Impact of probiotic strain of the non-pathogenic *Escherichia coli* “Nissle1917” on gene expression of shiga toxin *E. coli* O157:H7 *In vitro* and *In vivo*

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Enterohemorrhagic *Escherichia coli* (EHEC) O157:H7 are major food-borne pathogens responsible for serious infections ranging from mild diarrhea to hemorrhagic colitis and life threatening complications. Shiga toxins (Stxs) are the main virulence factor of *E. coli* O157:H7. The antagonistic effect of a prophylactic treatment with the probiotic *E. coli* Nissle 1917 (EcN) against *E. coli*O157:H7 was investigated *in vitro* and *in vivo*. Probiotic EcN strongly reduced EHEC numbers (cfu) of O157:H7 the probiotic treatment had effect on O157:H7. The Stx1 and Stx2 gene expression was estimated in mice before treatment with EcN and after 24 and 48 hrs of treatment post EHEC infection, probiotic treatment significantly decreased Stx mRNA levels. In conclusion, *in vitro* and *in vivo* data suggest that EcN probiotic offer strong inhibitory effects on growth, Shiga toxin gene expression of *E. coli* O157:H7, and useful in the fight against O157:H7 infection.

Keywords: *E. coli* O157:H7, STEC, *E. coli* Nissle 1917, Shiga toxin, *In vitro*, *In vivo*, gene expression

INTRODUCTION

*Escherichia coli* (*E. coli*) are known as a versatile bacterial species encompassing both commensal and intra intestinal and extra intestinal pathogenic strains. An *E. coli* genome contains between 4200 and 5500 genes (Touchon et al. 2009). *E. coli*O157:H7 is a particularly virulent serotype of Shiga toxin-producing *E. coli* (STEC) also known as vero cytotoxin-producing, recognized as a human pathogen in 1982 (Riley et al., 1983). The ability of *E. coli* O157:H7 to cause severe diseases in humans is related to their capacity to secrete shiga toxins (Stx1 and Stx2) or vero cytotoxins (VT1 and VT2) and variants of these toxins (Mora et al.2007).It is one of the causative pathogens of hemorrhagic colitis, which can be accompanied with life-threatening hemolytic uremic syndrome (HUS), thrombotic thrombocytopenic purpura, and neurologic damage (Mele et al. 2014).The spectrum of EHEC-associated disease ranges from mild watery diarrhea to hemorrhagic colitis and in severe cases to the hemolytic-uremic syndrome (HUS) that can lead to mortality, especially in children under 5 years of age. HUS occurs in 5–10% of EHEC infections as a result of exposure to Stx, the main virulence factor of the pathogen, and is characterized by a triad of acute kidney failure, micro angio pathic hemolytic anemia and thrombocytopenia (Karpman and Ståhl, 2014; Keir, 2015).

Since the first step of the infection process is colonization of the host, it is important to understand how *E. coli* pathogens successfully colonize the intestine. Results of Meador et al. (2014) indicate that development of probiotics to target multiple *E. coli* patho types will be problematic, as the factors that govern niche
occupation and hence stable colonization vary significantly among strains. *E. coli* Nissle1917 (EcN) is a nonpathogenic Gram-negative strain isolated by Prof. Alfred Nissle from Freiburg, Germany, in 1917 from the intestinal micro flora of a young soldier (Kruis et al. 2004), and it is the active component of microbial drug Mutafior® (Ardeypharm GmbH, Herdecke, Germany and EcN, Cadigroup, In Italy) used in many gastrointestinal disorder including diarrhea, uncomplicated diverticular disease and UC. It is the only probiotic recommended in ECCO guidelines as effective alternative to mesalazine in maintenance of remission in UC patients (Scaldaferri et al. 2016). EcN has long been recognized as having probiotic potential against dysentery and traveler’s diarrhea (Nissle,1959). A series of previous studies Autieri et al. (2007); Fabich et al. (2008); Jones et al.(2008) indicated that in the mouse intestine different pathogenic and commensal *E. coli* strains each use a different set of approximately 6 sugars of the more than 18 sugars that *E. coli* potentially is capable of using in vitro. This led us to hypothesize that invading *E. coli* pathogens overcome colonization resistance by taking advantage of nutrients that are available because they are not used by resident commensal *E. coli* strains (Fabich et al. 2008).

