Detection of *E. coli* O157: H7 from Local Beef Meat Products and Poultry Meat Products in Duhok Province Markets using Conventional Culture and PCR

Summary: The study included detection of *Escherichia coli* o157: H7 in local beef and poultry meats represented with minced meat, beef burger and kebab for beef while the whole chicken, chicken burger, drum stick, breast and wings represented poultry meat samples, during the period from November 2011 till June 2012. Detection of these organisms depends on culture characteristic of colonies and biochemical tests, as well as using of polymerase reaction assay (PCR) as recent technique for confirmation. The serotyping depends on the presence of the gens *rfbO*157 and *flicH*7. Out of the total 100 sample, *E. coli* was isolated *rfbO*157 gene in 1 sample (2%) of local poultry meat while beef samples was negative for this gene. Also we detected *flicH*7 gene in 2 samples (4%) in imported beef whereas poultry meat revealed this gene (2%). DNA concentration of *E. coli* strains isolated from beef and poultry meat was between 67.7 ng/μl and 53.8 ng/μl respectively. We attained specific duplicated band with molecular weight 625 bp for *flicH*7 gene while *rfbO*157 gene revealed 259 bp.

Introduction

Meat has traditionally been viewed as a vehicle for a significant proportion of human foodborne disease. Although the spectrum of meat – borne disease of public health importance has changed with changing production and processing systems, continuation of the problem has been well illustrated in recent years by human surveillance studies of specific meat-borne pathogens such as *Escherichia coli*, *Salmonella* spp., *Campylobacter* spp. and *Yersinia enterocolitica*. In addition to existing biological, chemical and physical hazards, new hazards are also appearing [18]. *Escherichia coli* O157: H7 is an important human pathogen causing haemorrhagic colitis, haemolyti c-uraemic syndrome and thrombotic thrombocytopenic purpura [10,19]. *E. coli* O157: H7 serotypes are identified as enterohaemorrhagic *E. coli* and categorized as verotoxin-producing *E. coli* [11]. Verotoxin is also known as shiga-like toxin [3]. Cattle, especially the young ones, have been implicated as a principal reservoir of *E. coli* O157: H7 [17,20,15]. Cattle frequently excrete these bacteria in their faeces [16,9]. The illness is often linked to the consumption of contaminated and undercooked beef meat. Shiga toxin-producing *E. coli* (STEC) is now a major cause of food-borne disease, mostly in the United States, Canada, Japan and Europe [5,10]. The objective of the present study was to isolate *E. coli* O157: H7 from our local Meat products samples by conventional culture method and confirm it by a serogroup-specific PCR assay in Duhok province.

Materials and methods

A hundred samples were collected from different markets in Duhok province, fifty samples of meat products are beef and fifty samples of poultry meat products, in the period between Nov. 2011 and March 2012.

Culture conditions

Meat samples (10 mg each) were enriched in 90 ml of modified Tryptic Soy broth (mTSB) both supplemented with novobiocin (Oxoid, UK) at 37℃ for 18 hours. The broth cultures were spread plated onto MacConkey agar (LABM, UK) 37℃ for 18 – 24 hours for isolation of Enterobacteriaceae. Then sub cultured on Eosin Methylene Blue agar(LABM,UK) as selective media for 18 – 24 hour at 37℃, the colonies appear in Green Metallic sheen color in presence of *Escherichia coli*, followed by sub culturing on Hemorrhagic colitis agar (HC) (Sifin, Germany)[21].

PCR assay

The PCR assay has been carried out in Duhok research center in, Faculty of Vet. Medicine, Duhok university. NSF colonies on HC agar that had been confirmed as *E. coli* employed as templates for PCR assay. A whole-cell suspension was prepared by suspending a NSF bacterial colony from HC agar in sterile distilled water. The Bacterial DNA was extracted using Genomic DNA purification kit (Fernentas, Germany). 2 μl of the supernatant was used as template for amplification by PCR. The presence or absence of *flic*H7 gene encoding the flagella antigen H7 and *rfb*O157 gene

encoding the somatic antigen O157 [2, 12] were examined.

Table 1 Describes oligonucleotide sequence of primers used in the PCR reaction mixture

Target gene	Primer sequence (name)	Product size (bp)	Thermocycling programme
*rfb*O157	F: 5′-CGG ACA TCC ATG TGA TAT GG-3′ R: 5′-TTG CCT ATG TAC AGC TAA TCC-3′	259	94℃ for 5 min;94℃ for 60 s, 56℃ for 30 s, 72℃ for 60 s, 35 cycles; 72℃ for 10 min[7]
*flic*H7	F: 5′-GCG CTG TCG AGT TCT ATC GAG-3′ R: 5′-CAA CGG TGA CTT TAT CGC CAT TCC-3′	625	

The PCR reaction was performed in a 25 μl amplification mixture consisting of 12.5 μl master mix (Cinnagen), 8.5 μl of water and 1 μl (10 pmol) of each primer and 2 μl of template DNA. The thermocycler program was started with an initial incubation at 94℃ for five min, followed by 35 cycles of denaturation at 94℃ for 60 sec, annealing at 56℃ for 30 sec and elongation at 72℃ for 60 sec, and a final extension at 72℃ for 10 min. The PCR products were separated by electrophoresis on 1% agarose gel at 100 V for 40 min in TBE buffer, visualized by ethidium bromide staining, illuminated by UV-transilluminator and documented by a gel documentation apparatus. One-hundred bp DNA ladder was used as a size reference for PCR assay.

