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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 isan 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 uppermostincidence of resistance towardtetracyc lines (100%), ampicillin (100%) and gentamicin (89.47%). All of isolates were resistant toward an antibiotic, while incidence of resistance towardover 6 antibiotics was 36.84%. *CITM* (89.47%), *CTX* (89.47%), *aac(3)-IV* (78.94%), *dfrA1* (63.15%), *sul1* (63.15%) and *tetA* (57.89%) were the most generallyidenti fied antibiotic resistance genes. Incidence of *ehlyA*, *stx2*, *stx1* and *eae A* were 100%, 42.10%, 100% and 100%, respectively. Boost contamination of hamburger samples with *E. coli* O157 bacteria which are exposedto 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.

Introduction

Food hygiene and safety are chiefissues in the food trades, and microbiological safety is anexplicitapprehension. In recent years, fast food consumption has publicizedincessant growth [1-4]. Much of this growth is credited to the subdivision of frozen meat products, particularlyhamburgers. 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*) issignificantcause of food-borne diseases [8, 9]. Meat and meat products are suchchiefsources 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

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(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 antibioticsincludingaminoglycosides, 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), chloramphenicol (cat1 and cmlA), sulfonamide (sul1), erythromycin (ereA) and aminoglycosides (aadA1) is animperativefactor for existence of antibiotic resistance in bacteria [13, 16, 17].

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

Materials and Methods

Moraldeliberation

The survey was allowed by the MoralPanel 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 superstores 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 ^{0}C.

E. coli O157 isolation

Bacterial isolation was performed rendering the protocols labeledbeforeh 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) includingceftazidime (30 µg/disk), trimethoprim (5 µg/disk), cefotaxime (30 µg/disk), enrofloxacin (5 µg/disk), ciprofloxacin (5 µg/disk), chloramphenicol (30 µg/disk), sulfamethoxazole (25 µg/disk), imipenem (30 u/disk), tetracycline (30 u/disk), ampicillin (10 u/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 (Flexrcycler^2, Germany) was used. Fifteen microliters of the PCR products were electrophoresed using 1.5% agarose gel [8, 9]. Runs werecomprised 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: AAATCGCCATTCGTTGACTACTTCT R: TGCCATTCTGGCAACTCGCGATGCA	366	1 cycle: ^{0C} ---------- 3 95 min. 34 cycle: ^{0C} ---------- 60 s 94 ^{0C} ---------- 45 s 56 ^{0C} ---------- 60 s 72 1 cycle: ^{0C} ---------- 10 72 min	5 µL PCR buffer 10X 2 mM $Mgcl_2$ 150 µM dNTP (Fermentas) 0.75 µM of each primers F & R 1.5 U Taq DNA polymerase (Fermentas) 3 µL DNA template
stx2	F: CGATCGTCACTCACTGGTTTCATCA R: GGATATTCTCCCCACTCTGACACC	282		
eaeA	F: TGCGGCACAACAGGCGGCGA R: CGGTCGCCGCACCAGGATTC	629		
ehly	F: CAATGCAGATGCAGATACCG R: CAGAGATGTCGTTGCAGCAG	432		
aadA1	F: TATCCAGCTAAGCGCGAACT R: ATTTGCCGACTACCTTGGTC	447		
tetA	F: GGTTCACTCGAACGACGTCA R: CTGTCCGACAAGTTGCATGA	577		
tetB	F: CCTCAGCTTCTCAACGCGTG R: GCACCTTGCTGATGACTCTT	634		
dfrA1	F: GGAGTGCCAAAGGTGAACAGC R: GAGGCGAAGTCTTGGGTAAAAAC	367		
qnr	F: GGGTATGGATATTATTGATAAAG R: CTAATCCGGCAGCACTATTTA	670	1 cycle: ^{0C} ---------- 8 94 min. 32 cycle: ^{0C} ---------- 60 s 95 ^{0C} ---------- 70 s 55 ^{0C} ---------- 2 72 min 1 cycle: ^{0C} ---------- 8 72 min	5 µL PCR buffer 10X 2 mM $Mgcl_2$ 150 µM dNTP (Fermentas) 0.75 µM of each primers F & R 1.5 U Taq DNA polymerase (Fermentas) 3 µL DNA template
aac(3)-IV	F: CTTCAGGATGGCAAGTTGGT R: TCATCTCGTTCTCCGCTCAT	286		
sul1	F: TTCGGCATTCTGAATCTCAC R: ATGATCTAACCCTCGGTCTC	822		
blaSHV	F: TCGCCTGTGTATTATCTCCC R: CGCAGATAAATCACCACAATG	768		
CITM	F: TGGCCAGAACTGACAGGCAAA R: TTTCTCCTGAACGTGGCTGGC	462		
cat1	F: AGTTGCTCAATGTACCTATAACC R: TTGTAATTCATTAAGCATTCTGCC	547		
cmlA	F: CCGCCACGGTGTTGTTGTTATC R: CACCTTGCCTGCCCATCATTAG	698		
CTX	F: ATGTGCAGTACCAGTAAGGT R: TGGGTAAAGTAGGTCACCAGA	594	1 cycle: ^{0C} ---------- 4 95 min. 30 cycle: ^{0C} ---------- 60 s 95 60^{0C} ---------- 60 s ^{0C} ---------- 60 S 72 1 cycle: ^{0C} ---------- 5 72 min	5 µL PCR buffer 10X 2 mM $Mgcl_2$ 150 µM dNTP (Fermentas) 0.75 µM of each primers F & R 1.5 U Taq DNA polymerase (Fermentas) 3 µL DNA template

