ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Genotypic Study of *Escherichia coli* O157:H7 Isolated from Stool Samples of Humans and Cattle

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Manuscript Info

Manuscript History:

Received: 15 April 2014
Final Accepted: 25 May 2014
Published Online: June 2014

Key words:
: Human, E.coli O157:H7, stool samples, PCR assay

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Abstract

The main objectives of this study were to determine the prevalence of *E.coli* O157:H7 in humans and cattle in province of Missan , and determine the genotypic relationship between the both isolates by PCR assay ,to achieve this goal, 198 human stools were collected from patients suffering from bloody and non-bloody diarrhea and urinary tract infections ,both genders, and variable age, during the period from the middle of October 2013 to middle of February 2014 in the province of Missan, also at the same period, 54 cattle fecal samples and 59 mucosal gallbladder swabs were collected from cattle slaughtering in Missan abattoir.

The samples were cultured aerobically on routine media and selective media at 37C for 24-48hrs,then the isolates were identified by biochemical tests and Api 20E test and they were confirmed diagnosis by PCR assay.

The results showed that 10(5%) out of 198 human stools samples were *E.coli* O157:H7 positive and 9(16.6%) out of 54 and 1(1.75) out of 59 of fecal samples and gallbladder swabs of cattle respectively were positive *E.coli* O157:H7 . PCR assay revealed that both human and cattle bacterial isolates expressed rfbO157 and flicH7 genes as well as stx1 and stx2 genes.

Introduction

Escherichia coli O157:H7 a food borne disease, were considered very important zoonotic disease that induced a clinical signs ranged from limited watery to severe bloody diarrhea and hemolytic colitis in the patients (**Armstrong *et al.*, 1996**), also it can be caused extra-intestinal infection generally hemolytic ureamic syndrome (HUS) and kidney failure especially in children (**Karmali *et al.*, 1985**) besides neurological disturbances and other complications of the infection (**Mariani-Kurkdjian and Bingen, 1999**).

E. coli O157:H7 infections were emergency disease (**Manning *et al.*,2008**) and showed highly virulence factors ,therefore low dose (100 organisms) may take place infection and express clinical signs in humans and furthermore the high virulence factors of *E.coli* O157:H7 which associated with severe illness of humans was shiga toxins (**Paton and Paton, 1998).**

Cattle and other ruminants were harbored *E.coli* O157:H7 without clinical symptoms and this animals may shed this pathogen with feces for long period that induced food contamination (**Grauke *et al.*,2002**) , **Flores *et al.*, (2006**) revealed that beef and dairy cattle were carried rates than 0.5 to 2% hence, beef and dairy products considered more important source for food – borne transmission of this organism to human beings (**Riley *et al.*, 2005**).

In Iraq, here were limited researches about the genotypic relationship between cattle and human serotype isolates ,therefore ,the present study aimed to determine the prevalence of *E.coli* O157:H7 in human and cattle as well as to determine the genotypic relationship of *E.coli* O157:H7 isolates from human and cattle.

Materials and Methods

Sample collection

198 stool samples were collected from patients suffering from diarrhea, bloody diarrhea and urinary tract impairment with both genders and different ages in Al-Sader teaching hospital, Al-zahrawi hospital for the period from the middle of October 2013 to middle of February 2014 in the province of Missan, as well as 54 fecal samples and 59 mucosal gallbladder swabs were collected from cattle slaughtered in Amarah abattoir at the same time of human collecting stools.

Sample culturing

The samples were transported with transport media cary & blair to public health laboratory and then inoculated on sorbitol MacConkey agar (SMAC) and eosin methylene blue (EMB) and chromagar O157 as well as on cefixime tellurite- sorbitol macConkey agar (CT- SMAC) at 37ºC for 24 hours and observed the growth of bacterial colonies.

