Introduction

Food hygiene and safety are chief issues in the food trades, and microbiological safety is an explicit apprehension. In recent years, fast food consumption has publicized incessant growth [1-4]. Much of this growth is credited to the subdivision of frozen meat products, particularly hamburgers. The hamburger consumption has augmented so much [5]. Thus, it is imperative to ensure the quality and safety of hamburger. Nevertheless, numerous outbreaks of food-borne diseases owing to the contaminated hamburger consumption have been testified [6,7].

Escherichia coli (E. coli) is significant cause of food-borne diseases [8, 9]. Meat and meat products are such chief sources of E. coli [10,11]. Enterohemorrhagic E. coli (EHEC) bacteria are a dangerous phenomenon originated from Shiga toxin-producing E. coli (STEC) [8-10]. They are accountable for hemolytic uremic syndrome (HUS), thrombotic thrombocytopenic purpura.

Genotypic and Phenotypic Assessment of Antibiotic Resistance and Recognition of Virulence Factors in Escherichia coli O157 Serogroup Isolated from Hamburger

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Escherichia coli (E. coli) O157 is an important food-borne pathogen. The existing survey was addressed to assess the incidence, phenotypic and genotypic resistance profile toward antibiotics and incidence of virulence factors amongst the E. coli O157 isolates recovered from hamburger. Two-hundred hamburger samples were collected from superstores of Mazandaran, Iran. Hamburger samples were cultured and phenotypic antibiotic resistance pattern was deliberated using disk diffusion test. Isolated E. coli O157 bacteria were identified by PCR. Nineteen out of 200 (9.50%) hamburger samples were contaminated with E. coli O157. Brand C (18%) had the uppermost contamination rate, while brand D (4%) had the lower most. E. coli O157 isolates displayed the uppermost incidence of resistance towards tetracycline lines (100%), ampicillin (100%) and gentamicin (89.47%). All of isolates were resistant toward an antibiotic, while incidence of resistance toward over 6 antibiotics was 36.84%. CITM (89.47%), CTX (89.47%), aac(3)-IV (78.94%), dfrA1 (63.15%), sulI (63.15%) and tetA (57.89%) were the most generally identified antibiotic resistance genes. Incidence of ehyA, stx2, stx1 and eaeA were 100%, 42.10%, 100% and 100%, respectively. Boost contamination of hamburger samples with E. coli O157 bacteria which are exposed to low microbial quality of raw meat samples and also unfitness of cooking time and temperature. Thoughtful antibiotic prescription and courtesies to the ideologies of food security can condense the hazard of resistant and virulent E. coli O157 in hamburger.

Keywords: Antibiotic resistance, Escherichia coli O157, Antibiotic resistance genes, Virulence factors, Hamburger.
(TTP), hemorrhagic colitis (HC) and diarrhea [12, 13]. O157 is a chief sero group of the above mentioned phenomena with boost clinical standing [8-13]. E. coli O157 is chiefly originated from cattle [8-10]. Meat, meat products and milk of contaminated animals are the most significant sources of human infection [13-15].

E. coli O157 bacteria harbored the boostincidence of intimin (eaeA), Shiga toxins (stx1 and stx2) and hemolysin (hlyA). They act as adhesive and invasive factors and are chiefly accountable for occurrence of attaching-effacing (A/E) lesions which mainly caused by the eaeA gene [8-10].

E. coli O157 bacteria are chiefly resistant toward numerous kinds of antibiotics including aminoglycosides, fluoroquinolone, tetracyclines, sulfonamides, and phenicols [16,17]. Attendance of confident antibiotic resistance genes, particularly those encode resistance toward β-lactamases (CTX), fluoroquinolone (qnr), cephalothin (blaSHV), trimethoprim (dfrA1), tetracycline (tetA and tetB), gentamicin (aac(3)-IV), ampicillin (CITM), sulfamethoxazole (sul1), erthyromycin (ereA) and aminoglycosides (aadA1) is an imperativefactor for existence of antibiotic resistance in bacteria [13, 16, 17].

Numerous re searches have been led on molecular epidemiology of E. coli O157 bacteria in food stuffs. Consequently, anexisting examination was performed to measure the phenotypic and molecular profiles of antibiotic resistance and delivery of virulence factors amongst the E. coli O157 bacteria recovered from hamburger samples in Iran.

Materials and Methods

Moral deliberation

The survey was allowed by the Moral Panel of the Islamic Azad University, Science and Research Branch, Iran.