Probiotics are viable agents conferring benefits to the health of the human host (Reid et al. 2003). They can provide a beneficial effect on intestinal epithelial cells in numerous ways. Some strains can block pathogen entry into the epithelial cell by providing a physical barrier or by creating a mucus barrier; other probiotics maintain intestinal permeability acting on tight junctions. Some probiotic strains produce antimicrobial factors; other strains modulate the immune response (Gareau et al. 2010). Currently, there is no specific treatment available for EHEC infections in human creating an urgent need for the development of alternative therapeutic strategies among them, one of the most promising approaches is the use of probiotic microorganisms (Cordonnier et al.(2016). The aim of the present study is to describe the effect of the first using in Iraq of EcN on gene expression of STEC O157:H7 in vitro and in vivo.

**MATERIALS AND METHODS**

**Sample Collection**

Current study included 364 clinical and food samples, 226 hospitalized patients (163 stool samples and 63 urine samples) suffering from hemorrhagic colitis (HC) and hemolytic uremic syndrome (HUS) comprises children’s age (less than 15 years) and from both gender, admitted to Al-Zaafarania hospital and central children’s hospital in Baghdad. And 138 food samples (85 samples of meat product and 53 vegetables samples of potatoes, tomatoes, onion, cucumber, Muskmelon, Cabbage, lettuce, radishes, leeks, celery, Parsley, Crest, basil, and mint) collected from markets in different areas of Baghdad city (AL-Dura, Al-Bayaa, and Al-Karada) during the period from April 2016 to April 2017. The collected samples were transferred directly to the laboratory in an ice box with a minimum of delay.

**Isolation and Enrichment**

Each food sample (25g) of meat and vegetables was weighed into sterile container and 225ml of modified tryptic soy broth (m TSB) were added supplemented with vancomycin (0.8 mg/L) or no according to Brando et al.(2008) and incubated for 6-24hr. at 42°C. After 24h. enrichment aliquots of 100μl were plated onto Eosine methylene Blue (EMB) to presumptively identify isolates as Gram-negative enteric bacteria, *E. coli* have (green-metallic colonies) and onto Sorbitol MacConkey(SMAC)agar test for sorbitol non-fermenting bacteria (colorless colonies) After that transferred onto modified Sorbitol MacConkeyagar supplemented with cefixime and tellurite (CT-SMAC)then culturing on to HiCrome *E. coli* O157:H7 Selective base agar, for 24 h. at 42°C. For stool and urine samples: Loop full of each stool and urine samples from humans patients was enriched in mTSB supplemented with vancomycin (0.8mg/L) or no according to Brando et al.(2008) then the same procedure of food samples were done.

**Conventional Biochemical Tests**

All bacterial isolates were examined morphologically by Gram’s stain also Colonies of presumptive *E. coli* O157:H7 were subjected to an important group of tests used IMVIC test, indole -methyl red -Voges- Proskauer-citrate (Adams and Moss, 2008). Also oxidase, motility, urea and Kliger’siron agar test.

**API 20E and VITEK 2 System**

Bacterial isolates suspected as *E.coli*O157:H7 according to conventional biochemical tests results were re-examined by API20E system, and VITEK 2 system (bio Merieux). The identification card for gram-negative bacilli (ID-GNB card) for the VITEK 2 system is a 64-well plastic card.
containing 41 fluorescent biochemical tests (Funke et al. 1998). The isolates were analyzed on the VITEK 2 system, in accordance with manufacturer’s instructions.

**Latex Agglutination Test (LAT)**

This test was used for more specific identification of *E. coli* O157:H7 by using commercial kit (*E. coli*O157:H7 latex agglutination kit /Product No. 541070, Abraxis, Warminster, PA, USA).

