nugroho, W. S.; Wibowo, M. H. and Suyasa, I. N. (2017):** Frequency and risk-factors analysis of *Escherichia coli* O157:H7 in Bali-cattle. *Acta Trop.*, 172: 223-228.

**World Health Organization (2017):** Diarrhoeal disease Key facts**, Available at:** https://www.who.int / news-room / factsheets / detail / diarrhoeal-disease. Accessed 20 December 2018**.**

**Yang, S. C.; Lin, C. H.; Aljuffali, I. A.; and Fang, J. Y. (2017):** Current pathogenic *Escherichia coli* foodborne outbreak cases and therapy development. *Arch. Microbiol.*, 199: 811-825.

**Appendix (1): Vitek2 Gram-negative card well contents according to BioMerieux, manufacturer manual**

| | |
|---|---|
| 5KG: 5-Keto-D-Gluconate<br>ADO: Adonitol<br>AGAL: α-galactosidase<br>AGLTP: GlutamylArylamidase-transferase<br>AGLU: α-glucosidase<br>APPA: Ala-Phe-Pro-Arylamidase<br>BAlap: β-Alanine arylamidasepNA<br>BGAL: β- Galactosidase<br>BGLU: β-Glucosidase<br>BGUR: β-glucuronidasE<br>BNAG: β-N-Acetyl-Glucoaminidase<br>BXYL: β-Xylosidase<br>CIT: Sodium Citrate<br>CMT: Coumerate<br>dCEL: D-cellobiose<br>dGLU: D-glucose<br>dMAL: D-maltose<br>dMAN: D-mannitol<br>dMNE: D-mannose<br>dSOR: D-Sorbitol<br>dTAG: D-Tagatose<br>dTER: D-Trehalose<br>ELLM: Ellman<br>GGT: γ-Gutamyl- Transferase | GlyA: Glycine arylamidase<br>$H_2S$: $H_2S$ production<br>IARL: L-arbitol<br>IHISa: L-histidine assimilation<br>ILATa: L-Lactate assimilation<br>ILATK: L-Lactate assimilation<br>IMLTa: L-Malate assimilation<br>LDC: Lysine decarboxylase<br>LIP: Lipase<br>MNT: Malonate<br>NAGA: β-N-Acetyl-Galactosaminidase<br>O129R: 0/129 resistance (comp.vibrio)<br>ODC: Ornithine decarboxylase<br>OFF: Fermentation Glucose<br>PHOS: Phosphate<br>PLE: Palatinose<br>proA: L-ProlineArylamidase<br>PyrA: L-Pyrrolydonyl-Arylamidase<br>SAC: Saccharose/Sucrose<br>SUCT: Succinate alkalinization<br>TyrA: Tyrosine Arylamidase<br>URE: Urease<br>STAG: D-Tagatose |

# التوصيف الجزيئى والسيرولوجي لميكروب الايسيريشيا كولاي الغير مٌفرزه لسموم الشيجا، المرتبطة بالأغذية المجمعة من السوق المحلى بمحافظة الفيوم ، مصر

**عمروعزت محمد محمود [١] ، غادة عمر الدمرداش [٢] ، محمد حسين حمدي روبي [٣] وسحر رشدي محمد [٢]**

١ قسم الكيمياء الحيوية ، كلية الزراعة ، جامعة الفيوم ، الفيوم ، مصر.

٢ معهد بحوث صحة الحيوان ، الدقى ، الجيزة ، مصر.

٣ قسم علوم وتكنولوجيا الأغذية ، كلية الزراعة ، جامعة الفيوم ، الفيوم ، مصر.

**الملخص:**

تعتبر الايسيريشيا كولاي واحدة من البكتيريا التي تسبب تفشي الإسهال في جميع أنحاء العالم ، وهي مسؤولة عن أمراض الإنسان والحيوان. تم تجميع ٥٠ عينه لبن خام و ٥٠ عينة لحم بقر من السوق المحلية بمحافظة الفيوم. خضعت هذه العينات إلي الاختبارات البكتيرية و السيرولوجيه و البيولوجيه الجزيئيه و كان معدل عزل الايسيريشيا كولاي من اللبن الخام وعينات لحوم البقر الخام ٥٨٪ و ١٤٪ على التوالي. تبين بالفحص السيرولوجي لعترات الايكولاي المعزوله من عينات اللبن الخام وجود O١٤٢، O٥٥، O١١١، O٢٧ وO٢٦ بنسبة ٢٠.٦٩٪ و ٢٠.٦٩٪ و ١٧.٢٤٪ و ١٧.٢٤٪ و ٣.٤٥٪ على التوالي. بينما أظهر الفحص السيرولوجي لعترات الايكولاي المعزوله من عينات لحم البقر وجود O١١١، O٢٧، O١٤٢، O٥٥ وO١٢٧ بنسبة ٢٨.٥٦٪ و ١٤.٢٨٪ و ١٤.٢٨٪ و ١٤.٢٨٪ و ١٤.٢٨٪ على التوالي. تم إستخدام تفاعل إنزيم البلمره المتسلسل (PCR) لاكتشاف جينات الضراوة لكل من *stx1* -*stx2* و *eae* في الايسيريشيا كولاي و كانت جميع العزلات سلبية لكل من جينات *stx1* و *stx2*.

وكانت عزلات اللبن الخام من O١٤٢، O٥٥، O١١١، O٢٧ إيجابية للجين *eae*. بينما كانت العزلة O٢٦ سلبية لهذا الجين. و كانت عزلات لحوم البقر من O١٤٢، O٥٥، O١١١، O٢٧ إيجابية للجين *eae*. بينما كانت العزلة O١٢٧ سلبية لهذا الجين.