Original article

Molecular identification of virulence and antibiotic-resistant genes in *Escherichia coli* O157 and non-O157 recovered from water samples

Olowe BM 1, Arogbodo JO 2

1- Department of Biological Sciences, Faculty of Basic Medicals and Applied Science, Lead City University, Ibadan, Oyo State, Nigeria.
2- Department of Animal Production and Health, Federal University of Technology, Akure, Ondo State, Nigeria.

**ARTICLE INFO**

**Article history:**
Received 30 April 2021
Received in revised form 31 May 2021
Accepted 5 June 2021

**Keywords:**
*Escherichia coli* O157:H7
Non-O157
Virulence genes
Pathogenic genes

**ABSTRACT**

**Background:** This research work focused on ascertaining the presence of virulence and antibiotic-resistant genes in *Escherichia coli* (E. coli) O157 and non-O157 recovered from drinking water sources. **Methods:** Identification of *E. coli* O157 and non-O157 was carried out using standard serological and PCR techniques. Virulence genes (*rfb* O157, *fliC* H7, *stx*1, *stx*2, *eae* and *hly* genes) and antibiotic-resistant gene (*Bla*TEM) were detected using PCR method on selected isolates (n= 15) from different water sources which demonstrated multiple antibiotic-resistance in a previous study.

**Results:** The serological identification result revealed that a total of 68 out of 382 *E. coli* isolates, recovered in a previous work, were identified as a presumptive *E. coli* O157. These included 19.1 %, 21.7 %, 33.3 %, 14.3 % and 9.1 % of *E. coli* isolates from wells, boreholes, sachets, streams and pipe-borne respectively. Statistical analysis revealed that there was no significant difference in the frequency of *E. coli* O157 from the different water sources (p > 0.05). Also, there was a statistically significant positive correlation between the *E. coli* isolates and *E. coli* O157 (Pearson’s r = 0.996).

Detection of virulence and antibiotic-resistant genes showed that only 46.7 %, 33.3 %, 33.3 %, 93.3 %, and 66.7 % carried *rfb* O157, *fliC* H7, *stx*1, *stx*2 and *rpoS* gene respectively. In contrast, all the isolates possessed *hly* and *Bla*TEM genes but none had *eae* gene. **Conclusion:** The presence of one or combination of these genes in these isolates depicts their virulence and resistance nature.

**Introduction**

*Escherichia coli* (E. coli) O157 is a pathogenic bacterium implicated to be a causative agent of human haemorrhagic colitis and haemolytic uraemic syndrome [1]. According to the reports of Rompre et al. and Juhna et al. [2, 3], pathogenic *E. coli* had been known to inhabit water from different sources. Food Safety Authority, Mull and Hill, Jokinen et al. and Mezule et al. [4-7], also corroborated the above as of community health importance. According to Ajayi et al. [8], the generic *E. coli* is considered as an intestinal pathogen and naturally exists as a commensal in the gut of humans and warm-blooded animals where it enjoys synergistic association with other members of the bacterial family. It also plays a beneficial role in the prevention of potentially pathogenic organisms in the gut. Studies revealed that *E. coli* which forms part of the normal intestinal flora of humans and warm blooded animals is capable of inhibiting the growth of other toxigenic strains of *E. coli* [9-11].

However, some strains of *E. coli* occasionally emerge as pathogens due to the...
presence of certain pathogenic features and virulence genes which are located on transmissible genetic elements and this distinguishes them from ordinary commensal strains as reported by Ronsengren et al. [12].

*Escherichia coli* strains causing enteric infections have been classified into various pathotypes which include enterotoxigenic (ETEC), enteropathogenic (EPEC), enteroinvasive (EIEC), enterohemorrhagic (EHEC), enteroaggregative (EAEC) and diffusely adherent *E. coli* (DAEC). This classification is based on the presence of different combinations of virulence factors such as production of haemolysins, colicins, haemagglutinins, enterotoxins, proteases, colonization factors and cell surface hydrophobicity [13,14]. Among these pathotypes, Shiga toxin-producing *E. coli* (STEC) is a group of enterohemorrhagic *E. coli* (EHEC) which produces Shiga toxins [15-18].