**In vitro Experiment (Agar Gel Diffusion Test)**

This method was adopted according to (Kavanagh, 1975), for assessing the antibacterial activity of the prepared extract. 0.1 ml of standardized bacterial stock suspensions (1.5×10⁸ CFU/ml) of *E. coli* O157:H7 was streaked on the surface of Muller-Hinton plate by a sterile cotton swabs and in each of these plates 3 well, 6 mm in diameter were cut using a sterile Pasteur pipette and the agar discs were removed by a sterile forceps, the wells were filled with 0.1ml of each concentration probiotics(10, 20 or 50 μl),and antibiotic (Doxycycline and azithromycin 10μg / ml) that allowed to diffuse at room temperature for two hrs. The plates were then incubated in the upright position at 37°C for 24 h. The activity of antibacterial was determined by measuring the diameter of inhibition zone around each well by millimeter against the *E. coli* O157:H7.

**Concentration of this bacterium also was brought to 1×10⁷ CFU/ml and then transferred to sterile syringe to feeding the mice.**

**In vivo Experiment (Molecular Study for Detection *E. coli*)**

**Bacterial DNA Extraction**

DNA extraction was done according to manufacturer’s instructions by using commercially available DNA extraction and purification kit (Geneaid Genomic DNA extraction Kit). Primers used for the PCR assays are shown in Table(1)

### Table 1. The sequences of forward and reverse primers for *E. coli*O157:H7.

<table>
<thead>
<tr>
<th>Primers</th>
<th>Sequence (5’→3’ direction)</th>
<th>Amplicon size(bp)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>16SrRNA-F</td>
<td>GCGGACGGGTGAGTAATGT</td>
<td>202bp</td>
<td>Kerrn et al. (2002)</td>
</tr>
<tr>
<td>16SrRNA-R</td>
<td>TCATCCTCTCAGACCAGTA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stx1- F</td>
<td>CATTACAGACTATTTCATCAGGAGGTA</td>
<td>140bp</td>
<td>Chui et al. (2010)</td>
</tr>
<tr>
<td>Stx1-R</td>
<td>TGTTCAACAATAAGCGTGGATTA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stx2- F</td>
<td>GCGGTITTTATTTGCAATTAGC</td>
<td>115bp</td>
<td>Hoffmann et al. (2006)</td>
</tr>
<tr>
<td>Stx2- R</td>
<td>TCCCGTCAACCTCCACCTGTA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IC-F</td>
<td>GACCACCCAGACAGAAC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IC-R</td>
<td>GTTGTACAGCCTCGTCCATGC</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
RESULTS

Results showed that out of 364 E. coli samples, 165 isolates were E. coli non ferment sorbitol suspected to be E. coli O157:H7 through colorless colonies appearance on CT-SMAC agar. Results of culturing on chromo agar clarified dark purple to magenta colored moiety appearance. Results of test performed for isolate E. coli O157:H7 exhibited that latex agglutination test gave positive results (40) isolates constituted 100% from total of suspected isolates by VITEK 2 system in imported meat products (minced meat) and human stool which similar to the results of amplifying of 16SrRNA gene using qPCR (Table 2).

**In vitro Effect of E. coli Nissle Against E. coli O157:**

The antibacterial activity of the EcN against pathogenic bacteria in vitro was determined using Well's Diffusion method, it showed the antibacterial activity against selected local E. coli O157 isolate. The higher inhibition zone diameter was 24 mm at the concentration of 50 μl E. EcN, while lower inhibition zone diameter was 11 mm at the concentration of 10 μl. Therefore, based on these results the concentration of 50 μl was chosen for the in vivo study.

Table 2. Results of tests performed for isolate E. coli O157:H7.

<table>
<thead>
<tr>
<th>Source of isolates</th>
<th>non- Ferment sorbitol</th>
<th>No. of presumptive isolates with chromo agar</th>
<th>No. of presumptive isolates with API 20E</th>
<th>No.(%)of positive isolates with VITEK2 system</th>
<th>No. (%)of positive isolates with E. coli O157:H7 LAT</th>
<th>No.(%)of positive isolates with q PCR for 16SrRNA gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stool</td>
<td>92</td>
<td>80</td>
<td>73</td>
<td>27(29.35)</td>
<td>29.3(27)</td>
<td>27 (29.3)</td>
</tr>
<tr>
<td>Urine</td>
<td>22</td>
<td>15</td>
<td>12</td>
<td>0(0)</td>
<td>0(0)</td>
<td>0(0)</td>
</tr>
<tr>
<td>Meat product</td>
<td>51</td>
<td>44</td>
<td>38</td>
<td>13 (25.4)</td>
<td>13(25.4)</td>
<td>13(25.4)</td>
</tr>
<tr>
<td>Vegetables</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0(0)</td>
<td>0(0)</td>
<td>0(0)</td>
</tr>
<tr>
<td>Total</td>
<td>165</td>
<td>139</td>
<td>123</td>
<td>40(24.2)</td>
<td>40(24.2)</td>
<td>40 (24.2)</td>
</tr>
</tbody>
</table>

