USING SESAME OIL TO TREAT THE INFECTION OF HEMORRHAGIC 
E. COLI O157 : H7 BACTERIA ISOLATION IN BAGHDAD : MOLECULAR AND HISTOLOGICAL STUDY

Mohamed B. Al-Jobory*, Amina N. Al-Thwaini** and Laith M. Najeeb*

*Department of Microbiology, College of Science, University of Anbar, Iraq.
**Department of Genetic Engineering, Institute of Genetic Engineering and Biotechnology for Post Graduate Studies, University of Baghdad, Iraq.

Abstract
A total of 200 stool samples bloody diarrhea and 150 urine samples were collected from children of both sexes, with age between 3 to 10 years. Aims of study including Isolation E.coli O157:H7 from bloody diarrhea and urine, Detection of virulence gene hly A, flic H7 and rfbO157 by (PCR), Study the pathogenicity of the isolated of E.coli O157:H7 in mice model, Study overlap effect of sesame oil and silver nanoparticles with antibiotic on pathogenicity E.coli O157:H7. All isolates were identified by culture using selective media, VITEK 2 and they were confirmed by latex agglutination test. Polymerase chain reaction (PCR) was employed to detect some virulence gene as hly A - flic H7 and rfbO157 in stool and urine isolates. The result revealed 11 isolates, 8 (4%) obtained from patients with bloody diarrhea and 3(2%) from patients with UTI. PCR amplification result revealed presence of hlyA, flicH7 and rfbO157 genes in all isolates. The pathogenicity of E.coli O157:H7 were study to recognize the alterations in some organs of mice after experimentally infected with this pathogen. The histopathological examination of intestine for infected mice after 24-48 hours of infection showed infiltration of inflammatory cells, focal lining epithelial stratification, then distention of villi appeared. The liver showed accumulation of lymphocyte, hemorrhage in central vein with sinuses expansion with increase nuclear size.

Trimethoprim, sesame oil, silver nanoparticles were used as treatment for infected mice. Sesame oil gave excellent result as treatment in comparison with antibiotic and silver nanoparticles, the result showed intestine degeneration with infiltration of inflammatory cells, at 2nd day post treatment. At 4th days post treatment the intestine showed slight inflammatory cells infiltration. when liver showed irregular chromatin distribution with bizarre looking some nuclei at 2nd day post treatment. Complete intestine and liver recovery and appeared as normal organs after 7 days post treatment.

Key words : Escherichia coli O157:H7, antibiotic, silver nanoparticles, sesame oil.

Introduction
Enterohemorrhagic E. coli (EHEC) belong to a group of bacteria known as attaching and effacing (A/E) pathogens that cause disease by adhering to the luminal surfaces of their host’s intestinal epithelium (Robyn et al., 2013). Shiga toxin-producing Escherichia coli (E. coli) (STEC), also called verocytotoxin-producing E. coli (VTEC), have emerged as pathogens that can cause food-borne infections and severe and potentially fatal illnesses in humans, such as haemorrhagic colitis (HC) and haemolytic uraemic syndrome (HUS) which is the main cause of acute renal failure in children (Kargar and Homayoon, 2015). E. coli, O157:H7 has increased importance worldwide as a public health problem in the past two decades. It is the predominant and most virulent serotype in a pathogenic subset of Vero toxin producing E. coli (Nateneal et al., 2016). The most prevalent EHEC serotype causing outbreaks in North America is O157:H7 with cattle serving as the primary reservoir for this highly infectious organism, thus making EHEC contamination a major issue in food and water safety (Croxen and Finlay, 2010). There are several virulence factors that contribute to E. coli pathogenicity, such as pili, enterotoxins (LT, ST), shiga-like toxins, endotoxin (lipopolysaccharide), hemolysin, aerobactin, cytotoxic shock syndrome toxins, intimin, and biofilm formation (Blanco et al., 1996; Wong et al.,
Endotoxin lipopolysaccharide (LPS) and Vero toxin (VT) are released and absorbed cross the gut epithelium. LPS, VT and maybe other virulence factors lead to an increase in pro-inflammatory cytokines from host cells and subsequent release of chemokines from inflammatory cells (Palermo et al., 2009).