E. coli O157:H7 identification

E. coli O157:H7 was identified by biochemical tests , Api-20E profile system and the isolates were confirmed by polymerase chain reaction (PCR) assay by using primers for four genes to identify and detect rfbO157, flicH7, stx1 and stx2 genes. table (1)

Results and Discussion

Baterial isolation and identification

The bacteriological culturing revealed a green metallic sheen colonies on eosin methylene blue agar and this colonies appear a smooth colorless on SMAC agar at 24-48hrs post-culturing at 37C,moreover, this colonies showed mauve color on ChromagarO157 agar (Fig:1), and the bacterial isolates expressed gram-negative stain, this features may be indicated that the bacterial colonies belonged to *E.coli* O157:H7,this result was similar to bacterial colonies of *E.coli* O157:H7 that recorded by **Adam *et al*.,(2008) and Son *et al*.,(2000).**

Table (1): primers and primer sequences and their product sizes used in multiplex PCR to detect *E. coli* O157:H7

Primers		Primer sequence (5' to 3')	Product size	Reference
flicH7	F	GCG CTG TCG AGT TCT ATC GAG	625 bp	Sarimehtoglu et al.,2009
	R	CAA CGG TGA CTT TAT CGC CAT TCC		
rfbO157	F	CGG ACA TCC ATG TGA TAT GG	259 bp	Jamshidi et al., 2011
	R	TTG CCT ATG TAC AGC TAA TCC		
stx1	F	ACA CTG GAT GAT CTC AGT GG	614bp	Gannon et al. (1992)
	R	CTG AAT CCC CCT CCA TTA TG		
stx2	F	CCA TGA CAA CGG ACA GCA GTT	779bp	Gannon et al. (1997)
	R	CCT GTC AAC TGA GCA CTT TG		

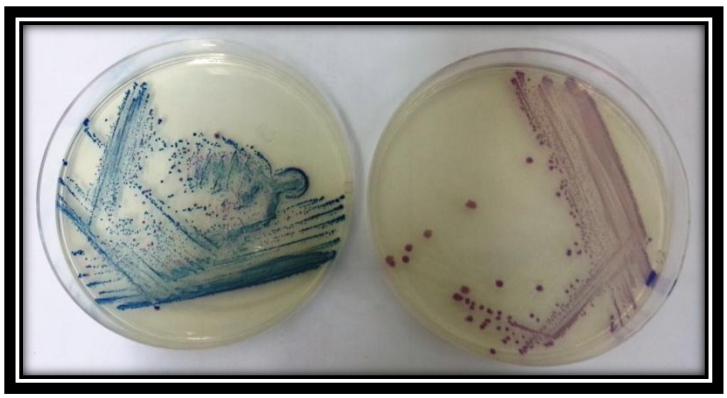

Figure (1) the right picture *E.coli* blue color colonies in chromagar O157 and the left picture is *E.coli* O157:H7 mauve color colonies in chromagar O157

Biochemical Test

The bacterial isolated were positive for indole test, MR test and catalase test, while they were given negative results for VP test, Simmon's citrate and urease test.

Biochemical results were supported that the present isolates may be *E.coli* O157 serotype ,this isolates were also identified by Api-20E system bacterial isolation and identification revealed that 33 isolates were suspected *E.coli* O157:H7 and this isolates were confirmed diagnosis by PCR assay.

Multiplex PCR Products

Twenty bacterial isolates from human stools and cattle samples were suspected *E. coli* O157:H7 and this isolates were confirmed diagnosis by PCR assay which showed that 20 isolates expressed (rfbO157) gene, 10 of them from human stool isolates and 9 of them from cattle fecal and 1of them from cattle mucosal gallbladder swabs, whereas 18 isolates out of 20 showed (flicH7) gene, 10 of them from human stool isolates and 8 from cattle fecal isolates,(Fig:2,3) and Table:2

PCR assay also revealed that 8 out of 20(40%) isolates revealed (stx1) gene, 3 of them from human stool isolates and 5 from cattle fecal isolates whereas 7 out of 20(35%) isolates expressed (stx2), 3 of them from human stool isolates and 4 from cattle fecal isolates as in figures (.4&5). And table:2.

