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. Mahmoud¹, Ghada O. El-demerdash², Mohamed H. H. Roby³ and Sahar R. Mohamed²

¹Biochemistry Department, Faculty of Agriculture, Fayoum University, Fayoum, Egypt.
²Animal Health Research Institute, Dokki, Giza, Egypt.
³Food 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 O₁₄₂, O₅₅, O₁₁₁, O₂₇, and O₂₆ 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 O₁₁₁, O₂₇, O₁₄₂, O₅₅, and O₁₂₇ 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 O₁₄₂, O₅₅, O₁₁₁, and O₂₇ were positive to *eae* gene. However, O₂₆ isolate was negative to this gene. Also, the raw beef meat isolates of O₁₄₂, O₅₅, O₁₁₁, and O₂₇ were positive to *eae* gene. But, O₁₂₇ 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; Fayoum J. Agric. Res. & Dev., Vol. 33, No.1, January, 2019
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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 Cary 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 °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
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 gel pilot 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.**

<table>
<thead>
<tr>
<th>Target Genes</th>
<th>Primers sequences</th>
<th>Amplified Segment (bp)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>eae</td>
<td>F: ATG CTT AGT GCT GGT TTA GG&lt;br&gt; R: GCC TTC ATC ATT TCG CTT TC</td>
<td>248</td>
<td>Bisi-Johnson <em>et al.</em>, 2011</td>
</tr>
<tr>
<td>stx1</td>
<td>F:ACACTGGATGATCTCAGTGG&lt;br&gt; R:CTGAATCCCCCCTCCATTATG</td>
<td>614</td>
<td>Shetty <em>et al.</em>, 2012</td>
</tr>
<tr>
<td>stx2</td>
<td>F:CCATGACAACGGACAGCAGTTT&lt;br&gt; R:CCTGTCAACTGAGCAGCACTTTG</td>
<td>779</td>
<td>Shetty <em>et al.</em>, 2012</td>
</tr>
</tbody>
</table>
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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.

<table>
<thead>
<tr>
<th>No. of examined Samples</th>
<th>Escherichia coli</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of +ve samples</td>
</tr>
<tr>
<td>50 raw milk samples</td>
<td>29</td>
</tr>
<tr>
<td>50 raw beef meat samples</td>
<td>7</td>
</tr>
</tbody>
</table>

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.

| **Vitek2 Gram-negative card well contents according to BioMerieux, manufacturer manual are indicted in Appendix 1.** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 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 | B Alap | - |
| 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 | dMLT | - | 62 | ELM | - | 64 | ILAT | - |   |

The results of serotyping of E. coli positive isolates

Serogrouping of the 29 E. coli isolates from raw milk samples revealed presence of O142, O35, O111, O27, and O26 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 O111, O27, O142, O35, and O127 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.

<table>
<thead>
<tr>
<th><em>E. coli</em> serotypes</th>
<th>Raw milk samples</th>
<th>Raw beef meat samples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of tested strain (29)</td>
<td>% of serotypes</td>
</tr>
<tr>
<td>O&lt;sub&gt;142&lt;/sub&gt;</td>
<td>6</td>
<td>20.69</td>
</tr>
<tr>
<td>O&lt;sub&gt;55&lt;/sub&gt;</td>
<td>6</td>
<td>20.69</td>
</tr>
<tr>
<td>O&lt;sub&gt;111&lt;/sub&gt;</td>
<td>5</td>
<td>17.24</td>
</tr>
<tr>
<td>O&lt;sub&gt;27&lt;/sub&gt;</td>
<td>5</td>
<td>17.24</td>
</tr>
<tr>
<td>O&lt;sub&gt;26&lt;/sub&gt;</td>
<td>1</td>
<td>03.45</td>
</tr>
<tr>
<td>O&lt;sub&gt;127&lt;/sub&gt;</td>
<td>----</td>
<td>-------</td>
</tr>
<tr>
<td>Untyped</td>
<td>6</td>
<td>20.69</td>
</tr>
</tbody>
</table>

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<sub>142</sub>, O<sub>55</sub>, O<sub>111</sub>, and O<sub>27</sub> were positive to the intimin gene (*eae*). However, O<sub>26</sub> isolate was negative to *eae* gene of *E. coli* (Figure 2). Also, the raw beef meat isolates of O<sub>142</sub>, O<sub>55</sub>, O<sub>111</sub>, and O<sub>27</sub> were positive to the *eae* gene. But, O<sub>127</sub> 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.](image-url)
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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).