Samples

From May to August 2018, a total of 200 hamburger samples were obtained from the supermarkets of Mazandaran, Iran. Hamburger samples were obtained from 4 dissimilar profitable brands (A, B, C and D). Samples (100 g) were relocated to the laboratory at 4 °C.

E. coli O157 isolation

Bacterial isolation was performed rendering the protocols labeled before and [18,19]. For this goal, 25 g of samples was normalized well and one of the achieved solution was blended with 5 mL of buffered peptone water (Merck, Germany). Media were then incubated at 37 °C for 24 h. MacConkey sorbitol agar (Merck, Germany) was applied for determination of O157 serogroup. Definitive detection of O157 serogroup was performed using the Latex agglutination examination in sorbitol negative bacteroid [18]. Diverse biochemical testssuch as indole, methyl-red, Voges–Proskauer and citrate (IMVC) and Triple Sugar Iron Agar (TSIS) were also applied for identification of bacteria [19].

Phenotypic profile of antibiotic resistance

Phenotypic profile of antibiotic resistance of O157 isolates were examined by disk diffusion test. Mueller–Hinton agar media (Merck, Germany) were applied for this goal. Protocols of the Clinical and Laboratory Standards Institute (CLSI) were applied for this goal [20]. Kirby-Bauer Disk Diffusion Susceptibility Test Protocol was applied for this goal. Diverse antibiotic disks (Oxoid, UK) including cefotaxime (30 µg/disk), enrofloxacin (5 µg/disk), ciprofloxacin (5 µg/disk), clavulanic acid (30 µg/disk), sulfamethoxazole (25 µg/disk), imipenem (30 µg/disk), tetracycline (30 µg/disk), ampicillin (10 µg/disk), gentamicin (10 µg/disk) and cotrimoxazole (30 µg/disk) and were applied for this goal.

Detection of virulence and antibiotic resistance genes

O157 isolates were cultured on nutrient broth (Merck, Germany) and further incubated at 37 °C for 24 h. Principles of producing factory of DNA extraction kit (Thermo Fisher Scientific, Germany) were applied for DNA extraction. Extracted DNA samples were subjected to quantification by NanoDrop device (Nano Drop, Thermo Scientific, Waltham, USA), qualification (2% agarose gel) and purity checking (A260/A280). PCR was conducted rendering beforehand documents (Table 1) [14,15]. Thermo-cycler device (Flexycycler 2, Germany) was used. Fifteen microliters of the PCR products were electrophoresed using 1.5% agarose gel [8, 9]. Runs were comprised a negative control (PCR grade water) and positive controls (E. coli O157:K88ac:H19).
### TABLE 1. PCR circumstances applied for detection of virulence and antibiotic resistance genes in the E. coli O157 isolates recovered from hamburger samples [14, 15].