Histopathological examination of tissue biopsies for the identification of infectious organisms is a very important diagnostic tool. Specifically, in clinical medicine, histopathology has contributed to advances in our understanding of disease leading directly to new and more effective treatment. Some new infection involving histopathology in their discovery, have also lead to fresh diagnostic challenges. Histopathology has become increasingly common in recent decades (James, 2017).

**Materials and Methods**

**Sample collection**

A total of 200 bloody diarrhea and 150 urine samples were collected in the period from beginning of September to end of December 2016 in Al-Eskan pediatrics hospital and children safe hospital from children of both sexes aged of 3 - 10 years. They were all suffered from bloody diarrhea and UTI.

**Isolation of bacteria**

Full loop was taken and enriched in modified tryptic soy broth (mTSB) supplemented with vancomycin (4mg/L) according to (Sanderson et al., 1995) and incubated in 37 °C for 24h. Then full loop was streaked onto CTSMAC to seek sorbitol non-fermenting bacteria (colorless colonies) after that onto Hicrome media and Eosin methylen blue (EMB) as differential Media. The bacteria were identified by VITEK2 (Biomerieux-france). The latex agglutination test (Oxoid-England) was done for confirmation the serotype.

**Molecular detection**

**Bacterial DNA Extraction**

DNA was extracted from 8 isolates from stool and 3 from urine by DNA extraction Kit (promega USA) and then detected by gel Electrophoresis. The extraction protocol carried out as recommended by the manufacture data sheet. DNA Purity and concentrations of all samples were determined by using Spectrophotometer 2600 uv/vis (Unico USA).

**Polymerase Chain Reaction (PCR)**

Oligonucleotide primers were identified genes design according to Paton and Paton(1998) and Gannon et al. (1997) and list as in table 1.

PCR tubes were transferred to the thermalcycler to start the amplification reaction according to a specific program for each gene.

**PCR program of rfbO157 including**

Initial denaturation at 94°C for 4minuts in 1 cycle, Denaturation at 94°C for 30 second, Annealing at 55°C for 30 second and Extention at 72°C for 1 minute in 35 cycle, final extention at 72°C for 4min. in 1 cycle.

**PCR program of flic H7 gene including**

Initial denaturation at 94°C for 30 sec. in 1 cycle, Denaturation at 94°C for 30 sec, Annealing at 55°C for 30 sec and Extention at 72°C for 1 minute in 35 cycle, final extention at 72°C for 4min. in 1 cycle.

**PCR program of hlyA gene including**

Initial denaturation at 94°C for 1 min. in 1 cycle, Denaturation at 94°C for 1 min., Annealing at 55  °C for 2 min. and Extention at 72°C for 2.5 min. in 35 cycle, final extention at 72°C for 5min. in 1 cycle.

**Histopathological study**

**Animals grouping**

For 48 mice BALB/c body weight (22-25gram) (age 9-15 weeks) that provided by animal house of AL-RAZI center in Baghdad which are used in basic research, were housed in previously sterilized and cleaned plastic cages. Eight groups of mice were used in this study each group consist of 6 mice:

1- The first group used as control was administered by normal saline only 0.2 ml.

2- The second group infected orally with 0.2ml E. coli O157:H7 of $1.8 \times 10^6$ cfu/ml (does lower than LD$_{50}$ $1.8 \times 10^7$) and sacrificed at two intervals (1st day and 2nd day post infection).

3- The third group infected administered 0.2 (ml/Kg) antibiotic (trimethoprim) after one day post infection.

4- The fourth group administered 0.1ml Sesame oil as treatment after one day post infection.