*Escherichia coli* O157 strains have been reportedly found in animals like cattle, horses, dogs and flies [19]. Human infection as well as water contamination can ensue, following defaecation by infected animals [20]. Contamination of drinking water sources with pathogenic *E. coli* can result to waterborne diseases such as dysentery with fever, urinary tract infection, severe bloody and watery diarrhoea, abdominal cramps, nausea, vomiting and possibly haemolytic uraemic syndrome (HUS) that may lead to kidney failure and eventual death [21,22]. Contamination of water sources with *E. coli* has long been a water quality issue due to the potential for disease transmission and antibiotic resistance. Investigations have identified antibiotics themselves in water sources [23-27]. The increasing rate and heavy use of antibiotics in health care, agriculture and animal husbandry contributes to the emergence of antibiotics in the environment and in turn contributes to the growing problem of antibiotic-resistant bacteria [28]. Furthermore, the spread of antimicrobial resistance between different bacterial strains and or species in different habitats has been reported [29-36]. The presence of virulence genes and increase in the number of resistant strains of *E. coli* is a major concern of health officials. The study site, Ado-Ekiti, is well known with cattle rearing and siting of abattoir near streams and river. These activities contribute to health risk of water sources available for the residents. And among the faecal contaminants, *Escherichia coli* O157 and non-O157 are highly pathogenic in human even at low infectious dose of $10^1$–$10^2$ cfu/ml. Therefore, this study aimed to detect virulence and antibiotic resistance gene in *E. coli* O157 and non-O157 recovered from drinking water sources in Ado-Ekiti, Ekiti State, Nigeria

**Materials and Methods**

**Bacterial isolates**

A total of 382 identified *E. coli*, from different drinking water sources from a previous published work were used in this study. They included 267 (69.89%) from well water samples, 33 (8.64%) from pipe borne water samples, 23 (6.02%) from borehole water samples, 56 (14.66%) from stream water samples and 3 (0.79%) from sachet (packaged) water.

**Serological identification of *E. coli* O157**

Detection and serotyping of toxigenic *E. coli* among the *E. coli* isolates were carried out using the standard method described by Khudor et al. [37]. Latex agglutination kit (DR0620M, Oxoid, UK) was used for conformity identification of *E. coli* O157 to detect the somatic antigen O157. The latex reagent (DR0620M, Oxoid, UK) was brought to room temperature. A drop of the test latex was dispensed onto the edge of a circle on the reaction card. Some loop-full drop of normal saline was added to the circle and it was ensured that the latex and saline did not mix at this stage. A portion of the test *E. coli* isolate was carefully emulsified in the saline drop giving a smooth suspension. Then the test latex and suspension was mixed and spread to cover the reaction area. The reaction card was rocked in a circular motion for exactly one minute and observed for agglutination reaction. *Escherichia coli* isolates positive at this stage was further confirmed by testing with the positive control latex reagent to ensure that the isolate is not auto-agglutinating. Identified *E. coli* which were positive for O157 latex agglutination were considered presumptive *E. coli* O157 based on latex agglutination.

**Detection of virulence and antibiotic resistance genes**

From among the serologically identified *E. coli*, *E. coli* O157 (n= 8) and *E. coli* non-O157 (n= 7) from different water sources were randomly selected for this study due to financial implication. These isolates were then tested for the presence of these genes: Virulence genes (*rfb* O157, *flIC* H7, *stx1, stx2, eae* and *hly* genes) and antibiotic-resistant gene (*blaTEM*) using PCR technique described below.
Bacterial genomic DNA extraction and purification

*Escherichia coli* O157 (n= 8) and *E. coli* non-O157 (n= 7) from different water sources were selected and grown overnight at 37°C. The bacterial cultures were transferred to eppendorf tubes and spun down at 14,000 rpm for 2 mins. The supernatant of each sample was discarded and 600 µL of 2X CTAB buffer was added to the pellet and it was incubated at 65°C for 20 mins. Then, it was allowed to cool to room temperature before the addition of chloroform. The resulting solution was mixed by gently inversion of the tube several times. Thereafter, it was spun at 14,000 rpm for 15 mins and the supernatant was transferred into a new eppendorf tube and equal volume of cold Isopropanol was added to precipitate the DNA. Then, it was incubated at -20°C in the freezer for 1 hr and later spun at 14,000 rpm for 10 mins and the supernatant was discarded and the pellet was washed with 70 % ethanol. Thereafter, it was air dried for 30 mins on the bench. The pellet was re-suspended in 100 uL of sterile distilled water as described by Akinyemi and Oyelakin [38]. The DNA concentration of each of the samples was measured on spectrophotometer at 260 nm and 280 nm and their genomic purity were determined.