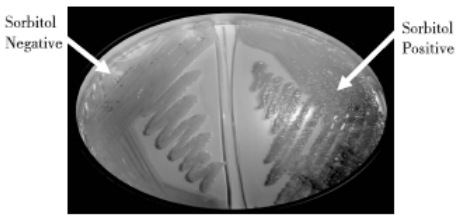
Result and discussion

The overall percentage of the Bacterial isolation had reached 1% of *Escherichia coli* O157: H7.

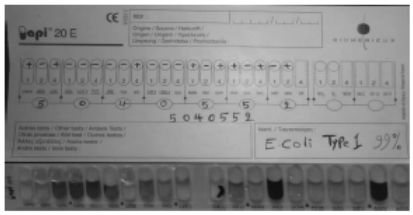
Table 2 Bacterial isolation rate from different source as shown down

Type of product	No.	Incidence %		
		Conventional	PCR	
			rfbO157	*flicH7*
Beef meat products	50	40% (20)	0% (0)	4% (2)
poultry meat products	50	16% (8)	2% (1)	2% (1)
Total incidence	100	28% (28)	1% (1)	3% (3)

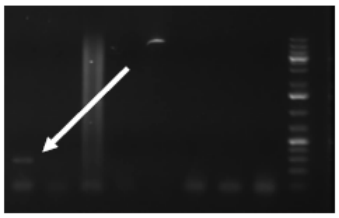
The overall percentage of bacterial isolation of *Escherichia coli* O157: H7 was 3%. Although *Escherichia coli* is part of the normal large-bowel flora of humans and animals. Most strains of *E. coli* are non-pathogenic in the intestine, some can produce diarrhea and by a number of distinct mechanisms. *E. coli* is usually considered to be an opportunistic pathogen which constitutes a large portion of the normal intestinal flora of humans. This organism can, however, contaminate, colonize, and subsequently cause infection of extra-intestinal sites and is a major cause of septicemia, peritonitis, abscesses, meningitis, and urinary tract infections in humans [1]. Although consider opportunistic pathogens, these organisms produce significant virulence factor such as endotoxins that can mediate final fatal infections. However, because they generally do not initiate disease in health, uncompromised human hosts, they are considering opportunistic. Additionally, in the case of Enterohaemorrhagic *E. coli* (EHEC), life threatening system disease can result from infection. Furthermore as the leading cause of nosocomial infections among enterobacteriaceae *E. coli* is likely to have greater virulence capabilities than the other species categorized as "opportunistic" Enterobacteriaceae [4]. In an earlier study, STEC O157 was isolated from 3.7% of retail beef and 1.5% of pork samples in the United States and Canada [3]. Although most sporadic cases and outbreaks have been reported from developed countries, human infections associated with STEC strains have also been described in Latin American countries, including Argentina, Chile and Brazil [10, 6]. It has also been reported from Kenya, Turkey and Iraq [13, 14]. We found that 28% of the samples were contaminated with the suspected *E. coli* O157 : H7 using conventional method and only 3% in using PCR, as it sensitive technique and more accurate. Our results suggested that cattle could be a reservoir of *E. coli* O157: H7 in Iraq, like many other countries and the products are contaminated during processing. [17, 20, 15]. Flagellar and somatic antigens can be detected by immunological assays. The main advantage of the employed PCR method is its ability to detect rough isolates or the isolates having a masked O antigen [2]. It has been described that the HC agar (Fluorocult HC Merck) The agar plates are clear and purple. Sorbitol fermentation and the pH-indicator, bromocresol purple, are used to detect sorbitol positive colonies yellow in colour. and MUG is split by the b-D-glucuronidase positive bacteria blue fluorescence under UV light 365 nm.. On HC Agar *E. coli* O157 : H7 colonies are colorless and show no fluorescence [8].



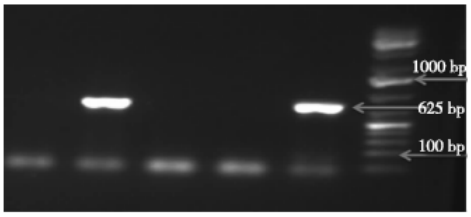
The picture shows the HC agar contain sorbitol negative *E. coli* and Sorbitol



The Picture above shows the positive result of *Escherichia coli* on API 20 E kit



Results of the PCR assay, amplifying 259 bp segment of rfbO157 of *E. coli* O157:H7. Lane 9 shows the sample.



Results of the PCR assay, amplifying 625 bp of flicH7 segment of *E. coli* O157:H7. Lane 1 and lane 2 shows the sample.

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