| Target gene | Primer sequence (5’-3’) | PCR product (bp) | PCR programs | PCR Volume (50µL) |
|-------------|--------------------------|------------------|--------------|------------------|
| stx1        | F: AAATCGCCATTGTGACTCTTCT<br> R: TGCCATTGTGCAACTCGCGGATGA | 366              | 1 cycle:<br> \( ^\circ \)--------- 3 95 min. | 5 µL PCR buffer 10X<br> 2 mM Mgcl\(_2\)<br> 150 µM dNTP<br> (Fermentas) |
| stx2        | F: CGATCGACTCTACTCGTTTCA<br> R: GGATATCTCCCCACTCTGGACCC | 282              | 34 cycle:<br> \( ^\circ \)--------- 60 s 94 | 2 mM Mgcl\(_2\)<br> 150 µM dNTP<br> (Fermentas) |
| eaeA        | F: TGGGCACAAACAGCGGCGGA<br> R: CGTCGCCGAACAGAGATTTTACCC | 629              | 1 cycle:<br> \( ^\circ \)--------- 60 s 56<br> \( ^\circ \)--------- 60 s 72 | 1.5 U Taq DNA polymerase<br> (Fermentas) |
| ehly        | F: CAAATGCAAGGACTGATCCGCCAC<br> R: CAGAGATGTGCTGGCAGAAC | 432              | \( ^\circ \)--------- 10 72 min. | 3 µL DNA template |
| aadA1       | F: TATCCAGCTAAGGCAGGACT<br> R: ATTTGCGCAGTACCTGGTC | 447              | | |
| tetA        | F: GGTTCAGTGAACGACGTCA<br> R: CTGTCCCGAAGTTCGATGA | 577              | | |
| tetB        | F: CCTCAGCTTTCTCAACCGGCTG<br> R: GCACCTTGCTGATGACCTTT | 634              | | |
| dfrA1       | F: GGAGGTGCCAAGGTTGACAC<br> R: GACCGGCAAGTCTGGGCTTACGA | 367              | 1 cycle:<br> \( ^\circ \)--------- 8 94 min. | 5 µL PCR buffer 10X<br> 2 mM Mgcl\(_2\)<br> 150 µM dNTP<br> (Fermentas) |
| qnr         | F: GGGTATGGATAATTGAAAG<br> R: CTAATCTGCAGCAGACTATTTA | 670              | 32 cycle:<br> \( ^\circ \)--------- 60 s 95<br> \( ^\circ \)--------- 70 s 55<br> \( ^\circ \)--------- 2 72 min | 0.75 µM of each primers F & R<br> 1.5 U Taq DNA polymerase<br> (Fermentas) |
| aac(3)-IV   | F: TCTCAGATGGACAGTTGTT<br> R: TCATCTGTCTTCTCGTCAT | 286              | | | |
| sul1        | F: TTCCGGATCTCCGATCTCAC<br> R: ATGATCTCACCCTGCTTC | 822              | 1 cycle:<br> \( ^\circ \)--------- 8 72 min. | 3 µL DNA template |
| blaSHV      | F: TGGCCATGTTATATTCCTCCC<br> R: CGCAGATATATCCACCACATG | 768              | | |
| CITM        | F: TGGCCAGAAGTCCAGGCAA<br> R: TTTCCTGCTAACGTGCTGCC | 462              | | |
| cat1        | F: AGTTTCTGCAATGTCGCTGACC<br> R: TTTGAATTCTTACCTAACCTTACC | 547              | | |
| cmlA        | F: CGCCACCGGTTGTGCTCTATAT<br> R: CACCTTGCTGCCCACATCATTAG | 698              | | |
| CTX         | F: ATGGTCAGTACCATCTAAGG<br> R: TGGGTAAAGGTAGGTCACCAGA | 594              | 1 cycle:<br> \( ^\circ \)--------- 4 95 min. | 5 µL PCR buffer 10X<br> 2 mM Mgcl\(_2\)<br> 150 µM dNTP<br> (Fermentas) |

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Statistical analysis

Data gotten from the experimentations were classified in the Excel software. SPSS/21.0 software was accompanied for statistical analysis. Chi-square and Fisher’s exact two-tailed tests were applied to measure any noteworthy association. Statistical significance was determined at a $P$ value < 0.05.

**Results**

Table 1 signifies the incidence of *E. coli* O157 bacteria amongst the hamburger samples. Nineteen out of 200 (9.50%) hamburger samples were contaminated with *E. coli* O157. Br and C (18%) were the most generally contaminated hamburger sample. Incidence of contamination of brand D (4%) was higher than others. Statistical remarkable variance was gotten amid brands of samples and incidence of *E. coli* O157 ($P$<0.05).

Table 3 embodies the phenotypic profile of antibiotic resistance of *E. coli* O157 bacteria isolated from hamburger samples. *E. coli* O157 isolates harbored the uppermost incidence of resistance toward tetracyclines (100%), ampicillin (100%), gentamicin (89.47%), ciprofloxacin (73.68%) and sulfamethoxazole (73.68%). *E. coli* O157 isolates harbored the lowest incidence of resistance toward imipenem (15.78%), chloramphenicol (15.78%), cotrimoxazole (31.57%), ceftazidime (36.84%) and cefotaxime (42.10%). Brand C of hamburger samples harbored the uppermost and most varied incidence of resistance toward antibiotic agents. Statistical remarkable variance was gotten amid brands of samples and incidence of molecular profile of antibiotic resistance ($P$<0.05).

Table 4 embodies the delivery of molecular profile of antibiotic resistance amongst the *E. coli* O157 bacteria. CITM (89.47%), CTX (89.47%), aac(3)-IV (78.94%), dfrA1 (63.15%), sul1 (63.15%) and tetA (57.89%) were the most generally perceived antibiotic resistance genes. CmlA (5.26%), cat1 (10.52%), tetB (31.57%) and blaSHV (36.84%) had more subordinate incidence amongst other perceived antibiotic resistance genes. Statistical remarkable variance was gotten amid brands of samples and incidence of molecular profile of antibiotic resistance ($P$<0.05). As well, numerical noteworthy variance were gotten amid the resistance of tetA and tetB ($P$<0.05) and cat1 and cmlA ($P$<0.05) antibiotic resistance genes.

