Direct Detection of pathogenic *Escherichia coli* from chicken meats by Real-Time PCR

Mohammed Abd Alabbas Molaghi

Department of public health, college of veterinary medicine, Al-Qadisiya University, Iraq

Abstract

Pathogenic *Escherichia coli* is one of major important Food-borne pathogens that cause of illness and in some time death in developing countries due to infectious gastrointestinal diseases by consumption of contaminated chicken meats. The objective of this study was goal to detection food-borne pathogens *E coli* from meat chicken samples obtained from different markets in Al-Diwanyia city. A total of 50 meat chicken samples were subjected to DNA extraction by using (AccuPrep® DNA Extraction Kit) than the extracted DNA subjected to Real-Time PCR technique to detection Pathogenic *E coli* based shiga toxin 1 (stx1) which consider important virulence factor gene. The results showed a high degree of contamination in meat chicken samples in different market in Al-Diwanyia city. Pathogens *E. coli* was appeared (16/50) at (32%) carried shiga toxin 1 (stx1) as positive samples. In conclusion, we conclude that contaminated chicken meat by Pathogenic *E coli* is causes potential problems for public health, and used the Real-Time technique was shown high specific and rapid method in direct detection of (stx1) gene in Pathogenic *E.coli*.

……

Keywords: *E.coli***,** shiga toxin, Real-Time PCR**,** chicken meats

Introduction

Escherichia coli O157:H7 is an important food-borne microorganism causing diarrhea, hemorrhagic colitis and serious infections such as hemolytic uremic syndrome (HUS) particularly in children and immune-compromised individuals [(1).] Cross contamination of meats and other types of foods during processing, handling, marketing and inadequate cooking all contribute to food-borne infections caused by *E. coli* O157:H7 [(2).] Infections caused by Shiga Toxigenic *E. coli* (STEC) are so severe that it is important and essential to detect the infections at the onset and adopt necessary preventive measures. For many years, laboratory detection of food–borne pathogens has relied on direct isolation, which is still the preferred method, as it represents the gold standard as compared to newly developed rapid detection methods. However several methods available for rapid detection are discussed that shortens the detection time and initiate therapeutic measures [(3).]

The detection of pathogens in food is an important component of any integrated program to ensure the safety of foods throughout the food supply chain. Microbiological analysis for each food type was used to monitor the state of contamination at all times and analyze its trends so as to detect emerging risks. These analyses are based on the detection of microorganisms by visual, biochemical, immunological, or genetic means, either before enrichment (quantitative methods) or after enrichment (qualitative methods, also known as presence/absence tests [(4)]

The real-time PCR assay is a rapid, specific and sensitive method for determination of *E. coli* O157:H7 concentrations in beef and chicken meat samples. The use of rapid detection methods such as real-time PCR has a great potential for the detection of *E. coli* O157:H7 in food with respect to the monitoring of food hygiene and it does not require post-PCR procedures to detect amplification products as in conventional PCR-based methods [(5,6).]

The testing methodology must be as rapid as possible. The tests must also have a low rate of false positive results so that wholesome product is not needlessly discarded. False-negative results must be avoided to prevent the release of contaminated products that have the potential to cause diseases [(7).]

Materials and Methods

Meat samples collection: 50 chicken meat samples from different market in Diwanyia city were collected in 25ml sterile containers transported into laboratory and stored in a refrigerator until use for genomic DNA extraction.

Genomic DNA extraction: Bacterial genomic DNA was extracted from meat tissue samples by using (AccuPrep® DNA Extraction Kit. Bioneer. Korea). 200 mg meat tissue sample was placed in 1.5 ml microcentrifuge tube and 20 ul 10 mg/ml Proteinase K and 200ul tissue lysis buffer was added and mixed by vortex, then incubated at 60°C for 60 minutes. Then the tubes transferred in to centrifuge at 10000rpm for 5 min, after that, the supernatant was transferred in to new 1.5ml microcentrifuge tube and genomic DNA extraction was done according to company instruction. After that, the extracted DNA was checked by Nanodrop spectrophotometer, then store in -20°C at refrigerator until perform PCR assay.