5- The fifth group administered both 0.1ml Sesame oil and 0.2 ml antibiotic (trimethiprime).

6- The sixth group administered 0.1 ml of 0.5mg/kg silver nano-particles 80nm for seven days after infection.

7- The seventh group administered 0.1 ml of 0.5mg/kg silver nano-particles 80nm and 0.1ml Sesame oil after infection.

8. The Eighth group administered 0.1ml of 0.5 mg/kg silver nano-particles 80nm and 0.2ml antibiotic after infection.

All mice in each group were sacrificed at three
Using Sesame Oil to Treat the Infection of Hemorrhagic E. coli O157: H7 Bacteria Isolation

Table 1: Oligonucleotide primers sequences used for PCR amplification.

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence (5'-3')</th>
<th>Amplicon size (bp)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>fliC157</td>
<td>F: GCCTGTGCTGATGTCGACG R: CAACGGTGACTTTA TCGCCAATTC</td>
<td>625</td>
<td>Gannon et al. (1997)</td>
</tr>
</tbody>
</table>

Table 2: Frequency of Non sorbitol fermenting (NSF) and CT-SMAC confirmed E. coli in Human stool and urine.

<table>
<thead>
<tr>
<th>Isolates Source</th>
<th>Total samples</th>
<th>Sorbitol fermenting E.coli</th>
<th>No. of E. coli O157 positive isolates for CT-SMAC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human stool</td>
<td>200</td>
<td>120 (60%)</td>
<td>8 (4%)</td>
</tr>
<tr>
<td>Human urine</td>
<td>150</td>
<td>87 (58%)</td>
<td>3 (2%)</td>
</tr>
<tr>
<td>Total</td>
<td>350</td>
<td>207</td>
<td>11 (6%)</td>
</tr>
</tbody>
</table>

Table 3: Frequency of latex agglutination positive E. coli O157:H7.

<table>
<thead>
<tr>
<th>Isolates source</th>
<th>Isolates No.</th>
<th>O157 positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human stool</td>
<td>200</td>
<td>8 (4%)</td>
</tr>
<tr>
<td>Human urine</td>
<td>150</td>
<td>3 (2%)</td>
</tr>
<tr>
<td>Total</td>
<td>350</td>
<td>11</td>
</tr>
</tbody>
</table>

intervals (2nd days post treatment, 4th days post treatment and 7th days post treatment).

**Histological examination**

The intestine removed from control and infected mice and cut in long pieces, then fixed in 10% neutral buffered formalin. Further routine processing for hematoxylin and eosin staining was performed in the biotechnology research center at the AL-Nahrain university according to Musa (2017).

**Results**

The result revealed that 120 out of 200 stool sample were positive to E. coli. only 8 isolates were diagnosed as E. coli O157:H7 from stool and 3 isolates from urine that appeared on SMAC as small, circular and colorless colonies with smoky center and (1-2) mm in diameter and on Hicrome media that appeared dark purple to magenta colored moiety (table 2).

**Detection of virulence factors genes**

All isolates (11) were investigated for some virulence factors that responsible for pathogenicity of this pathogen and found that all isolates have these genes as showing in table 4 and figs. 1,2,3,4,5,6.

**Pathogenicity investigation of E. coli O157: H7 isolates by experimental Infection of mice (in vivo study)**

The symptoms appeared in all mice with morbidity (100%) after 24-48 hrs post infection. The clinical sings represented by bloody diarrhea, raised fur, shivering labored breathing. This finding also described by Ko et al. (2005) and Musa (2017). No signs of disease or mortality were observed in animals of control group that administrated with normal saline.

The intestines of infected mice were examined macroscopically after two days post infection, it appeared congested and sticky in comparison with intestine of control group (figs. 7,8). The liver macroscopically appeared congested after 2 days post infection in comparison with control.