DNA electrophoresis

Agarose gel electrophoresis was used to determine the quality and integrity of the DNA by size fractionation on 1.0 % agarose gels. Agarose gels were prepared by dissolving and boiling 1.0 g agarose in 100 mL 0.5X TBE buffer solution. The gels were allowed to cool down to about 45°C and 10µL of 5 mg/mL ethidium bromide was added, mixed together before pouring it into an electrophoresis chamber set with the combs inserted. After the gel has solidified, the PCR amplicon was loaded in the well created. Electrophoresis was done at 100 V for 2 hours. The DNA was visualized and photographed on UV light source.

**Polymerase chain reaction analysis using SSR primers**

Polymerase chain reaction analysis was run with the specific primers (purchased from Integrated DNA Technologies, Inc., Coralville, Iowa) (Table 1) by a GeneAmp PCR thermocycler (Model 2400, Perkin Elmer, USA). The PCR mix comprises of 1µL of 10X buffer, 0.4 µL of 50 mM MgCl2, 0.5 µL of 2.5 mM dNTPs, 0.5µL of 5 mM forward and reverse primers, 0.05 µL of 5units/µlTaq with 2 µL of template DNA and 5.05 µL of distilled water to make-up 10 µL reaction mix. The PCR profile used has an initial denaturation temperature of 94°C for 3 mins, followed by 30 cycles of 94°C for 30 sec, 60°C for 40 sec, 72°C for 60 sec and the final extension temperature of 72°C for 5 mins and the 10°C hold forever.

**Gel electrophoresis**

Polymerase chain reaction amplicon electrophoresis was carried out by size fractionation on 1.2 % agarose gels. Agarose gels were prepared by dissolving and boiling 2.4 g agarose in 200 mL 0.5X TBE buffer solution. The gels were allowed to cool down to about 50°C and 10 µL of 5 mg/mL ethidium bromide was added, mixed together before pouring it into an electrophoresis chamber set with the combs inserted. After the gel has solidified, the PCR amplicon was loaded in the well created. Electrophoresis was done at 100 V for 2 hours. The DNA was visualized and photographed on UV light source.

**Results**

The serological identification result revealed that a total of 68 out of 382 *E. coli* isolates were identified as a presumptive *E. coli* O157. These included 19.1 %, 21.7 %, 33.3 %, 14.3 % and 9.1 % of *E. coli* isolates from wells, boreholes, sachets, streams and pipe-borne respectively. Statistical analysis revealed that no significant difference in the frequency of *E. coli* O157 from the different water sources (p>0.05). Also, there is a statistically significant positive correlation between the *E. coli* isolates and *E. coli* O157 (Pearson’s r = 0.996) (Table 2).

The presence or absence of O157 antigen, Shiga-toxins, virulence, antibiotic-resistant and stress response genes is summarized in table (3). The result in plate (1) confirmed the presence of O157 antigens (259 bp) in isolates in lane 10, 11, 12 and 15 which were identified as presumptive *E. coli* O157 serologically. Also isolates in lane 13 and 14 has O157 antigen of 1.3 kbp and 3 kbp respectively. However, isolate (S33 from borehole sample) in lane 9 which was serologically identified as *E. coli* O157 did not carry the gene for O157 antigens. While the presumptive *E. coli* O157 isolates in lane 1 to 8 were negative for O157 antigen except for isolates in lane 4 which was positive for O157 antigens of 1.6 kbp. All the isolates in lane 1 to 8 were negative for *flc* H7 antigen while from
among those positive for O157 antigen, only isolates in lane 10 to 14 were positive for the presence of flic H7 antigen (> 625 bp). This shows that isolates in lane 10 to 14 were E. coli O157:H7 while isolates in lane 4 and 15 were E. coli O157: H- (Plate 2).

