PCR Detection of Intimin Gene in Shiga-Like Toxin Producing *Escherichia Coli* Isolated from Animal Carcasses

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Abstract

The Shiga- like toxin-producing *Escherichia coli* (STEC) is important group of food-borne pathogens. These bacteria can cause severe health problems in humans like diarrhea, hemorrhagic colitis and hemolytic uremic syndrome. Cattle, sheep and goat are thought to be a reservoir for *E. coli (*STEC). Number of samples collected 117 and 94 animals carcasses swaps (cattle and sheep) respectively.

Total number n of sorbitol fermented *E coli* 85 (40.284%) out of 211 samples from cattle and sheep. Sheep carcasses has the higher rate (50%). 17 samples (20%) STEC out of 85 NSREC isolates were identified. Cattle carcass has the higher rate (28.94%).Total 17 of STEC samples tested by serological latex test, 14 (6.635% was serotypes O157 and O157:H7 STEC. All 14 isolates tested with PCR technique were - ve for the presence of intemin gene (eaeA) that responsible to attachment of bacteria to intestinal cells.

Introduction

The Shiga toxin-producing *Escherichia coli* (STEC) of the O157 serotype is the cause of a serious human alimentary infection associated with haemorrhagic or watery diarrhoea which may, particularly in children, be complicated by life threatening haemolytic uremic syndrome[(1)]. *Escherichia coli* O157:H7 is a foodborne pathogen that has been associated with meat-, produce-, and water-related disease outbreaks [(2, 3, 4)]. Shiga toxin-producing *Escherichia coli*(STEC) is one of the most important group of foodborne pathogens which are responsible for many serious diseases. STEC, with their most common serotype O157:H7 possess several virulence factors - among them the most important is Shiga toxin (Stx) existing in two main variants: Stx1 and Stx2[(5)]. Shiga like toxin produced by STEC are very similar to those produced by *Shigella dysenteriae* type 1and those also known as Shiga-like toxins (SLTs). SLT1 and SLT2 are different proteins and encoded by different sets of genes, but their active molecular structure and biological functions are similar. Verotoxins inactivate ribosomal RNA, inhibit protein synthesis and eventually result in the host cell's death [(6)]. It therefore seems reasonable to assume that any food contaminated with Shiga-like toxin *E. coli*, which possess accessory virulent factors, could be at risk for public health. In addition to toxin production, another virulence-associated factor expressed by STEC is a protein called intimin(*eaeA*), which is responsible for intimate attachment of STEC to the intestinal epithelial cells, causing attaching and effacing (A/E) lesions in the intestinal mucosa [(7)].

Several epidemiological studies have revealed that O157 VTEC strains are prevalent in the gastrointestinal tract of healthy cattle, and therefore cattle are regarded as the principal reservoir of these pathogens[(8)]. Polymerase chain reaction

(PCR) offers the great possibility for the detection and molecular typing of food-borne pathogens, farm ruminants such as cattle and sheep are major reservoirs of the bacteria and can excrete them in their feces[9]. At present, there is limited information on the prevalence of O157 STEC in meat. Also, there is limited information on the prevalence of O157 STEC in live chickens [10].
The aim of this study was to carry out a health status monitoring in order to evaluate the presence of STEC in animal carcasses.

Materials and methods

I. Collection of Sampling:

The samples were collected according to method described in [11] 117, 94, swabs samples were collected from cattle and sheep respectively , have been rubbed to an area of 25cm² in three different area at high risk contamination (neck, shoulder and abdomen) aseptically placed in to modified trepticase- soy broth (mTSB).

II. Isolation : After enrichment period of samples a loopfull of materials from the mTSB broth was streaked on the surface of sorbitol MacConkey agar which composed of 1% sorbitol instead of lactose in standard MacConkey agar, this medium also supplemented with cefixime (0.05mg/1^{-1}), potassium tellurite (2.5 mg/1^{-1}) and rhamnose (5 g/1^{-1}). Colorless colonies transferred onto eosin methlene blue (EMB) and MacConkey agar incubated for additional overnight to identify lactose fermentation and metallic sheen.