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 noteworthyassociation. Statistical signification 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. Statisticalremarkable variance was gottenamid 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 uppermostincidence of resistance towardtetracyclines (100%), ampicillin (100%), gentamicin (89.47%), ciprofloxacin (73.68%) and sulfamethoxazole (73.68%). *E. coli* O157 isolates harbored the lowest incidence of resistance towardimipenem (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 variedincidence of resistance toward antibiotic agents. Statistical remarkable variance was gottenamid brands of samples and incidence of resistance of *E. coli* O157 (*P*<0.05).

Figure 1 demonstrations the incidence of multi-drug resistant *E. coli* O157. *E. coli* O157 isolatesentirelydis played resistant to an antibiotic, while incidence of resistance towardmore than 6 antibiotics was 36.84%.

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 perceive dantibiotic 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 gottenamid brands of samples and incidence of molecular profile of antibiotic resistance (*P*<0.05). As well, numerical noteworthy variance were originate damid the incidence 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 isolatesre covered from hamburger samples. Incidence of *stx1*, *stx2*, *eaeA* and *ehlyA* were 100%, 42.10%, 100% and 100%, respectively. All *E. coli* O157 isolates concurrently harbored all three *stx1*, *eaeA* and *ehlyA* virulence genes (100%). Concurrentincidence of *stx1*, *stx2* and *eaeA* virulence genes was 31.57%. Statistical remarkable variancewas gottenamid brands of samples and incidence of virulence genes (*P*<0.05). As well, numerical noteworthy variance were gottenamid 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 (%)
Hamburger	A	50	5 (10)
	B	50	3 (6)
	C	50	9 (18)
	D	50	2 (4)
	Total	200	19 (9.50)

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

Samples (N positive for *E. coli* O157)		N positive for each antibiotic agent (%)											
		Cfz*	**Cft**	**Cip**	**Tet**	**Imp**	**Amp**	**Sul**	**Gen**	**Trt**	**Enr**	**Cot**	**C30**
Hamburger	A (5)	2 (40)	2 (40)	4 (80)	5 (100)	1 (20)	5 (100)	3 (60)	5 (100)	3 (60)	4 (80)	2 (40)	1 (20)
	B (3)	2 (66.66)	2 (66.66)	2 (66.66)	3 (100)	-	3 (100)	2 (66.66)	2 (66.66)	2 (66.66)	2 (66.66)	1 (33.33)	-
	C (9)	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)	2 (22.22)
	D (2)	1 (50)	1 (50)	1 (50)	2 (100)	-	2 (100)	1 (50)	1 (50)	1 (50)	1 (50)	1 (50)	-
	Total (19)	7 (36.84)	8 (42.10)	14 (73.68)	19 (100)	3 (15.78)	19 (100)	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 u/disk), imp: imipenem (30 u/disk), amp: ampicillin (10 u/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)		N positive for each antibiotic resistance genes (%)											
		aadA1	***tetA***	***tetB***	***dfrA1***	***qnr***	***aac(3)-IV***	***sul1***	***blaSHV***	***CITM***	***cat1***	***cmlA***	***CTX***
Hamburger	A (5)	2 (40)	3 (60)	2 (40)	3 (60)	3 (60)	4 (80)	4 (80)	2 (40)	5 (100)	1 (20)	-	5 (100)
	B (3)	2 (66.66)	2 (66.66)	1 (33.33)	2 (66.66)	2 (66.66)	2 (66.66)	2 (66.66)	1 (33.33)	2 (66.66)	-	-	2 (66.66)
	C (9)	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)	1 (11.11)	1 (11.11)	9 (100)
	D (2)	1 (50)	1 (50)	1 (50)	1 (50)	1 (50)	1 (50)	1 (50)	1 (50)	1 (50)	-	-	1 (50)
	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)	2 (10.52)	1 (5.26)	17 (89.47)