**In vivo Effect of E. coli Nissle against E. coli O157:**

Results of this study showed after treatment out of 40 isolates were 8 isolates that Stx producing by the isolates of E. coli O157:H7 were positive for Stx-1 or Stx-2 genes or both where two isolates shown positive results for Stx-1 and Stx-2 genes, and six isolates have positive results for Stx-1 gene (meat products and minced meat), while one isolate gave positive result for Stx-2 gene (stool). Ct values for Stx-1 and Stx-2 genes ranged from (21.92-34.7)(Tables 3,4), also The quantification of copy numbers of Shiga toxin and mRNA levels of the Stx genes after 24 and 48h of treatment were determined using RT-PCR. The results of ΔCT, ΔΔCT and 2^-ΔΔCt showed there was a significant difference in the mean values between the different studied groups. RT-PCR results indicated that mice infected with E. coli O157:H7 then treated with doxycycline antibiotic treatment responded after 24 and 48 hours by increasing the ΔΔCT results by -0.77 and -1.47 Ct or approximately 1.7 and 2.7 fold-differences, respectively for Stx1, and 3.74 and 3.59 Ct or approximately 0.07 and 0.08 fold-differences, respectively for Stx2, comparing with control treatment (infected mice untreated) (Tables 3, 4).
Table 3. Stx1 gene expression after treatment.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Time(h)</th>
<th>IC average CT</th>
<th>Stx1 Average CT</th>
<th>ΔCT</th>
<th>ΔΔCT</th>
<th>Fold gene expression 2^ΔΔCt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infected mice(untreated)</td>
<td>--</td>
<td>25.95±1.3</td>
<td>26.07±1.7</td>
<td>0.12±0.04</td>
<td>0.00±0.00</td>
<td>1.0±0.03</td>
</tr>
<tr>
<td>Infected with E. coli O157:H7 then treated with E. coli Nissle</td>
<td>24</td>
<td>26.03±1.5</td>
<td>33.2±1.47</td>
<td>7.18±0.62</td>
<td>7.06±0.54</td>
<td>0.007±0.0</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>23.25±1.2</td>
<td>34.31±2.6</td>
<td>11.06±0.7</td>
<td>10.94±0.8</td>
<td>0.0005±0.0</td>
</tr>
<tr>
<td>E. Coli O157H7 and E. Coli Nissle gives together then treated with E. coli Nissle</td>
<td></td>
<td>--</td>
<td>--</td>
<td>--</td>
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<td></td>
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</tr>
<tr>
<td>Infected with E. coli O157:H7 then treated with doxycycline antibiotic</td>
<td>24</td>
<td>23.45±1.6</td>
<td>22.8±1.6</td>
<td>0.65±0.03</td>
<td>0.77±0.04</td>
<td>1.7±0.02</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>25.42±1.4</td>
<td>24.07±1.6</td>
<td>1.35±0.04</td>
<td>1.47±0.02</td>
<td>2.7±0.04</td>
</tr>
</tbody>
</table>