The results shown on plate (3) revealed that only isolates in lane 4, 10, 12 to 14 were positive for stx1 (>348 bp) while Plate 4 showed that all the isolates except isolate in lane 15 possess stx2 gene (246 bp). All the 15 isolates (E. coli O157 and non-O157) were positive for the virulence plasmid encoding the enterohaemolysin gene, hly A, (165 bp) (Plate 5) but were negative for the intimin-coding eae gene (Plate 6). The amplification of antibiotic gene (BlaTEM), encoding ampicillin resistance, was used to confirm that all the isolates possessed this resistance gene (1.8 kbp) coding for BlaTEM (Plate 7).

Table 1. Polymerase chain reaction (PCR) primers as used in this study.

<table>
<thead>
<tr>
<th>Primer set</th>
<th>Target Gene</th>
<th>Sequence (5′→3′)</th>
<th>Size of PCR Product (bp)</th>
<th>References</th>
</tr>
</thead>
</table>
| A          | stx1        | 5′CAGTTAATGTCGTGGCGAAGG3′
                  3′CACCAGACAATGTAAACCGTG5′ | 348 | Osek [39] |
              | stx2        | 5′AGGCCCACTCTTTAATACATCC3′
                  3′CGTCATTCTCTTGTTAACTGTGCG5′ | 246 | Beutin et al. [40] |
| B          | eaeA        | 5′GGGATCGATTACCGTCAT3′
                  3′TTTATCAGCCTTAATCTC5′ | 837 | Osek [39] |
              | HlyA        | 5′ACGATGTGTTTTATTCTGGA3′
                  3′CTTCACGTGACCATACATAT5′ | 165 | Selim et al. [18] |
| C          | Rfb O157    | 5′CGGACATCCCATGTGATATGG3′
                  3′TTGCTATGTACAGCTAATCC5′ | 259 | Khudor et al. [37] |
              | flic H7     | 5′GCCTCTCGAGTTTCTATCGAGC3′
                  3′CAACCGTGACTTATCGCCCATCC5′ | 625 | Khudor et al. [37] |
| D          | E. coli O157 RpoS | 5′GCCTTTGGCTGGACCTTATC3′
                  3′GAATAGTACCGTGGTTGCTAAT5′ | 250 | Parry-Hanson et al. [41] |

Table 2. Serological identification of E. coli O157.

<table>
<thead>
<tr>
<th>Sources (n)</th>
<th>Serotype O157 (%)</th>
<th>Serotype non-O157 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wells (267)</td>
<td>51 (19.1)</td>
<td>216 (80.9)</td>
</tr>
<tr>
<td>Boreholes (23)</td>
<td>5 (21.7)</td>
<td>18 (78.3)</td>
</tr>
<tr>
<td>Sachets (3)</td>
<td>1 (33.3)</td>
<td>2 (66.6)</td>
</tr>
<tr>
<td>Streams (56)</td>
<td>8 (14.3)</td>
<td>48 (85.7)</td>
</tr>
<tr>
<td>Pipe-borne (33)</td>
<td>3 (9.1)</td>
<td>30 (90.1)</td>
</tr>
<tr>
<td>TOTAL (382)</td>
<td>68 (17.8)</td>
<td>314 (82.2)</td>
</tr>
</tbody>
</table>

\[ p>0.05 \]

\[ \chi^2 = 0.4675, \text{ df} = 4, \ r = 0.996 \]

n = the total number of E. coli recovered from each water source.
Table 3. Detection of pathogenic, antibiotic resistant, and stress response genes with polymerase chain reaction (PCR) technique.

<table>
<thead>
<tr>
<th>Lane</th>
<th>Isolates</th>
<th>Sources</th>
<th>Genes Detection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td><strong>rfb O157</strong></td>
</tr>
<tr>
<td>1</td>
<td>S71</td>
<td>Well</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>S177</td>
<td>Stream</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>S31</td>
<td>Boreholes</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>S70</td>
<td>Well</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>S89</td>
<td>Well</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>S152</td>
<td>Well</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>S202</td>
<td>Pipe-borne</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>S34</td>
<td>Pipe-borne</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>S33</td>
<td>Borehole</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>S3B</td>
<td>Stream</td>
<td>+</td>
</tr>
<tr>
<td>11</td>
<td>S2</td>
<td>Well</td>
<td>+</td>
</tr>
<tr>
<td>12</td>
<td>S35</td>
<td>Well</td>
<td>+</td>
</tr>
<tr>
<td>13</td>
<td>S82R</td>
<td>Stream</td>
<td>+</td>
</tr>
<tr>
<td>14</td>
<td>S30</td>
<td>Well</td>
<td>+</td>
</tr>
<tr>
<td>15</td>
<td>S10</td>
<td>Well</td>
<td>+</td>
</tr>
</tbody>
</table>