III.Identification of bacterial isolates

Primary identification:STEC isolate were identifical accently to morphological and biochemical characterization that mention that including

1. Gram stain, 2.Oxidase test [12], 3.Catalase test [13].

Biochemical confirmation

Classical biochemical confirmation

Triple sugar iron agar [13], Indol test [13].Uriase test [12].Simmon's citrate test [13].

Specific Biochemical confirmation

Cellobiose fermentation test [12], Potassium cyanide test (KCN) [12].

IIII.Serological confirmation

By used latex agglutination kit. Colonies that exhibited positive reaction to non sorbitol fermented *E. coli* andon biochemical testing were further tested by a rapid latex agglutination test using (Wellcolex E.coli O157:H7/Remel) according to the manufacturer's instruction.

IV.Polymerase chain reaction

The *eae* gene was studied according to [14]. Genomic DNA was amplified by using the primers displayed in table 1.
PCR reaction and condition are displayed in tables 2 and 3.

Results

Table (1): Primer sequence that used for PCR detection of *eae.*

eae	Primer sequence (5´-3´)	Product size(bases)	Reference
Forward Reveres	GTGGCGAATACTGGCGAGACT CCCCATTCTTTTTCACCGTCG	890	(14)

Table (2): The PCR condition for *eaeA*

Stage	Setps	Temperature	Time	No. of cycles
First	Denaturation 1	94c˚	3minuts	1
Second	Denaturation 2	95c˚	20 se	35
	Annealing	58c˚	40se	
	Extension1	72c˚	90se	
Third	Extension2	72c˚	5min	1

Table (3): The reaction mixture (25 µl) for *eae*

DNA templates	2.5 µl
Mastermix	12.5 µl
Primer forward	1µl
Primer reverse	1 µl
DW	8 µl

The frequency percent of non-sorbitol fermentation E.coli(NSF *C. coli*) was in the cattle, sheep (38.47, 50) respectively based on fermentation and biochemical test.

Table (4): Frequency of NSF *E.coli* isolates in cattle and sheep.

Isolates sources	Total	Nonsorbitol fermenter *E .coli*	%	Another bacteria NSF	%
Cattle	117	38	.4732	79	67.53
Sheep	94	47	50	47	50
Total	211	85	40.284	126	59.716
				X^2=6.652	P< 0.01

Proportion Shiga toxin producing *E. coli*. isolates

The frequency percent of Shiga toxin producing *E. coli* was in the cattle, sheep. (28.94, 12.76) respectively based on non-sorbitol fermentation and biochemical test.

Table (5): Frequency of non -sorbitol fermented STEC isolates, cattle, Sheep.

Isolates Sources	Non sorbitol fermented	STEC	%	*E. coli* Spp.+NSF Bacteria	%
Cattle	38		28.94	106	90.6
Sheep	47	6	12.76	88	93.6
Total	85	17	20	194	91.942
X^2= 0.641 P<0.05					

Proportion of *E.coli* O157:H7 and O157 isolates

Serological examination for NSF E.coli isolates revealed that the 6.8 %, 6.4% of isolates in cattle, sheep respectively were STEC (O157:H7 and O157. Results in diagnosis of STEC depend on serological tests in cattle, sheep,

Table (6): Number and percentage of (O157:H7 and O`57) isolates.

Isolates sources	Total	O157 and O157:H7 serology			
		No. positive	%	No. negative	%
Cattle	117	8	6.8	109	93.2
Sheep	94	6	6.4	88	93.6
Total	211	14	6.635	197	93365
X^2= 0.017 P<0.05					

Polymerase chain reaction technique (PCR)

A- DNA-extraction and purification

The DNA of all isolates (17) that suspected *E. coli* O157 and O157:H7 from biochemical and serology tests was extracted and purificated by using DNA extraction and purification kit. The product were analyzed on 1 % agarose gel containing ethidium bromide,visualized under UV transillumination, the DNA appear as fluorescent band.