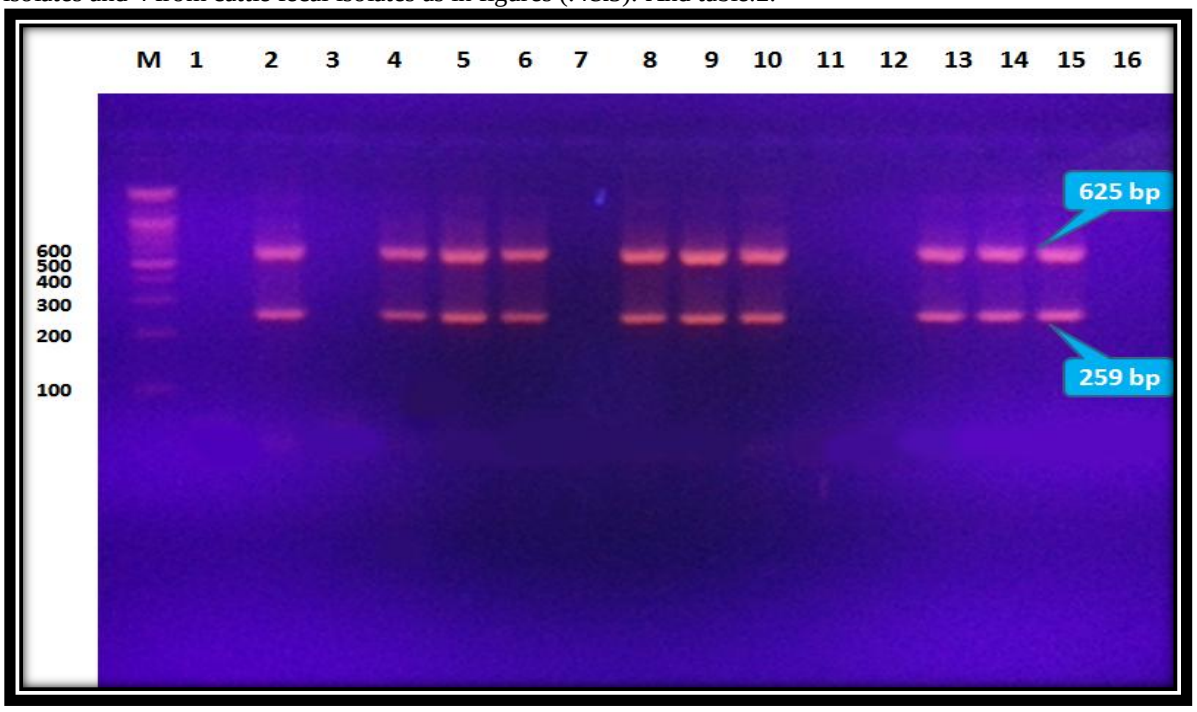

Figure (2): Agarose gel (2%) electrophoresis shows amplification of 259 bp & 625 bp fragments of (rfbO157) and (flicH7) genes by multiplex PCR. Lanes: 2,4,5,6,8,9,10,13,14&15 positive amplification of *E. coli* O157:H7 for human. Lane M: 100 bp DNA marker.