Real-Time PCR

Real-Time PCR technique was performed by using qPCR Syber green dye kit for detection and amplification of (shiga toxin 1 gene) virulence factors gene in *E coli*. The primes were designed in this study by using NCBI-GenBank recorded sequence for *E coli* partial stx1 gene for shiga toxin 1, strain EHEC FE94076 GenBank: (:FR875155.1) and by using primer3 plus design online. The primers were provided by (Bioneer Company. Korea) as show in the following table:

Primer	Sequence		Amplicon
Stx1	F	TGCGGTTACATTGTCTGGTG	87bp
	R	TGGCGATTTATCTGCATCCC	

The Real-Time PCR amplification reaction was done by using (AccuPower™ 2X Green star qPCR master mix kit, Bioneer. Korea) and the qPCR master mix were prepared for each sample according to company instruction as following table:

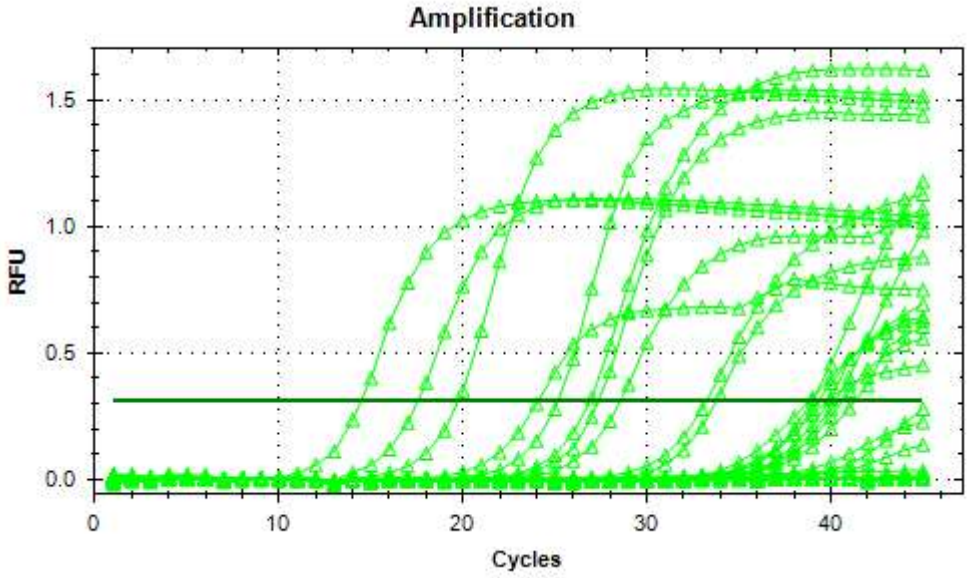
qPCR master mix	Volume
Genomic DNA template	2.5µL
2X Green star master mix	25µL
Stx1 gene Forward primer (10pmol)	1µL
Stx1 gene Reverse primer (10pmol)	1µL
DEPC water	20.5µL
Total volume	50µL

These qPCR master mix reaction components that mentioned in table was placed in sterile white qPCR strip tubes and transferred into Exispin vortex centrifuge for 3minutes, the place in MiniOpticon Real-Time PCR system and applied the following thermocycler conditions as the following table:

qPCR step	Temperature	Time	Repeat cycle
Initial Denaturation	95 °C	3 minute	1
Denaturation	95 °C	10 sec	45
Annealing\ Extension	57.2 °C	30 sec	
Detection(scan)			
Melting	60-95°C	0.5 sec	1

Results:

Real-Time PCR technique based SYBR Green dye for detection of Shiga toxin-producing *E coli* (STEC) were show in (16/50) at (32%) positive samples by amplification of stx1 gene in extracted DNA from meat chicken samples as shown in Figures 1,2.

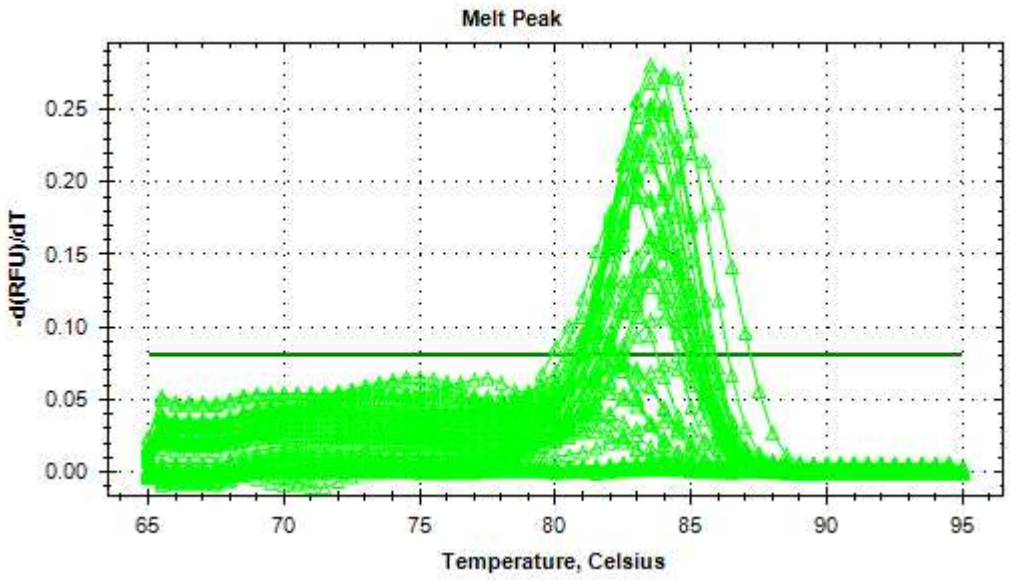


(Fig. 1): Real-Time PCR amplification plots for stx1 gene that show the positive samples at CT:13 to CT:39 cycle. The samples with amplification at 13 cycles contained very large amount of DNA while the samples with the amplification appeared at 39 cycles contained lower quantity of DNA for *E. coli*.