Table 5 labels the delivery of virulence factors in the O157 isolates covered from hamburger samples. Incidence of stx1, stx2, eaeA and ehyA were 100%, 42.10%, 100% and 100%, respectively. All *E. coli* O157 isolates concurrently harbored all three stx1, eaeA and ehyA virulence genes (100%). Concurrent incidence of stx1, stx2 and eaeA virulence genes was 31.57%. Statistical remarkable variance was gotten amid brands of samples and incidence of virulence genes ($P$<0.05). As well, numerical noteworthy variance were gotten amid the distribution of stx1 and tetB ($P$<0.05) and cat1 and cmlA ($P$<0.05) antibiotic resistance genes.

**TABLE 2. Incidence of *E. coli* O157 serogroup amongst the hamburger samples.**

| Types and brands of samples | No. samples collected | No positive bacteria (%) |
|----------------------------|----------------------|--------------------------|
|                            | A                    | 50                       | 5 (10)                   |
|                            | B                    | 50                       | 3 (6)                    |
| Hamburger                  | C                    | 50                       | 9 (18)                   |
|                            | D                    | 50                       | 2 (4)                    |
| Total                      |                      | 200                      | 19 (9.50)                |

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TABLE 3. Incidence of antibiotic resistance of *E. coli* O157 isolates recovered from hamburger samples.

| Samples (N positive for *E. coli* O157) | Cfz* | Cft | Cip | Tet | Imp | Amp | Sul | Gen | Trt | Enr | Cot | C30 |
|----------------------------------------|------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| A (5)                                  | 2 (40) | 2 (40) | 4 (80) | 5 (100) | 1 (20) | 1 (100) | 5 (60) | 3 (100) | 5 (60) | 3 (80) | 4 (40) | 2 (20) |
| B (3)                                  | 2 (66.66) | 2 (66.66) | 2 (66.66) | 3 (100) | - (100) | 3 (66.66) | 2 (66.66) | 2 (66.66) | 2 (66.66) | 1 (33.33) | - (1) |
| Hamburger                              | 2 (22.22) | 3 (33.33) | 6 (66.66) | 9 (100) | 2 (22.22) | 9 (100) | 8 (88.88) | 9 (100) | 7 (77.77) | 5 (55.55) | 2 (22.22) | |
| C (9)                                  | 1 (50) | 1 (50) | 2 (50) | 1 (100) | 1 (50) | 5 (55.55) | 1 (100) | 1 (100) | 1 (100) | 1 (100) | - (1) |
| D (2)                                  | 1 (50) | 1 (50) | 2 (50) | - (100) | 2 (100) | 1 (66.66) | 1 (100) | 1 (100) | 2 (100) | 1 (100) | - (1) |
| Total (19)                             | 7 (36.84) | 8 (42.10) | 14 (73.68) | 19 (100) | 15 (78.94) | 19 (94.74) | 14 (73.68) | 17 (89.47) | 13 (68.42) | 12 (63.15) | 6 (31.57) | 3 (15.78) |

*Cfz: ceftazidime (30 µg/disk), cft: cefotaxime (30 µg/disk), cip: ciprofloxacin (5 µg/disk), tet: tetracycline (30 µg/disk), imp: imipenem (30 µg/disk), amp: ampicillin (10 µg/disk), sul: sulfamethoxazole (25 µg/disk), gen: gentamicin (10 µg/disk), trt: trimethoprim (5 µg/disk), enr: enrofloxacin (5 µg/disk), cot: cotrimoxazole (30 µg/disk), c30: chloramphenicol (30 µg/disk)*

TABLE 4. Profile of antibiotic resistance genes amongst the *E. coli* O157 isolates recovered from hamburger samples.