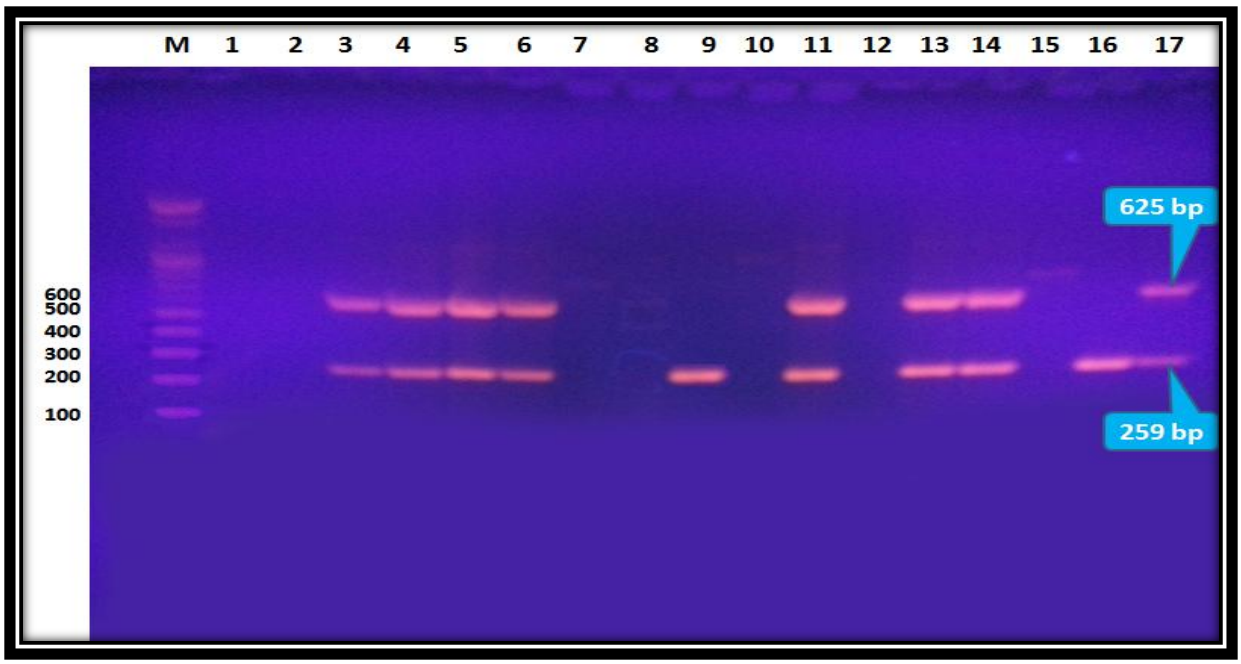

Figure (3): Agarose gel (2%) electrophoresis shows amplification of 259 bp & 625 bp fragments of (rfbO157) and (flicH7) genes by multiplex PCR. Lanes: 3,4,5,6,9,11,13,14 &17 positive amplification of *E. coli* O157:H7 for cattle feces. Lane: 16 positive amplification of *E. coli* O157:H7 for cattle gallbladder. Lane M: 100bp DNA marker

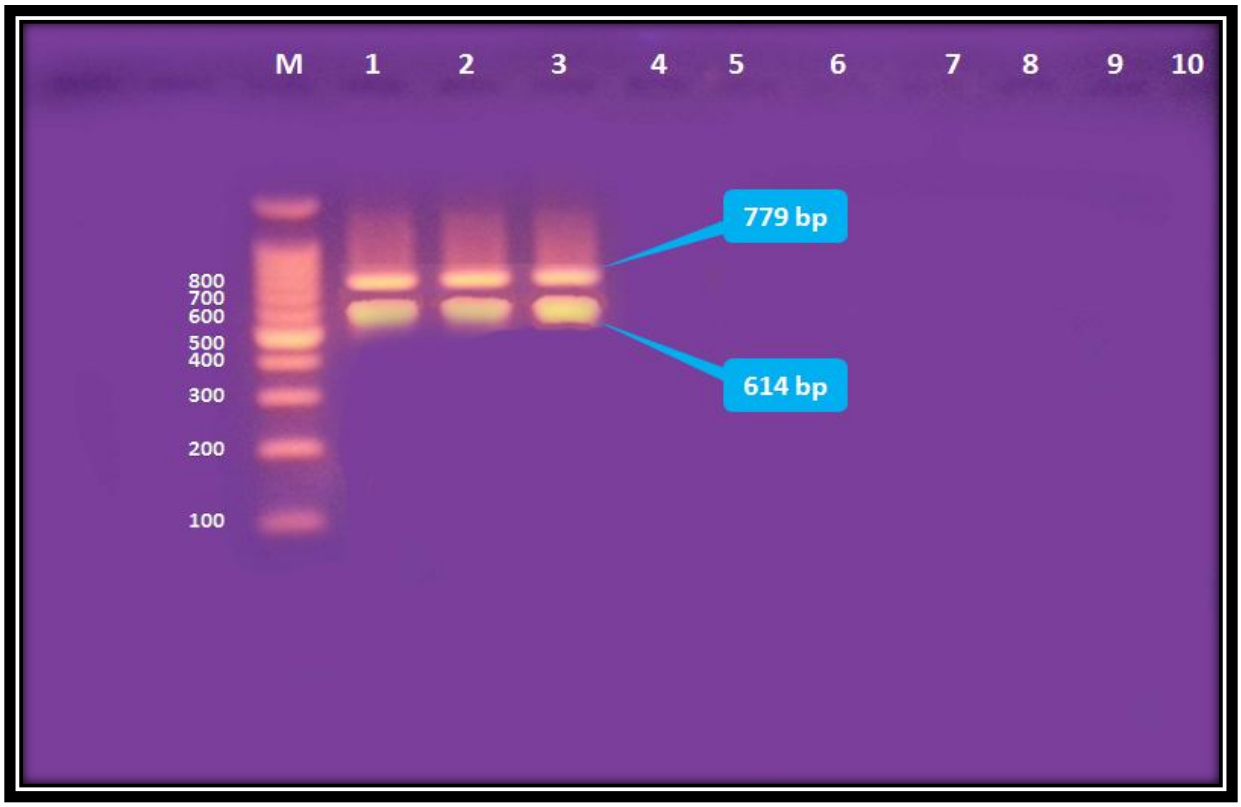

Figure (4): Agarose gel (2%) electrophoresis shows amplification of 614 bp and 779 bp fragments of stx1 & stx2 genes by multiplex PCR. Lanes: 1&2 show positive amplification of E. coli O157:H7 for human, Lane 3 shows positive *E. coli* O157:H7 for cattle. Lane M: 100 bp DNA marker