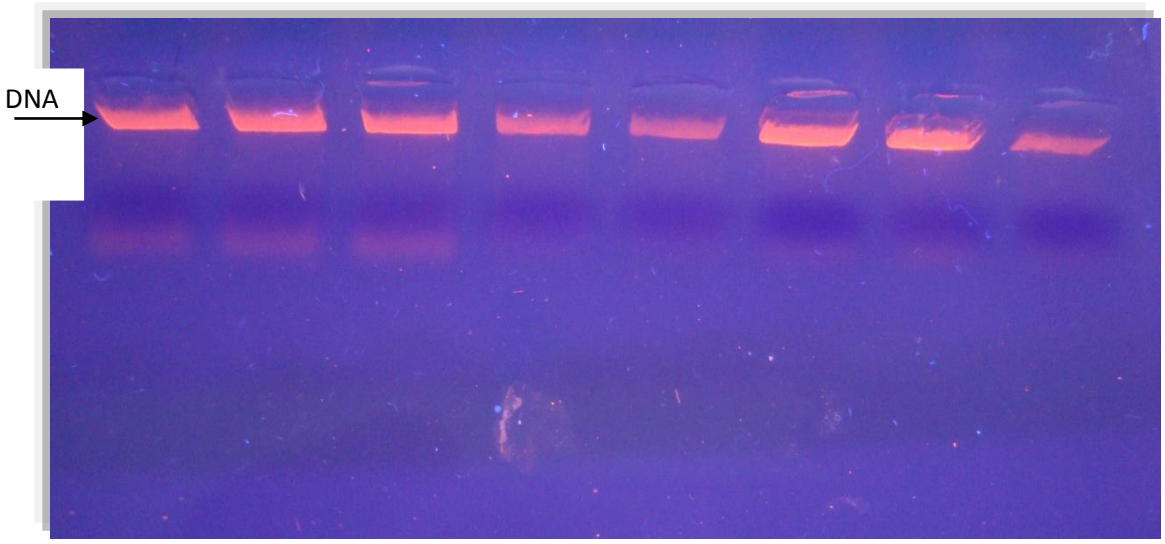

Figure (1): Total genomic DNA extracted from isolates using 1% agarose gel electrophoresis.

B- DNA amplification

The amplification of bacterial DNA was performed in 25 µl volumes, the total DNA of all isolates inter PCR running in specific program for *eaeA* gen ,the primers that used in PCR for detected characteristic virulence factors (*eaeA*) in the VTEC .this primers binding with specific sequence for *eaeA* gene that responsible on production intimine . The amplification product analyzed on (1,4)% agarose gel containing ethidium bromide, successful binding appear as fluorescent band , this band compared with DNA marker .if the amplification product for *eaeA* gene positive appear at level 890 bp when compared with DNA marker. All isolates from cattle and sheep not give PCR positive product for *eaeA* gen in this study see fig. 2.

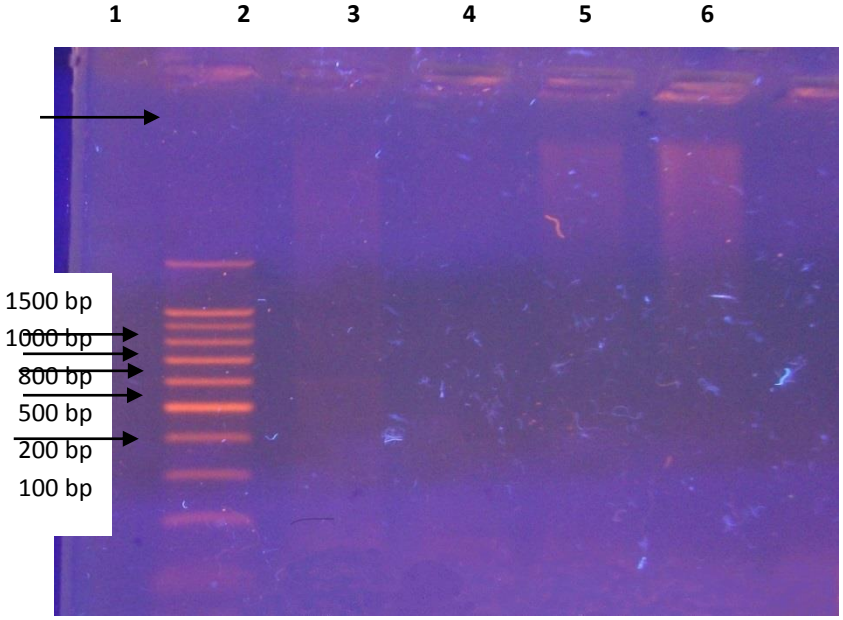

Figure 2: Polymerase chain reaction (PCR) amplification of eae gen 890 bp fragment of STEC, O157 and O157:H7, line 2, 3, 4,5,6 negative specimens, and line 1 (100 bp ladder).