Well	Fluor	Sample	End RFU	Call
A02	SYBR	chicken meat	0.420	(+) Positive
A03	SYBR	chicken meat	0.469	(+) Positive
A05	SYBR	chicken meat	1.00	(+) Positive
B05	SYBR	chicken meat	1.05	(+) Positive
C03	SYBR	chicken meat	0.702	(+) Positive
C05	SYBR	chicken meat	0.864	(+) Positive
D02	SYBR	chicken meat	0.474	(+) Positive
D05	SYBR	chicken meat	1.44	(+) Positive
E01	SYBR	chicken meat	0.575	(+) Positive
E04	SYBR	chicken meat	1.62	(+) Positive
E05	SYBR	chicken meat	1.02	(+) Positive
F01	SYBR	chicken meat	0.757	(+) Positive
F05	SYBR	chicken meat	1.50	(+) Positive
G02	SYBR	chicken meat	1.08	(+) Positive
G03	SYBR	chicken meat	0.565	(+) Positive
H03	SYBR	chicken meat	0.595	(+) Positive
A01	SYBR	chicken meat	0.00688	
A06	SYBR	chicken meat	0.00639	
B01	SYBR	chicken meat	0.164	
B02	SYBR	chicken meat	0.171	

(Fig. 2): Real-Time PCR endpoint analysis that the some positive and negative samples.

The specificity of stx1 gene primers that amplification by Syber green based Real-Time PCR was determined by dissociation curve (Melt Curve). Where the positive amplification product samples show specific amplification at melt peak mainly at (Tm: 82C°) without primer dimer or nonspecific products. (Figure-3)



(Fig. 3): Real-Time PCR Melt curve that shows the melting point for *E. coli* stx1 gene ranged from 82.5°C to 85°C for all samples, and the line from the highest peak to the button was detected that the melting point at 83°C slightly range above or low which represent the specific primers amplification.

Discussion

Pathogenic *E.coli* is recognized as an important pathogen in outbreaks of acute diarrhea especially in developing countries [8]. *E coli* isolates frequently contaminate food of animal origin; in our investigation, this microorganism was recovered from 16 out of 50 (32%) tested chicken meat samples. The STEC strains are mostly commensal bacteria in animals, with a high potential for foodborne transmission to humans [9]. Our study revealed highest prevalence of STEC 1 (stx1) strains isolated from chicken meat samples. *E coli* has been isolated from wide ranges of raw foodstuffs including dairy cattle and their products [10], milk [11], beef cattle [12], meat products [13], retail fresh meats and poultry [14], and eggs [15]. Our study similar to a previous study in Iran performed on poultry meats showed that 146 of 422 poultry samples (34.59%) were *E. coli* positive [16]. Other study in Korea showed that 41 of 900 poultry samples (4.6%) were *E. coli* positive and there

was no O157 serogroup detected [17]. Previous study Czech republic showed that out of 987 samples, 22 strains (2.2%) were identified as *E. coli* O157 and only 9 poultry meat samples were positive for all *stx1*, *stx2*, *eaeA*, and *ehxA* virlence genes [18]. Along with, most studies focused on STEC strains of *E. coli* evealed that chicken meat is a major source for exposure of these strains to human population [19]. STEC strains vary in their capacity to cause serious diseases in humans or animals, and this is associated with the type or amount of *stx* produced [20]. Therefore, the type of *stx* toxin produced by STEC isolated from human infections has been extensively studied [21]. In our study we revealed that Real-Time PCR technique high specific and rapid method accurate and safe diagnostic method for detection of pathogens in meat samples and indicated that the *E. coli* virulence genes especially *stx1*is well distributed in poultry meat in investigated regions. Besides the O157 serogroup are the predominant serogroup of bacterium in chicken meat in Al-Diwanyia city in Iraq because high temperature, and this city also had the highest prevalence of *E. coli* in poultry meat samples (*E. coli* is more active in warm places). It seems that sanitation conditions, especially in poultry slaughterhouses and supermarkets, help to reduce the contamination rate of poultry meat.