Table 4. Stx2 gene expression after treatment.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Time (h)</th>
<th>IC average CT</th>
<th>Stx2 Average CT</th>
<th>ΔCT</th>
<th>ΔΔCT</th>
<th>Fold gene expression 2^ΔΔCt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infected mice (untreated)</td>
<td>--</td>
<td>25.95±1.3</td>
<td>18.02±1.8</td>
<td>-7.93±2.6</td>
<td>0.00±0.0</td>
<td>1±0.05</td>
</tr>
<tr>
<td>Infected with E. coli O157:H7 then treated with E. coli Nissle</td>
<td>24</td>
<td>20.25±1.3</td>
<td>21.5±0.97</td>
<td>1.27±0.84</td>
<td>9.2±0.54</td>
<td>0.001±0.00</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>23.10±1.4</td>
<td>22.16±1.2</td>
<td>0.94±0.33</td>
<td>6.99±0.4</td>
<td>0.007±0.00</td>
</tr>
<tr>
<td>E. Coli O157H7 and E. coli Nissle gives together then treated with E. coli Nissle</td>
<td>24</td>
<td>24.80±1.62</td>
<td>22.93±1.14</td>
<td>1.87±0.74</td>
<td>6.06±0.31</td>
<td>0.01±0.00</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>24.80±1.09</td>
<td>25.36±1.09</td>
<td>0.56±0.06</td>
<td>8.49±0.57</td>
<td>0.002±0.00</td>
</tr>
<tr>
<td>Infected with E. coli O157:H7 then treated with doxycycline antibiotic</td>
<td>24</td>
<td>20.6±0.96</td>
<td>16.41±0.7</td>
<td>4.19±1.45</td>
<td>3.74±0.3</td>
<td>0.07±0.02</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>23.25±1.4</td>
<td>18.9±0.85</td>
<td>4.34±1.07</td>
<td>3.59±0.4</td>
<td>0.08±0.02</td>
</tr>
</tbody>
</table>
DISCUSSION

EHEC serotype O157:H7 has traditionally been associated with severe disease conditions and epidemics. Due to the risk of serious disease complications such as HUS caused by increased release of Stx after exposure to certain antimicrobial agents and growing concerns about antibiotic resistance (Bielaszewska et al., 2012), antimicrobial agents are not recommended in EHEC mediated infections. Probiotics may offer an alternative therapeutic option. In this study the meat products, stool and urine isolates were examined. The presumptive colonies of (165) E. coli O157:H7 on SMAC were small, circular and colorless with a weak pale brownish appearance on this selective media, sorbitol serves as a carbon source which cannot be fermented by E. coli O157:H7. Colonies of E. coli O157:H7 appear colorless, whereas other E. coli appear red (March and Ratnam, 1986). Results of selective media Hi Crome O157:H7 showed a dark purple to magenta colored moiety. These results are in agreement with other researchers (Baqer, 2013; Jabur et al., 2016). All the isolates were Gram negative rods and therefore satisfied that presumptive characteristic for E. coli. Also identified by conventional biochemical tests and API20E system (Table 2). The LAT was used for more specific identification of E. coli O157:H7. It is used to detect the somatic antigen O and flagellar antigen H. The E. coli O157:H7 latex agglutination kits are available from Abraxis, and its sensitivity and specificity was reported as 100% and 99.0%, respectively. All tested isolates were confirmed as E. coli using 16S rRNA-PCR, similar results found by Abu-Duhier (2015). The pair wise comparison of PCR versus VITEK-2 indicated that there was no difference between these two techniques in the identification of E. coli.

When healthy human volunteers are fed E. coli strains isolated from their own feces, those strains do not colonize (Anderson et al. 1973). This is an example of colonization resistance (Appelroo-Renkema et al. 1990). The mechanisms of the antibiotic-induced production and release of Stx by STEC have extensively been characterized in vitro for the most frequent STEC strain, O157:H7. Previous studies have reported that antibiotic treatment significantly increases the propagation of Stx phages (Cornick et al. 2006; McGannon et al. 2010; Safdar et al. 2002) did not reveal a correlation between the use of antibiotics and the frequencies of the development of HUS. In this study, the antibiotic doxycycline inhibited the growth of E. coli O157 more than azithromycin with the inhibition zone diameter of 41 mm at the concentration 10 μg/ml, therefore it has been chosen for the in vivo study. The antibiotic doxycycline has high antibacterial activity against E. coli by numbering of inhibition zone than azithromycin due to this drug binds reversibly to the 30S subunit of the bacterial ribosome, thereby blocking access of the amino acyl-tRNA to the mRNA-ribosome complex at the acceptor site, and it has a broad-spectrum as a bacteriostatic antibiotic (Hady et al. 2014). Coroceanu et al. (2012) suggested, the response of the respective epidemiologic STEC strain to antibiotics should be rapidly characterized in order to identify antibiotics that do not enhance the release of Stx.