Discussion

Contaminated ground beef and other bovine products are particularly important in transmitting Shiga toxin producing *E. coli* since 1982; more than 100 outbreaks of Enter hemorrhagic *E. coli* O157 have been documented. Of these outbreaks, 52% have been attributed or linked to foods derived from cattle [(15)].

In present study collected sample from cattle and sheep the numbers of each sample (117, 94) respectively. The frequency percent of non sorbitol fermentation *E .coli* was in the cattle, sheep (32.47% and in cattle 50%) respectively in cattle, Iraqi study [(16)] who found that 21.88% isolates were NSF *E. coli*. Another study [(17)] reported lower isolation rate in comparison with the present study which was reported in this study the isolation was 7 %. In sheep the results in present study (50%) was very closely from another study on sheep meat in India reported the isolation rate (49%) [(18)].

Shiga toxin-producing *E. coli* (STEC) is now a major cause of food-borne disease, mostly in the United States, Canada, Japan and Europe [(19)].In present study the frequency percent of non sorbitol fermentation (O157andO157:H7) was in cattle, sheep (6.85% , 6,4%) respectively based on fermentation and biochemical and serological tests .

In present study found STEC O157:H7and O157 isolates in the sheep carcasses a rate lower than that found in cattle. .At this result similar resultes that reported by [(20)] among meat samples examined, the highest prevalence (8%) was recorded in beef, followed by lamb and mutton (2.5%). In the UK, a survey of 1000 sheep at a slaughterhouse found STEC O157:H7 in the feces of 22 (2%), a rate lower than that obtained from cattle (16%) at the same site. [(21)]

Total isolation rate in present study was consistent with Adem *et al* (2008) who reported that the total isolation rate O157:H7 in cattle, sheep meat samples was (31from738).

The overall variations in the prevalence of *E. coli O157:H7 and O157* may be a result of different sampling techniques employed and laboratory methodologies used, and might also be due to the reason that the studies were conducted in different countries at different times.

Depending on PCR technique the study show all isolate not contain *eae* gene that responsible on attachment of bacteria on the lumen intestine.While the same isolates (7 out of 14) was PCR positive for shiga toxin one(*STX1)* and shiga toxin two(*STX2*) genes in other study. [(22)]

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الخلاصة

تعتبر جرثومة الاشركيا القولونية المنتجة للذيفان المشابه للذيفان المنتج من قبل جرثومة الشكلا من أهم المسببات المرضية المحمولة بالغذاء. يمكن انْ تسبب مشاكل الصحة الحادّةِ في البشر مثل الإسهالِ، التهاب القولون النزفي ومتلازمة البول الدموي وتعتبر الأبقار و الأغنام خازن لجرثومة. جمعت 117 مسحة من ذبائح الأبقار و94 مسحة من ذبائح الأغنام .اظهرت النتائج إن عدد الشريكا القولونية غير المخمرة لسكر السوبتول 85 بنسبة 40.284% من مجموع 211 عينة, حيث سجلت اعلى نسبة في ذبائح الاعنام 50%. ومن مجموع 85 عزلة كانت غير مخمرة للسوبتول حدد 17 عزلة بنسبة 20% كانت منتجة لذيفان الشيكا , حيث اظهرت ذبائح الأبقار اعلي نسبة 28.94.جميع العزلات ا لمنتجة لذيفان الشيكا(17) تم إختبارها بفحص التلازن حيث وجد ان 14عزلة (6.635%) منتجة لذيفان الشيكا وضمن الانواع المصلية O157, O157: H7 ، وبتقنية تضاعف سلسلة الدنا تم اختبار 14 عزلة للكشف عن وجود الجين المسؤول عن التصاق البكتريا بخلايا الامعاء العزلات واظهر الكشف كانت لا تحمل ذلك الجين.