Lane 1-8 (E. coli non-O157), Lane 9-15 (E. coli O157), Lane M (100bp DNA size marker)
Lane 9 (S33), Lane 10 (S3B), Lane 11 (S2), Lane 12 (S35), Lane 13 (S82R), Lane 14 (S30) and Lane 15 (S10) were positive for rfb O157 gene.

Plate 2. Gel electrophoresis for flic H7.

Lane 1-8 (E. coli non-O157), Lane 9-15 (E. coli O157), Lane M (100bp DNA size marker)
Lane 10 (S3B), Lane 11 (S2), lane 12 (S35), Lane 13 (S82R) and lane 14 (S30) were positive for flic H7 gene.
Plate 3. Gel electrophoresis for stx1.

Lane 1-8 (*E. coli* non-O157), Lane 9-15 (*E. coli* O157), Lane M (100bp DNA size marker) 
Lane 4 – (S70), Lane 10- (S3B), Lane 12 (S35), Lane 13 (S82R), Lane 14 (S30) and Lane 15 (S10) were positive for stx1 gene.

Plate 4. Gel electrophoresis for stx2 gene.

Lane 1-8 (*E. coli* non-O157), Lane 9-15 (*E. coli* O157), Lane M (100bp DNA size marker) Lane 1 (S71), Lane 2 (S177), Lane 3 (S31), Lane 4 (S70), Lane 5 (S89), Lane 6 (S152), Lane 7 (S202) from pipe-borne water sample), Lane 8 (S34), Lane 9 (S33), Lane 10 (S3B), Lane 11 (S2), Lane 12 (S35), Lane 13 (S82R) and Lane 14 (S30) were positive for stx2 gene.

Plate 5. Gel electrophoresis for hlyA gene.

Lane 1-8 (*E. coli* non-O157), Lane 9-15 (*E. coli* O157), Lane M (100bp DNA size marker) All isolates in all the lanes were positive for hlyA gene.
Plate 6. Gel electrophoresis for eae gene.

Lane 1-8 (E. coli non-O157), Lane 9-15 (E. coli O157), Lane M (100bp DNA size marker). Isolates in Lane 1-15 were negative for eae gene.

Plate 7. Gel electrophoresis for BlaTEM gene.

Lane 1-8 (E. coli non-O157), Lane 9-15 (E. coli O157), Lane M (100bp DNA size marker). Isolates in Lane 1-15 were positive for BlaTEM gene.

Discussion

The serological identification of E. coli O157 which was carried out by latex agglutination assay for O157 antigens have greatly facilitated the screening of STEC O157. This latex kit is accurate and easy to use in clinical laboratory setting. This method was used to eliminate other serotypes of pathogenic E. coli which have the cultural and biochemical characteristics [42]. The recovery of E. coli O157 from the drinking water sources was contrary to the findings by Hamieh et al. [43]. They did not recover any E. coli O157 from the water samples in Lebanon using the latex agglutination kit. The serologically identified isolates were considered presumptive E. coli O157 and were further confirmed using PCR technique. The PCR is a rapid technique with high specificity and sensitivity [44]. The applications of singleplex and multiplex PCR for detection of STEC have been reported [45]. The detection of rfbO157 and flicH7 genes in the selected E. coli isolated from the water samples are characteristics of STEC O157: H7 and this has been associated with water related outbreaks. This study corroborates with the study by Hamner et al. [46] and Thenmozhi [47], who documented that E. coli O157: H7 was isolated and identified from drinking water sources. STEC O157:H7 is mainly transmitted to humans by the consumption of contaminated food and water. From among the identified E. coli O157 in this study, two were found to be negative for flicH7 and thus were non-motile type of the pathotype [10]. In a similar study, Osek and Gallein [48] also reported that H7 negative strains have been identified and they represent a distinct clone within E. coli O157 serogroup. However, E. coli O157: H- shares several
virulence characteristics with other STEC of the O157: H7 serotype.