| Samples (N positive for *E. coli* O157) | aadA1 | tetA | tetB | dfrA1 | qnr | aac(3)-IV | sul1 | blaSHV | CITM | catl | cmlA | CTX |
|----------------------------------------|-------|------|------|-------|-----|-----------|------|--------|------|------|------|-----|
| A (5)                                  | 2 (40) | 3 (60) | 2 (60) | 3 (60) | 3 (80) | 4 (80) | 4 (40) | 2 (100) | 5 (20) | 1 (100) | - (100) | - (100) |
| B (3)                                  | 2 (66.66) | 2 (66.66) | 2 (66.66) | 2 (66.66) | 2 (66.66) | 2 (66.66) | 1 (33.33) | 2 (66.66) | - (1) | - (1) | - (66.66) | |
| Hamburger                              | 3 (33.33) | 5 (55.55) | 2 (22.22) | 6 (66.66) | 4 (44.44) | 8 (88.88) | 5 (55.55) | 3 (33.33) | 9 (100) | 11 (11.11) | 11 (11.11) | 100 (100) |
| C (9)                                  | 1 (50) | 1 (50) | 1 (50) | 1 (50) | 1 (50) | 1 (50) | 1 (50) | 1 (50) | 1 (50) | 1 (50) | - (1) | - (1) |
| D (2)                                  | 1 (50) | 1 (50) | 1 (50) | 1 (50) | 1 (50) | 1 (50) | 1 (50) | 1 (50) | 1 (50) | 1 (50) | - (1) | - (1) |
| Total (19)                             | 8 (42.10) | 11 (57.89) | 6 (31.57) | 12 (63.15) | 10 (52.63) | 15 (78.94) | 12 (63.15) | 7 (36.84) | 17 (89.47) | 10 (52.63) | 5 (26.26) | 4 (21.05) |
### TABLE 5. Profile of virulence factors amongst the *E. coli* O157 isolates recovered from hamburger samples.

| Samples (N positive for *E. coli* O157) | N positive for each virulence genes (%) |
|----------------------------------------|----------------------------------------|
|                                        | stx1 | stx2 | eaeA | ehlyA | stx1 + eaeA | stx2 + eaeA | stx1 + eaeA + ehlyA | stx1 + stx2 + eaeA |
| A (5)                                  | 5 (100) | 2 (60) | 5 (100) | 5 (100) | 2 (80) | 5 (100) | 2 (80) |
| B (3)                                  | 3 (100) | 1 (66.66) | 3 (100) | 3 (100) | 1 (66.66) | 3 (100) | 1 (66.66) |
| C (9)                                  | 9 (100) | 4 (55.55) | 9 (100) | 9 (100) | 3 (88.88) | 9 (100) | 2 (22.22) |
| D (2)                                  | 2 (100) | 1 (50) | 2 (100) | 2 (100) | 1 (50) | 2 (100) | 1 (50) |
| Total (19)                             | 19 (100) | 8 (42.10) | 19 (100) | 19 (100) | 7 (36.84) | 19 (100) | 6 (31.57) |

**Fig. 1.** Incidence of multidrug resistant *E. coli* O157 bacteria recovered from hamburger samples.

**Discussion**

*E. coli* O157 is measured as hazardous cause of gastrointestinal disorders associated with consumption of foods with animal origin. Disease is recurrently labeled the ‘burger bug’ because of the boost of influence of hamburger in *E. coli* O157 transmission. Protagonist of hamburger in broadcast of *E. coli* O157 to human has been documented in roughly literature works [21-23].

An existing investigation is the most inclusive report of phenotypic and genotypic description of antibiotic resistance and incidence of virulence factors in the *E. coli* O157 isolates recovered from raw hamburger samples in Iran. Our discoveries exposed that 9.50% of samples were positive for *E. coli* O157. In 1993, 623 people were infected with *E. coli* O157 owing to undercooked hamburgers consumption [24]. In 1994, the U.S. Department of Agriculture (USDA) professed *E. coli* O157 to be an alloy of raw ground beef and hamburger. Nevertheless, hamburger-based *E. coli* O157 outbreaks endure to happen [25]. There were roughly expected clarifications for the boost incidence of *E. coli* O157 in hamburger samples. In the beginning, the likelihood of cross contamination occurrence in the abattoirs among meat carcasses and feces. It is because of ruminants, particularly their feces are documented as the chief source of *E. coli* O157. At second, using low-slung quality and contaminated meat for hamburger preparation. At third, deficiency of passable time and temperature applied for hamburger processing. Thus, it is not astonishing...
that 9.50% of samples were positive for *E. coli* O157. Alterations in the hygienic circumstances of diverse hamburger producing units caused alterations in the occurrence of *E. coli* O157 in dissimilars assessed brands. Concurrent presence of diverse virulence and antibiotic resistance genes showed high pathogenicity of isolated bacteria and also emergence of transmission of antibiotic resistance. *E. coli* O118 was also predominant EHEC bacteria which was not our approached in an existing survey.