As recommended by FAO/WHO, probiotic bacteria can be defined as live microorganisms which, when administered in adequate amounts, confer a health benefit on the host. The present study supports the proposed idea that, although these microorganisms may exert their beneficial effects due to their well-known ability to modulate intestinal microbiota, these effects are not restricted to the gastrointestinal tract. Considering this, we have selected the probiotic strain EcN, which has shown efficacy in different intestinal conditions including diarrhea (Henker et al., 2008). Reverse transcription PCR (RT-PCR) is technique which uses reverse transcriptase enzyme to produce DNA from RNA. RT-PCR has shown great potential for detecting viable pathogens such as when mRNA is detected (Priyanka et al., 2016). It is a rapid and sensitive method that can be used for the quantification of microorganisms (Liu and Mustapha, 2014) and used for examine the presence of specific virulence genes of the pathogenic strains which included Stx1 and Stx2 (Abu-Duhier, 2015). Results of current study revealed that The Ct values of Stx1cDNA amplification exhibited significant variation after 24 and 48h, Ct values of Stx1mRNA in untreated mice was 26.07 and in treated ranged from (21.92-34.7), Fratamico and DebRoy (2010) stated that E. coli O157 strains isolated from food may be harbor both Stx1 and Stx2 or one of two type, and possibility that will give a stronger signal than strains that possess only one of the explored toxins. Also Qin et al. (2015) illustrated that E. coli O157 strains isolated from stool were positive or negative for both genes; otherwise, either Stx1 and Stx2 is indicated. Gilani et al. (2012) stated that probiotic EcN displays strong inhibitory effects on growth.

Shiga toxin release and adhesion of major EHEC serotypes. Thus, EcN is a putative therapeutic candidate during EHEC-mediated diseases and caused decrease in growth viability by 39~82 % of EHEC strains determined by plate count and qRT-PCR. EcN possesses multiple features that might contribute to its ability to colonize the intestine. In this study, the amount of Stx1 and Stx2 after 24 and 48 h co-incubation with EcN tested reduced significantly. The $2^{\Delta\Delta CT}$ method is the method of relative quantification that is most frequently found in popular software packages for qPCR experiments. The threshold cycle (CT) is the cycle at which the fluorescence level reaches a certain amount (the threshold). This method directly uses the Ct information generated from a qPCR system to calculate relative gene expression in target and reference samples (Rao et al. 2013). The results of ΔCT, ΔΔCT and $2^{\Delta\Delta CT}$ showed there was a significant difference in the mean values between the different studied groups (Tables 3, 4). EcN by itself being able to use glycolysis and gluconeogenesis simultaneously to keep E. coli EDL933 at relatively low levels when pre colonized in the mouse large intestine, and suggest that, since the micro biota itself isn’t able to out compete E. coli EDL933 in the mouse large intestine, a commensally E. coli strain such as the probiotic EcN seems to be strongly needed to be able to nutritionally compete against E. coli EDL933. (Schinner, 2013). In conclusion, this study showed that probiotic EcN is an effective agent. To the best of our knowledge, this is the first report in Iraq that investigates the efficacy of EcN by E. coli O157. The in vitro and invivo data suggest that EcN probiotic offer strong inhibitory effects on growth, Stx gene expression of E. coli O157:H7.

CONCLUSION
EHEC have been recognized as one of the most important food-borne pathogens. As current treatment of EHEC infections is essentially symptomatic, alternative therapeutic options, such as the use of probiotic microorganisms, have been considered in an attempt at decreasing infection outcomes. EcN is a well-known probiotic, used in several countries, registered as a drug in certain European countries. The in vitro and invitro data of this paper suggest that E. coli Nissle 1917 probiotic offer strong inhibitory effects on growth, Shiga toxin gene expression of E. coli O157:H7. To the best of our knowledge, this is the first report in Iraq that investigates the efficacy of EcN by E. coli O157.

CONFLICT OF INTEREST
The authors declared that present study was performed in absence of any conflict of interest.

ACKNOWLEDGEMENT
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AUTHOR CONTRIBUTIONS
KMJ supervision and follow-up experiments and also wrote the manuscript. MTM supervision and follow-up experiments and also reviewed the manuscript. SHA performed the invitro and invivo experiments.

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