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 (stxl) and (stx2) (Figure 1). Meanwhile, the raw milk isolates of O27, O55, O111, and O142 were positive to the intimin gene (eae). These results agreed with the results of Blanco et al., (2006) who found that O55, O111 and O142 are negative for both shiga-toxin genes (stxl) and (stx2) and positive to the intimin gene (eae). However, O26 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


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

<table>
<thead>
<tr>
<th>Card Name</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>5KG: 5-Keto-D-Gluconate</td>
<td>GlyA: Glycine arylamidase</td>
</tr>
<tr>
<td>ADO: Adonitol</td>
<td>H₂S: H₂S production</td>
</tr>
<tr>
<td>AGAL: α-galactosidase</td>
<td>IARL: L-arbitol</td>
</tr>
<tr>
<td>AGLTP: GlutamylArylamidase-transferase</td>
<td>IHISa: L-histidine assimilation</td>
</tr>
<tr>
<td>AGLU: α-glucosidase</td>
<td>ILATa: L-Lactate assimilation</td>
</tr>
<tr>
<td>APPA: Ala-Phe-Pro-Arylamidase</td>
<td>ILATK: L-Lactate assimilation</td>
</tr>
<tr>
<td>BAalap: β-Alanine arylamidase</td>
<td>IMLTa: L-Malate assimilation</td>
</tr>
<tr>
<td>BGAL: β- Galactosidase</td>
<td>LDC: Lysine decarboxylase</td>
</tr>
<tr>
<td>BGLU: β-Glucosidase</td>
<td>LIP: Lipase</td>
</tr>
<tr>
<td>BGUR: β-glucuronidasE</td>
<td>MNT: Malonate</td>
</tr>
<tr>
<td>BXYL: β-Xylosidase</td>
<td>O129R: 0/129 resistance (comp.vibrio)</td>
</tr>
<tr>
<td>CIT: Sodium Citrate</td>
<td>ODC: Ornithine decarboxylase</td>
</tr>
<tr>
<td>CMT: Coumerate</td>
<td>OFF: Fermentation Glucose</td>
</tr>
<tr>
<td>dCEL: D-cellobiose</td>
<td>PHOS: Phosphate</td>
</tr>
<tr>
<td>dGLU: D-glucose</td>
<td>PLE: Palatinose</td>
</tr>
<tr>
<td>dMAL: D-maltose</td>
<td>proA: L-ProlineArylamidase</td>
</tr>
<tr>
<td>dMAN: D-mannitol</td>
<td>PyrA: L-Pyrrolydonyl-Arylamidase</td>
</tr>
<tr>
<td>dMNE: D-mannose</td>
<td>SAC: Saccharose/Sucrose</td>
</tr>
<tr>
<td>dSOR: D-Sorbitol</td>
<td>SUCT: Succinate alkalinization</td>
</tr>
<tr>
<td>dTAG: D-Tagatose</td>
<td>TyrA: Tyrosine Arylamidase</td>
</tr>
<tr>
<td>dTER: D-Trehalose</td>
<td>URE: Urease</td>
</tr>
<tr>
<td>ELLM: Ellman</td>
<td>STAG: D-Tagatose</td>
</tr>
<tr>
<td>GGT: γ-Gutamyl- Transferase</td>
<td></td>
</tr>
</tbody>
</table>
التصنيف الجزيئي والسيروЛОجي لميكروب الإسبريشيا كولاي الغير مفرّز له لموم الشجا،
المربطة بالأغذية المجمعة من السوق المحلي بمحافظة الفيوم، مصر.

أمرو عصمت محمد محمد، غادة عمار الدمرداش، محمد حسين حمدي روبى، وسحر شادي محمد
1 قسم الكيمياء الحيوية، كلية الزراعة، جامعة الفيوم، الفيوم، مصر.
2 معهد بحوث صحة الحيوان، الدقى، الجيزة، مصر.
3 قسم علوم وتقنية الأغذية، كلية الزراعة، جامعة الفيوم، الفيوم، مصر.

الملخص:
تعتبر الإسبريشيا كولاي واحدة من البكتيريا التي تسبب تفشي الإسهال في جميع أنحاء العالم، وهي مسؤولة عن أمراض الإنسان والحيوان. تم تجميع عينات من فارس وموزعه لحم
بقر من السوق المحلية بمحافظة الفيوم. خضعت هذه العينات إلى الاختبارات البكترية والسيروولوجية، وكان معدل إسبريشيا كولاي من اللحم الخام عبئ
لحم البقر الخاص 58% و24% على التوالي. تم تجميع الفحص السيرولوجي لعُنورات الإسبريشيا كولاي
من عينات اللحم الخام وجود 0.05، 0.03، 0.01، 0.001، 0.0001 النسبة 20.19% و 20.24% و
17.24% و 17.2% و 17.45% على التوالي. بينما أظهر الفحص السيرولوجي لعنورات الإسبريشيا
المعزولون من عينات لحم البقر وجد 0.05، 0.03، 0.01، 0.001، 0.0001 النسبة 28.56% و
14.28% و 14.28% و 14.28% و 14.28% على التوالي. تم استخدام تفاعل أنزيم البيلمر المتسلسل
各国 3fectiv في الإسبريشيا كولاي و كانت 3ae و 3stx2 و 3stx1 و 3stx1
جميع العزلات سلبية لكل من جينات 3stx2 و 3stx1 و 3stx1 و 3stx1
و كانت عزلات اللحم الخام من 3eae 0.05، 0.03، 0.01، 0.001، 0.0001 إيجابية للجين.
بينما كانت 3eae 0.05، 0.03، 0.01، 0.001، 0.0001 إيجابية للجن. 3eae 0.05، 0.03، 0.01، 0.001، 0.0001 إيجابية
للجن. 3eae 0.05، 0.03، 0.01، 0.001، 0.0001 إيجابية للجن. 3eae 0.05، 0.03، 0.01، 0.001، 0.0001 إيجابية
للجن.