Hossain et al. (2012) [26] conveyed that the incidence of *E. coli* O157:H7 in raw mutton, beef, turkey, chicken, ground beef, burger, sausage samples collected from Saudi Arabia were 2.50%, 2%, 0%, 2.50%, 5%, 10%, and 0%, respectively. Comparable to our research, they disclosed that beef burger was the most generally contaminated sample. Correspondingly, De Giusti et al. (2010) [27] conveyed that the incidence of *E. coli* O157 in raw meat samples collected from Italy was 2.61%. Advanced incidence rates of *E. coli* O157 than those perceived in the existing research have been conveyed from South Africa (74.50%) [28] and Malaysia (36%) [29]. Reversely, beef products have been perceived to be exclusively barren from *E. coli* O157 [30], while still others perceived subordinate inciden rates in meat products and hamburger [21, 31, 32]. Montaz et al. [15] conveyed that the incidence of *E. coli* O157 amongst the raw camel, sheep, goat and beef meat samples were 25%, 28.57%, 36% and 31.34%, respectively. Ranjbar et al. [9] conveyed that the incidence of *E. coli* O157 sero group amongst the cooked meat, raw meat, soup and raw chicken samples were 33.33%, 25%, 33.33% and 25%, respectively. The incidence of *E. coli* O157 in meat products demonstrated to be changeable in various area sowing tovariation number of livestock, season of sampling, hygienic circumstances in each farm, levels of farm management, sampling oddness, discrepancy in kind of samples, and departure in methods of pathogenic detection.

Our discoveries exposed that *E. coli* O157 isolates had totally resistance toward one antibiotic agent. Additionally, incidence of resistance toward more than 6 antibiotics was 36.84%. Mainstream of *E. coli* O157 isolates were resist toward ampicillin, tetracyclines, gentamicin, ciprofloxacin and sulfamethoxazole antibiotic agents. Unlawful and imprecise antibiotic prescription particularly in veterinary is may be the chief reason for the boost incidence of resistance in the *E. coli* O157. Boost incidence of resistance of *E. coli* bacteria toward gentamicin, tetracyclines, ciprofloxacin, ampicillin, and sulfamethoxazole antibiotic agents was also conveyed from Estonia [33], USA [34] and Saudi Arabia [35]. Outcomes of the existing research were also disclosed that phenotypic profile of antibiotic resistance of O157 bacteria was invertebrate by the genotypic pattern. Molecular phenomena of antibiotic resistance, particularly CITM, CTX, aac(3)-IV, dfrA1, sul1 and tetA was also perceived. Momtaz et al. [15] conveyed that the O157 isolates recovered from raw beef meat samples harbored both phenotypic and genotypic antibiotic resistance patterns with advanced incidence of resistance toward penicillin (100%), tetracycline (80.59%), gentamicin (55.22%) and trimethoprim (40.29%) and higher distribution of tetA (58.20%), blaSHV (70.14%), CITM (46.26%), aac(3)-IV (64.17%) and dfrA1 (43.28%) antibiotic resistance genes. Hemmatinezhad et al. [10] also conveyed that the incidence of dfrA1, sul1, aac(3)-IV, blaSHV, aadA1, qnr, tetB, cat1, cmlA, adaA and adaA1 genes in O157 isolates of poultry meat samples were 22.22%, 22.22%, 71.11%, 57.77%, 88.88%, 11.11%, 37.77%, 91.11%, 4.44%, 13.33% and 88.88%, respectively. They also disclosed that O157 isolates exhibited the upper most incidence of resistance toward tetracycline (93.33%), ampicillin (91.11%), gentamicin (51.11%), ciprofloxacin (68.88%) and penicillin (68.88%) antibiotic agents. Chloramphenicol is prohibited antibiotic in Iran. Nevertheless, 15.78% of *E. coli* O157 bacteria of the existing survey had whole resistance toward chloramphenicol. As well, incidence of cat1 and cmlA chloramphenicol encoding genes were 10.52% and 5.26%, respectively. The moderately boost incidence of resistance toward chloramphenicol may display its uneven and unofficial prescription in Iranian farms. Moderately boost incidence of resistance toward chloramphenicol was also conveyed in investigations conducted on Iran [8-17, 36], Iraq [37], Ethiopia [38] and USA [39]. Montaz and Jamshidi (2013) [40] conveyed that *E. coli* O157 isolates recovered from chicken meat samples displayed the uppermost incidence of resistance toward tetracycline (96.77%), chloramphenicol (96.77%), and sulfamethoxazole (80.64%) and sul1 (93.54%), aadA1 (64.51%), qnr (58.06%) and dfrA1 (54.83%) antibiotic resistance genes. Mooljuntee et al. [41] stated that the incidence of SHV, CITM, tetA, sul1, dhfrV and ereA...
markers of antibiotic resistance amid the O157 isolates of broilers were 86.40%, 93.30%, 90%, 100%, 100% and 73.30%, respectively. Comparable phenotypic and genotypic profiles of antibiotic resistance in the E. coli bacteria were also report from India [42] (boost incidence of resistance toward cephalothin, gentamicin, erythromycin, amikacin, and kanamycin), South Africa [43] (boost incidence of blaSHV, CITM, and tetA markers of resistance), Korea [44] (boost incidence of resistance toward ampicillin, streptomycin, tetracycline and amikacin) and Mexico [45] (boost incidence of blaSHV, aac(3)-IV, tet, qnr markers of resistance and also significant incidence of resistance toward cephalothin, ampicillin, trimethoprim-sulfamethoxazole, and chloramphenicol). Alterations found in the phenotypic and molecular incidence of antibiotic resistance in assorted investigations are may be owing tovariances in the kind of assessed samples, availability of antibiotic agents, rules about antibiotic prescription and even season.