In this study, majority of the isolates possessed either stx1 or stx2 singly while few possessed both. It has been shown that human isolates of E. coli O157: H7 that have stx1 and stx2 and those with only stx2 are very common but isolates having only stx1 are uncommon [49].

According to Brooks et al. [50], human illnesses have been aggravated due to combinations of virulence factors in genes. It was found that all the isolates were negative for eaeA gene but positive for hlyA. This finding is similar to the study by Sajida [51] who also recorded that all the isolates in his study were negative for eaeA gene but possessed the hlyA gene. Although eaeA carrying STEC strains are frequently associated with severe infection, outbreaks of HUS by eaeA negative STEC strains have been reported by Paton and Paton [52]. All the isolates screened for the β-lactam (Blastem) resistance gene were positive. This finding shows the distribution of antibiotic resistance genes which can possibly be carried on E. coli plasmid.

Resistance genes are often associated with integrons or mobile DNA elements such as plasmids and transposons that facilitate the spread of resistance genes [53]. More often, there is a linkage between many of these resistance genes on mobile elements and the distribution of antibiotic resistant bacteria in the environment [54]. Reports have indicated that the resistance genes currently present in human or animal associated microbiota are found in environment without antibiotic pollution [55,56]. This strongly supports the fact that resistant genes can persist and spread in the environment via horizontal gene transfer, thus increasing the chances of pathogens acquiring resistance.

The presence of rpoS gene in majority of the isolates screened suggested their ability to withstand environmental stress. This is in concordance with the study by Parry-Hanson et al. [43] who reported rpoS gene expression in acid-adapted E. coli O157:H7 during lactoperoxidase and lactic acid challenge in tryptone soy broth. RpoS is the primary sigma factor for global regulation of genes associated with environmental stresses [57]. Bacterial cells exposed to a large variety of harmful agents respond by increasing the cellular concentration of a stress sigma factor which replaces the house keeping sigma factor on the RNA polymerase enzyme, thereby changing its regulatory properties [57]. Since these isolates with virulence and resistance genes are recovered from water environment, further studies can be carried out to know the effect of different water treatments on these genes in the isolates.

**Conclusion**

The molecular studies carried out on selected environmental isolates (E. coli O157 and non-O157) revealed the presence of one or more virulence and antibiotic resistance genes. This therefore signifies that water sources contaminated with substances laden with these organisms is of high health risk to the consumer. This is because the virulence and resistance genes have more significant relation to human diseases. Also, the long survival of E. coli O157 in the environment and its low infectious dose are contributing factors to occurrences of water and food borne diseases which occurs as a result of consumption of water contaminated with these organisms. Moreover, these genes could be transferred horizontally in the environment to non-pathogenic bacteria leading to growing problem of clinical therapy.

**Acknowledgements**

The Management of Science Laboratory Technology Department, Federal Polytechnic, Ado-Ekiti, Nigeria is appreciated for the permission granted to carry out this work in their research laboratory.

**Funding:** None declared

**Competing interests:** There are no competing interests to declare.

**References**

1. Sanchez S, Sanchez DS, Martinez R, Lorente MT, Herrera-Leon S, Vidal D. The new allelic variant of the subtilase cytotoxin (sub AB2) is common among Shiga toxin producing Escherichia coli strains from large animals and their meat and meat products. Veterinary Microbiology 2013; 166: 645-649.


4-Food Safety Authority of Ireland. The consumption of bottled water containing certain bacteria or group of bacteria and the implications for public health 2009. Report of the Scientific Committee of Food Safety Authority of Ireland.


14-Mody R, O’Reilly CE. Infectious diseases related to travel. Centre for Disease Control and Prevention 2016; www.cdc.gov/e.coli


34-Kawane RS. Studies on antibiotics and heavy metal resistance profiling of Escherichia coli from drinking water and clinical specimens, Bioscience Discovery 2012; 3(3): 292-295.


36-Sharma VK, Yu X, McDonald TJ, Jinadatha C, Dionysiou DD, Feng M. Elimination of