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***
Hamburger	A (5)	5 (100)	2 (60)	5 (100)	5 (100)	5 (100)	2 (80)	5 (100)	2 (80)
	B (3)	3 (100)	1 (66.66)	3 (100)	3 (100)	3 (100)	1 (66.66)	3 (100)	1 (66.66)
	C (9)	9 (100)	4 (55.55)	9 (100)	9 (100)	9 (100)	3 (88.88)	9 (100)	2 (22.22)
	D (2)	2 (100)	1 (50)	2 (100)	2 (100)	2 (100)	1 (50)	2 (100)	1 (50)
	Total (19)	19 (100)	8 (42.10)	19 (100)	19 (100)	19 (100)	7 (36.84)	19 (100)	6 (31.57)

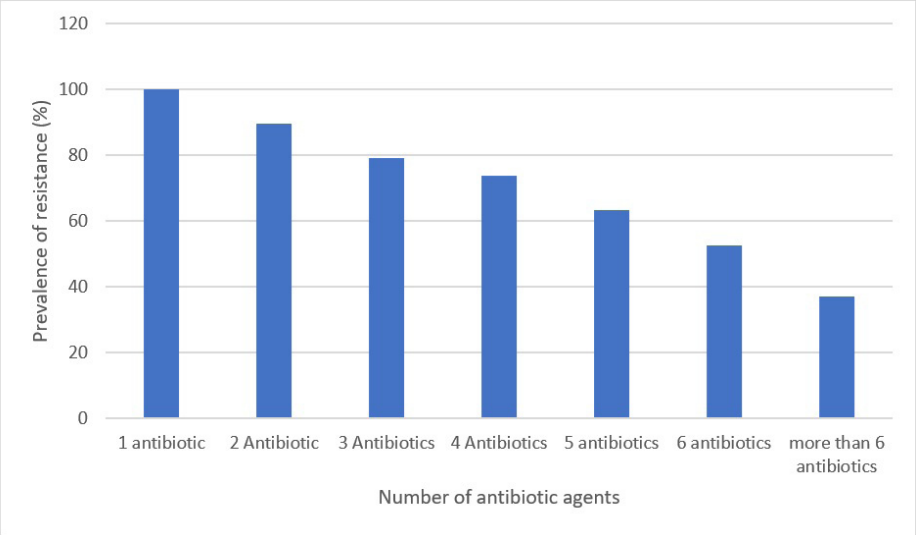

Fig. 1. Incidence of multidrug resistant *E. coli* O157 bacteria recovered from hamburger samples.

Discussion

E. coli O157 is measured as hazardous cause of gastrointestinal disorders associated with consumption of foods with animal origin. Disease is recurrentlylabeled the 'burger bug' because of the boostinfluence of hamburger in *E. coli* O157 transmission. Protagonist of hamburger in broadcast of *E. coli* O157 to human has been documented in roughlyliterature works [21-23].

An existing investigation is the most inclusive report of phenotypic and genotypic description of antibiotic resistance and incidence of virulence factorsin 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 peopleswere 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 abattoirsamid meat carcasses and feces. It is because of ruminants, particularly their feces are documented as the chiefsource 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 dissimilara ssessed 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 exclusivelybarren from *E. coli* O157 [30], while still others perceived subordinateinci dencerates in meat products and hamburger [21, 31, 32]. Momtaz *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 variousarea sowing tovariationin number of livestock, season of sampling, hygienic circumstances in each farm, levels of farm management, samplingoddness, 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 towardampicillin, 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. Boostincidence of resistance of *E. coli* bacteria towardgentamicin, 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 inveterate 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 towardpenicillin (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*, *tetA*, *cmlA*, *cat1* and *aadA1* 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 mostincidence of resistance towardtetracyc line (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 existingsurveyhad whole resistance towardchloram phenicol. As well, incidence of *cat1* and *cmlA* chloramphenicol encoding genes were 10.52% and 5.26%, respectively. The moderatelyboostincidence 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]. Momtaz and Jamshidi (2013) [40]conveyed that *E. coli* O157 isolates recovered from chicken meat samples displayed the uppermostincidence of resistance towardtetracycline (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] (boostincidence of resistance towardcephalothin, gentamicin, erythromycin, amikacin, and kanamycin), South Africa [43] (boost incidence of *blaSHV*, *CITM*, and *tetA*markers of resistance), Korea [44] (boost incidence of resistance towardampicillin, 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 towardcephalo thin, ampicillin, trimethoprim-sulfamethoxazole, and chloramphenicol). Alterations found in the phenotypic and molecularincidenceof antibiotic resistance in assortedinvestigationsare 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 isolatesinstantaneously harbored all *stx1*, *ehlyA* and *eaeA* virulence genes composed. Anexisting discoverydisclose 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 labeledbeforehand [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 comesexposed 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 isolatedis played the uppermostincidence of resistance towardtetracyclines, 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 boostincidence of resistance toward some antibiotic agentshighpoint 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, supplementaryexplorations are obligatory to determined supplementary epidemiological and microbiological features of *E. coli* O157 in hamburger.

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Conflict of interest

The author has no conflict of interests to declare regarding the publication of this paper.

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