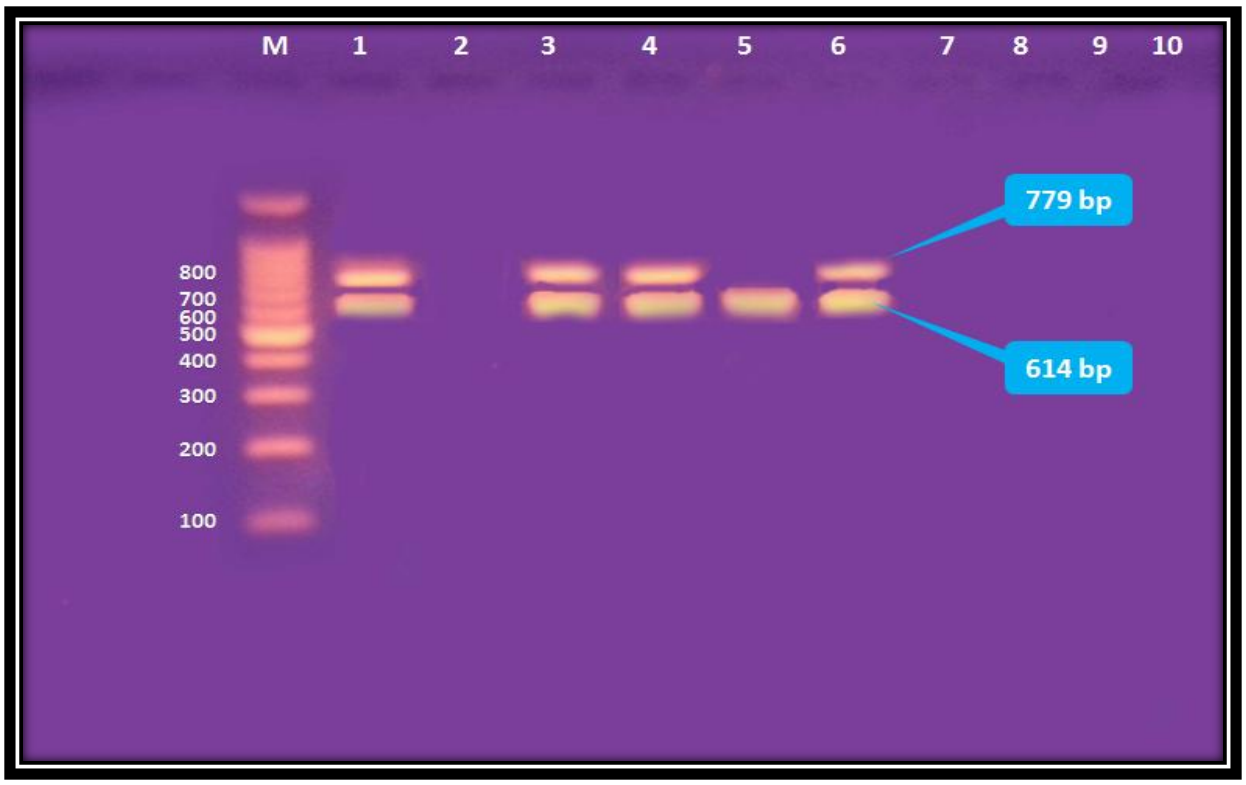

Figure (5): Agarose gel (2%) electrophoresis shows amplification of 614 bp and 779 bp fragments of (stx1 & stx2) genes by multiplex PCR. Lane: 1shows positive amplification of *E. coli* O157:H7 for human, Lanes: 3,4,5&6 show positive *E. coli* O157:H7 for cattle. Lane M: 100 bp DNA marker.

Table (2): the genetic markers of *E.coli* O157:H7isolates from human stool samples by PCR assay

Source	No. of isolate by PCR	rfbO157	flicH7	stx1	stx2
Human S	10	10	10	3	3
Cattle S	9	9	8	5	4
Cattle G	1	1	0	0	0
Total	20	20	18	8	7

S=stool sample, G=gallbladder swab sample

Prevalence of *E. coli* O157:H7 in Human

The present study showed that 10(5%) isolates out of 198 human stool samples were positive *E.coli* O157:H7 , also it was recorded that the number of positive *E.coli* O157:H7 isolates from the children with age <1 to 4 years (6 out of 10 , 3%)were more than the adults with age 15 to 45 years and >45 years(4 out of 10, 2%).