**Histopathological examination**

The pathogenicity of the stool recovered isolates were appeared in the infected animals as different lesions in different organs especially intestine and liver that attributed to virulence factors of this isolates which leads to the development of these histological alterations.

**Histological examination of intestine**

The macroscopic and microscopic examination of intestinal section of control group animals showed normal intestinal architecture (fig. 9).

Histopathological examination of Intestine of experimentally infected mice showed that intestine was...
Fig. 1: The PCR product of bacterial isolates from human stool (1,2,3,4,5,6,7,8), the amplified of \textit{hly} \textit{A} gene (534 bp) electrophoresed in 2% agarose gel.

Fig. 2: The PCR product of bacterial isolates from human urine (1,2,3), the amplified of \textit{hly} \textit{A} gene (534 bp) electrophoresed in 2% agarose gel.

Fig. 3: The PCR product for bacterial isolates from human stool (1,2,3,4,5,6,7,8), the amplified of \textit{flic} gene (625 bp) electrophoresed in 1.5% agarose gel.

Fig. 4: The PCR product for bacterial isolates from human urine (1,2,3), the amplified of \textit{flic} gene (625 bp) electrophoresed in 1.5% agarose gel.

Fig. 5: The PCR product for bacterial isolates from human stool (1,2,3,4,5,6,7,8), the amplified of \textit{rfb} gene (259 bp) electrophoresed in 1.5% agarose gel.

Fig. 6: The PCR product for bacterial isolates from human urine (1,2,3), the amplified of \textit{rfb} gene (259 bp) electrophoresed in 1.5% agarose gel.
affected with variable lesions with infiltration of inflammatory cells, focal lining epithelial stratification and basal lamina degeneration, fig. 10 at one day post infection. This agreement with Jennifer et al. (2010) who found symptoms appeared on all mice after one day post infection with E. coli O157:H7 that administered orally and changes appeared in the intestine when they examined histopathology. On the other hand (Romina et al., 2012) recorded sticky, considerably empty, flimsy, of mice intestine when macroscopic examination after 1 day post infected with E. coli O157:H7.

At, 2nd day post infection, intestine of infected mice showed distention of villi with increase inflammatory cell infiltration, fig. (11) multiple areas show stratification with mixed inflammatory cell (lymphocyte, plasma and eosinophil cell). Stratification of lining epithelial cell with destruction of basal lamina, reactive epithelial atypia secondary to inflammation with multiple layer (fig. 12).
Histological examination of liver

Microscopic examination of liver section of mice in control groups presented in fig. 13. At second day post infection, liver sections showed accumulation of lymphocyte, hemorrhage in central vein with sinuses and Hepatocyte cells appeared, degenerative cells with sinuses expansion (figs. 14, 15). Increase nuclear size and increase hyperchromasia with irregular chromatin distribution, with bizarre looking some nuclei and some have prominent nucleoli. increase N/C ratio with area of necrosis (fig. 16). The congestion and hemorrhage noted in this study accounted for activation of endothelial cells by inflammatory mediators.

Treatment

Trimethoprim

Experimental treatment by trimethoprim (0.2ml/kg) for the third group (n=6) after the symptoms showed clearly on mice at day1 post infection. Two mice were dead after two days post treatment, while other animals recovery was recorded after 4 days of treatment until the end of experiment. Similarly no morbidity or mortality was observed in control group animals throughout experiment. After seven days of treatment with antibiotic the intestine of scirfied mice appeared response to this treatment with few congested (fig. 18).

Histological examination of intestine treatment with trimethoprim

At 2nd day post treatment with antibiotic, intestine showed stratification epithelial cell, cell infiltration with...
Using Sesame Oil to Treat the Infection of Hemorrhagic *E. coli* O157 : H7 Bacteria Isolation

At 4th and 7th days post treatment with antibiotic, intestine showed inflammatory cell infiltration with degeneration of basal lamina (fig. 19).