Additional imperative section of our research was about the incidence of virulence factors. E. coli O157 isolated spontaneously harbored all stx1, ehlyA and eaeA virulence genes composed. An existing discovery disclose dextensive pathogenicity of E. coli O157 isolates. Thus, consumption of contaminated hamburger samples comprise virulent E. coli O157 may cause unadorned clinical diseases. The stx2 and stx1 markers were originated to be connected mostly with unsophisticated diarrhea or asymptomatic excretion [15]. Furthermore, they are accountable for pathogenicity of E. coli bacteria and occurrence of with HC and HUS [15]. The eaeA and hlyA genes have been originated in over than 90% of diseases caused by STEC bacteria including HUS [15]. There is an indication that bacteria comprising stx2 and eaeA virulence factors are accompanied with unadorned clinical illness [15]. Coexisting occurrence of stx2 and stx1 and eaeA markers has been labeled beforehand [46-48]. Koochakzadeh et al. [49] conveyed that of 18 E. coli isolates from meat samples, one isolate harbored the stx2 and ehly genes composed, and another one possessed stx2, eae and ehly concurrently. Shojaei (2017) [50] conveyed that E. coli O157 bacteria were concurrently positive for stx1, eaeA and ehly genes together which was comparable to our discoveries. It has been predictable that grouping of numerous virulence markers such as stx1, eaeA and ehlyA in E. coli bacteria may cause occurrence of more separate diseases in humans [51, 52].

Conclusions

In deduction, we implied boost incidence of multidrug resistant E. coli O157 with substantial distribution of markers of antibiotic resistance and virulence in hamburger samples. Our out comes exposed that hamburger was imperative source of E. coli O157 bacteria. Additionally, we originated that contamination with E. coli O157 was depend on brands of hamburger samples with higher incidence rate in brand C. E. coli O157 isolated played the uppermost incidence of resistance toward tetracyclines, ampicillin, gentamicin, ciprofloxacin and sulfamethoxazole. Moreover, all of the E. coli O157 bacteria were resistant to an antibiotic. Mainstream of the E. coli O157 bacteria were also positive for CITM, CTX, dfrA1, aac(3)-IV, sul1 and tetA markers of antibiotic resistance. E. coli O157 isolates concurrently concealed all stx1, eaeA and ehlyA virulence genes. Concurrent attendance of manifold virulence and antibiotic resistance markers and boost incidence of resistance toward some antibiotic agents highpoint an imperative epidemiological hazard regarding the consumption of hamburger. By means of high quality meat and appropriate thermal dispensation can lessen the hazard of E. coli O157 in hamburger. Nevertheless, supplementary explorations are obligatory to determined supplementary epidemiological and microbiological features of E. coli O157 in hamburger.

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Conflict of interest

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