The current study revealed that the percentage of male positive *E.coli* O157:H7 isolates (5.6%) was higher than those from female isolates (4.3%) (Table:4). In the present study we found a discrepancy by residence, positive *E.coli* O157:H7 isolates from the patients who lived in rural areas were 7(70%) isolates especially raw milk consumers and in urban areas were 3(30%) out of 10 isolates as in table (3).

Table (3): Prevalence level of *E.coli* and *E.coli* O157:H7 by residence

Age Group	E.coli +ve	Urban	Rural	O157:H7 +ve	Urban	Rural
<1	5	2	3	4	1	3
1_4	4	1	3	2	1	1
5_14	2	1	1	2		2
15_45	5	1	4	1		1
>45	2	1	1	1	1	
Total	18	6	12	10	3	7

Table (4): *E.coli* O157:H7 isolated from human stool samples according to age and gender:

Age Group	Total Samples	Male Samples	Female Samples	E.coli +ve	%	O157:H7 +ve	%	Male O157:H7+ve	Female O157:H7+ve
<1	45	24	21	5		4		3	1
1_4	51	22	29	4		2		1	1
5_14	38	21	17	2		2		1	1
15_45	30	20	10	5		1		1	
>45	34	20	14	2		1			1
Total	198	107	91	18	9	10	5	6	4

The present study demonstrated that among 198 human stools who suffering from diarrhea, bloody diarrhea and urinary tracts infections ,10(5%) of samples were *E.coli* O157:H7 positive, this result may be indicated this pathogen was considered one causes of gastrointestinal tract impairment and urinary tract infection in human this evidence was agreement with **AlWgaa,(2014**) who recorded that 8(3.50%) out of 228 urine samples of humans were *E.coli* O157:H7 positive isolates, also the present study found that the percentage of *E.coli* O157:H7 isolates from stool samples of young patients was higher than those recorded in adults patients, this result may be due to development acquired immunity in adults or due to difference in concentration of Gb3 in digestive system according to age, this result was agreement with **Paton and Paton,(1998)** who showed the ratio of *E.coli* O157:H7 infection in children was higher as compared with adult.

Also **Faten, (2013)** collected 230 stool samples from diarrheatic children and she showed that 14(6%) out of 230 were positive *E.coli* O157:H7.

It was reported in the present study that the ratio of bacterial isolates from male patients was more than those isolated from the female ,this result was agreement with results of the CDC Atlanta surveys 2009 that found *E.coli* O157:H7 in males were more than in females

We Suggested that the males were more exposure to sources of infection by this pathogens ,this evidence was coincidence with result of bacterial distribution among rural and urban patients, in the present study , that investigated a high number of *E.coli* O157:H7 isolates in the former as compared with later one .this result may be due to the rural individuals were more contact with source of infections such as carrier animals and their dairy or meat products.

This evidence was agreement with **Giacometti *et al*.,(2012**) who recorded that 10% of systemic STEC infection was associated with consumption raw milk, also **Ijaz,(2013**) recorded that unpasteurized milk was considered essential route of STEC O157 infection,

The present finding of PCR demonstrated that the PCR assay found that all human isolates expressed (rfbO157, flicH7 genes) ,this result was given indication that all isolates were *E.coli* O157:H7 .In Busra,, **Bassam *et al*., (2012**) found that among 52 isolates of E.coli from human stools, 6 of them showed rfb0157 gene and 3 of this isolates expressed FlicH7 gene.

Also the PCR assay showed that (stx1 and stx2) genes were present in 3 isolates of human samples , for each one ,this result may be indicated that Stx genes do not present in all serotype of *E.coli* O157 H7 and this

observation was agreement with **Masoumeh *et al*.,(2012)** who found that 5 out 9 of EHEC strains expressed Stx genes also **Pina *et al*., (2010**) , analyzed by multiplex PCR , reported that (3) of *E.coli* O157:H7 strains were gave (stx1 and stx2).

The result showed that *E.coli* O157:H7 isolated from patient suffering from bloody and non-bloody diarrhea as well as urinary tracts infection ,this result may be due to damage of intestinal epithelium and renal tissue by Stx (**Niedergang *et al*.,2004; Page and Liles 2013)** ,particularly human isolates, in the present study , expressed both Stx1 and Stx2 that cause kidney failure and hemorrhagic colitis (**Karmali *et al*.,2014**).