At 4th and 7th days post treatment with antibiotic, intestine showed inflammatory cell infiltration with degeneration of basal lamina (fig. 20).

**Histological Examination of intestine treatment with trimethoprim**

At 2nd day post treatment with antibiotic, liver showed irregular chromatin distribution and bizarre with nuclear enlargement (fig. 21).

At 4th and 7th day post treatment with antibiotic, liver showed nuclear enlargement (fig. 22).

**Treatment by sesame oil**

The fourth group consist of six mice (n=6). After 1 day of administered by pathogen no one of mice were dead. Sesame oil used when symptoms appeared after 1 day post infection. No morbidity was recorded after day 3 post infection until the end of experiment. After 5 days of treatment the mice return active. When mice killed after seven days of treatment with oil the intestine appeared great response to this treatment that return without congested as normal (fig. 23).

**Histological examination of intestine treatment with sesame oil**

At 2nd day post treatment with oil, intestine showed focal lining stratification epithelial cell, degeneration with infiltration of inflammatory cells (fig. 24).

At 4th days post treatment with oil, intestine showed slight inflammatory cells infiltration (fig. 25).

At 7th days post treatment with oil, intestine showed normal (fig. 26).
**Histological examination of liver treatment with sesame oil**

At 2\textsuperscript{nd} day post treatment with oil, liver showed irregular chromatin distribution with bizarre looking some nuclei (fig. 27).

At 4\textsuperscript{th} and 7\textsuperscript{th} days post treatment with oil, liver showed normal hepatocyte cells (fig. 28).

**Discussion**

*Escherichia coli* O157:H7 is well documented Shiga toxin–producing (STEC) serotype, it is one of hundreds of *E. coli* strains. Although, the most strains are harmless and live in the intestine of healthy human and animals, STEC strain produces a powerful toxin and can cause severe illness (Baquir *et al.*, 2008; JiYoun *et al.*, 2010). The primary reservoir of this strain is the cattle. Cattle feces are a major source of contamination of beef, other food products, and water. The direct contact with animals and material contaminated with animal feces, will increase the chances of infection.

In the present study, the virulence of *E. coli* O157:H7 was assessed in bloody diarrhea and urine samples using three genes including: flicH7, rfbO157 and hlyA.

Patients selection was restricted to those children suffered from bloody diarrhea and UTI because *E. coli O157:H7* is mostly associated with this clinical features. In the current study 8 (4\%) of Non Sorbitol Fermenting (NSF) revealed from bloody diarrhea samples which is similar to the study conducted by Al-awwadi *et al.* (2013).

*E. coli* O157:H7 isolated from 3 (2\%) samples of urine, this result less than Wong *et al.* (2012).

The confirmation of *E. coli O157:H7* was done by latex agglutination which confirmed identification of 8 isolated from stool and 3 from urine.

Many studies done about this bacteria in Iraq and world, that isolated it from different sources such as Ibrahim *et al.* (2014) isolated *E coli O157:H7* from Tigris River and children stool, also Baqer (2013), who detected this bacteria in food and patients in Baghdad, while sheikh *et al.* (2013) isolated of *Escherichia coli O157:H7* from beef hamburgers in Khuzestan Province, Iran.
The differences between the current result and other studies depending on site of collection, season, number of hospitals surveyed and medication especially exposure to antibiotics, in addition to the use of significant parameters and methods to detect these pathogens. High percentage result in some study may be due to the life style, rural individuals were more contact with source of infections such as carrier animals and their dairy or meat products (Khanjar and Alwan, 2014).

The molecular study was done by PCR for detection of 3 genes (hlyA – rfbO157 – flic H7) responsible for some virulence factors and the result revealed that all isolates (11) were harbored these genes through amplification the specific fragments of these genes. The hly A was chosen in this study as enterohemolysin protein that cause lysis of red blood cells by destroying their cell membrane that lead to cause bloody diarrhea when rfb O157 gene choose as lip polysaccharide the O antigen and flic H7 gene (encoding fimbria) that help bacteria to attachment. These results confirmed that this pathogen considered as an important causes of gastrointestinal tract impairment and urinary tract infection in human.