Prevalence of E. coli O157:H7 in Cattle

A. Fecal Samples

The result demonstrated that 9(16.6%) out of 54 fecal samples were positive *E.coli* O157:H7, 17.7% of this isolates were from male fecal samples while only one isolate was get from female fecal sample this may be due to the mals were the most slaughter animals in a battoir. (Table:5)

Table (5): the prevalence level of *E.coli*O157:H7 in male and female cattle fecal samples

Gender	Samples	E.coli +ve	%	O157:H7+ve	%
Male	45	10		8	
Female	9	2		1	
Total	54	12	22.20%	9	16.60%

The results of these isolates were confirmed by PCR assay that recorded all 9 isolates were positive *E.coli* O157 and this assay found that all 9 isolates were carried (rfbO157) gene, but 8 of them were expressed (flicH7) gene as well as 5 isolates out of 9 showed (stx1) gene and 4 isolates out of 9 showed (stx2).

B. Gallbladder Mucosal Swabs

The results revealed that 3(5%) out of 59 gallbladder mucosal swabs were positive *E.coli* while 1(1.7%) out of 59 gallbladder mucosal swabs were positive *E.coli* O157:H7 (table (6).)

Table (6): Prevalence level of *E.coli*O157 in gallbladder mucosal swabs

Gender	Samples	E.coli +ve	%	O157:H7+ve	%
Male	52	3		1	
Female	7				
Total	59	3	5%	1	1.70%

This result showed that ,16.6% of examined cattle slaughtering in Missan Province harbored *E.coli* O157:H7 in their intestinal tracts without clinical symptom and this animal shaded this organisms with their feces ,this result may be indicated that cattle were important reservoir of *E.coli* O157:H7 in this region of Iraq, ,this evidence was agreement with observation of **Wang *et al*.,(1996)** who detected that healthy cattle harbored E.coliO157:H7 in their gastrointestinal tracts (GITs) and shed this organism asymptomatically in their feces .

Also in Pakistan, **Shahzad *et al*., (2013**) found that Sorbitol non-fermented *E.coli* present in (12.9%) fecal samples of cattle, that harbor E.coli157:H7 without clinical signs may due to lack of Gb3 receptor on their intestinal (**Kaper *et al*., 2004**).

PCR assay showed similarity between genes (rfbO 157,flicH7) of human and cattle bacterial isolates , this result may indicated that cattle play important source of human infection by this pathogens, through contamination of the carcasses during slaughtering the animals or through cattle products , this idea was agreement with result of **Bollinger (2004)** who showed that 146 STEC outbreaks and sporadic cases of human

disease occur due to consumption beef contaminated with *E.coli* O157:H7,also **Sima *et al*.,(2011)** found that peak prevalence of *E.coli* O157:H7 in beef cattle.

In current study, a genotypic study demonstrated no differences in virulence factors between human and cattle isolates and both of this isolates carried stx1 and stx2 genes ,this result suggested a bloody diarrhea and urinary tract infection of humans in the present study, may be due to virulence *E.coli* O157:H7 strains that zoonotically transmitted from cattle, this idea was agreement with **McNally *et al*.,(2005)**who compared 30 *E. coli* serotype O157 strains from human disease cases with similar number of strains isolated from asymptomatic cattle for production of hemolysin, EspP, Tir, and EspD ,in Scotland, and they found few genotypic difference among the two sources of the strains ,also the present investigation was supported by **Rabbi *et al*.,(2013)** who mentioned that Shiga toxin producing *E. coli* (STEC) , food-borne pathogens, induced hemolytic colitis and a serious sequel, HUS.

The isolation of *E.coli* O157:H7 from gallbladder of cattle may be indicated that this pathogen may avoid host defense mechanism through resident in this organs and the gallbladder may be considered a site for persistence and continuous shedding of *E.coli* O157:H7,this evidence was supported by result of **Ertas *et al*.,(2003)** who said that the gallbladder was considered a site of persistence for fecal shedding of certain enteric bacteria also the present result was in consistent with results of **Stoffregen *et al*.,(2004)** who demonstrated *E.coli* O157:H7 in gallbladder post experimental infection of the calves and result of **Jeong *et al*.,(2006)** who isolated this pathogen from gallbladder of cattle.

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