The serotype O157:H7 positive for gene (hly A) isolated from urine, may indicated that this pathogen play crucial role in urinary tract infection in the humans and cause renal failure in the infected individuals. This result similar with Samy et al. (2017), who identified 2 E. coli O157:H7 isolates positive for hly A from patients with UTI, when less than Al-wgaa (2014), who reported 8 isolates from urine have hly gene in the urinary tract infections of humans in Baghdad.

In this study, intestinal sections showed lymphocyte aggregation below the mucosa and distention of villi with increase inflammatory cell infiltration. multiple areas shows stratification and destruction of basal lamina. Most of these finding are described by other researchers as (Mansour et al., 2014) and (Longa et al., 2008). The intestinal histopathological changes observed in this study could in part be explained by the effect of endotoxin (lipopolysaccharides), the destruction of epithelial layer of mucosal glands and infiltration of inflammatory cells observed in this study also mentioned by (Milan et al., 2012), who inject lipopolysaccharides of E. coli O157:H7 intra peritoneally into mice.

Histopathological changes of liver observed in this study were similar to that mention by Das et al. (2012) who found congestion in liver of mice that infected by E. coli O157:H7 that isolated from rectal swab of pet dogs and cats in West Bengal.

Pankaj et al. (2013) showed necrosis in liver of pigeons after infected by E. coli O157:H7 in India. Hepatic parenchymal necrosis has been reported a consequence of tissue anoxia resulting from vasoconstriction or by extracellular bacterial toxin (Brenden and Huizingat, 1986).

Treatment E. coli O157:H7 with trimethoprim appeared good result due to inhibits bacterial growth by inhibiting the synthesis of dihydrofolic acid of E. coli O157:H7 (Tarun and Chi, 2017).

Sesame oil appeared as treatment more effective and quicker than trimethoprim because contain vitamin k – proteins and antioxidant materials that may inhibition all biological activity of bacteria that lead to prevent production protein and inhibit the effect of lipopolysaccharide.

**Acknowledgements**

I would like to thanks all staff members of biotechnology research center of Al-Nhrain university for their kind help. Furthermore I would also like to thanks with much appreciation the crucial role of the staff of children safe hospital, teaching children hospital in Ramadi and Central Children’s Hospital who gave the permission to use all required equipment and the necessary materials to complete the research.

**References**


---

**Table 4**: Frequency of hly A, flicCH7 and rfbO157 genes of isolates in the samples.

<table>
<thead>
<tr>
<th>Isolates source</th>
<th>No. of positive isolates with SMAC media</th>
<th>hly A gene</th>
<th>flic H7 gene</th>
<th>rfbO157 gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human stool</td>
<td>8</td>
<td>8(100%)</td>
<td>8(100%)</td>
<td>8(100%)</td>
</tr>
<tr>
<td>Human urine</td>
<td>3</td>
<td>3(100%)</td>
<td>3(100%)</td>
<td>3(100%)</td>
</tr>
<tr>
<td>Total</td>
<td>11</td>
<td>11</td>
<td>11</td>
<td>11</td>
</tr>
</tbody>
</table>

---
producing E. coli (O157:H7) infection in children diarrhea in nasseriya city. GIPAST, 3(1): 1-06.


Nateneal Tamerat, Yimer Muktar and Daniel Shiferaw (2016). Application of Molecular Diagnostic Techniques for the


Pankaj Dutta, Manoj Kumar Borah, Ratul Sarmah and Rakhi Gangil (2013). Isolation, histopathology and antibiogram of *Escherichia coli* from pigeons (Columba livia), DOI: 10.5455/vetworld.2013.91-94.