References:

1-Dorn, C.R.D., Francis, H., Angrick, C.J., Willgohs, J.A., Wilson, R.A., Collins, J.E., Jenke, B.H. and Shawd, S.J. (1993). Characteristics of Vero cytotoxin producing *Escherichia coli* O157:H7 from bovine faeces. *J. Med. Microbiol*. 40: 424–427

2-Radu, S., Ling, O.W., Rusul, G., Abdul Karim, M.I., and Nishibuchi, M. (2001). Detection of *Escherichia coli* O157:H7 by multiplex PCR and their

characterization by plasmid profiling, antimicrobial resistance, RAPD and PFGE analyses. *J. Microbiol. Methods* 4:131–139.

3- Growther, L. and Andrew, N.S. (2011). Diagnostic methods for Shiga Toxin producing *E. Coli*. Indian Journal of Fundamental and Applied Life Sciences 1 (3) : 337-342

4-Lopez, C.G., Martinez J.V.S., Aguado, U.M., and Lopez, A.V. (2012). Microarray Detection and Characterization of Bacterial Foodborne pathogens. *http://www.springer.com/*978-1-4614-3249-4.

5- Jinneman, K.C., Yoshitomi, K.J. and Weagant, S.D. (2003). Multiplex real-time PCR method to identify shiga toxin genes *stx1* and *stx2* and *Escherichia coli* O157:H7/H-serotype. *Appl. Environ. Microbiol.* 69(10): 6327–6333.

6- Suria, M.S., Adlin Azlina A.K., Zamri I. , Mohd Afendy A.T., Lau H.Y., Mariana N.S. and Raha, A.R. (2012). Real-time PCR for detection of *fliC* gene of *E. coli* O157:H7 in beef and chicken meat. *J. Trop. Agric. and Fd. Sc*.40(1):81-88.

7-**Arthur,** T.M., Bosilevac, J.M., Nou, X. and Koohmaraei, M. (2005). Evaluation of culture and PCR-based detection methods for *Escherichia coli* O157:H7 in inoculated ground beef. *J. Food Prot*. 68(8): 1566–1574.

8-EI-Rami, F. E., Elias, A. R. Fawwak, T. S. and M. A. Alexander. (2012) Identification of virulence genes among antibacterial resistance *Escherichia coli* isolated from poultry," *Advanced Studies in Biology*, vol. 4, no. 8: 385–396.

9- Caprioli, A., S. Morabito, H. Brugere, and E. Oswald.(2005). Enterohaemorrhagic *Escherichia coli*: Emerging issues on virulence and modes of transmission. Vet. Res. 36:289–311.

10- Hussein, H. S., and T. Sakuma. (2005). Prevalence of Shigatoxin producing *Escherichia coli* in dairy cattle and their products. J. Dairy Sci. 88:450–465.

11-Burk, C., I. G. Braumiller, H. Becker, and E. Martlbauer. (2002). Nuclease fluorescence assay for the detection of verotoxin genes in raw milk. Lett. Appl. Microbiol. 35:153–156.

12-Galland, J. C., D. R. Hyatt, S. S. Crupper, and D. W. Acheson. (2001). Prevalence, antibiotic susceptibility, and diversity of *Escherichia coli* O157:H7 isolates from a longitudinal study of beef cattle feedlots. Appl. Environ. Microbiol. 67:1619–1627.

13-Chapman, P., Chapman, A., Cerdan Malo, A.T., Ellin, M., Ashton, R. and Harkin, M.A. (2001) Escherichia coli O157 in cattle and sheep at slaughter, on beef and lamb carcasses and in raw beef in raw beef and lamb products in South Yorkshire, UK Int. J. Food Microbiol., 64:139-150.

14-Doyle, M. P., and J. L. Schoeni. (1987). Isolation of *Escherichia coli* O157:H7 from retail fresh meats and poultry. Appl. Environ. Microbiol. 53:2394–2396.

15-Chiueh, L. C., W. H. Shiang, and D. Y. C. Shih. (2001). Characterization of *Escherichia coli* serotype O157 strains isolated in Taiwan by PCR and multilocus enzyme analysis. J. Food Drug Analysis 9:12–19.

16- Momtaz, H, and A. Jamshidi. (2013). Shiga toxin-producing *Escherichia coli* isolated from chicken meat in Iran: Serogroups, virulence factors, and antimicrobial resistance properties. Poultry Science 92:1305–1313.

17-Lee, G. ,**Jang,**Y., H. I., I. G. Hwang, and M. S. Rhee. (2009). Prevalence and classification of pathogenic *Escherichia coli* isolated from fresh beef, poultry, and pork in Korea. Int. J. Food Microbiol. 134:196–200.

18-Lukasova, J., B. Abraham, and S. Cupakova. (2004). Occurrence of *Escherichia coli* O157 in raw material and food in Czech Republic. J. Vet. Med. B Infect. Dis. Vet. Public Health 51:77–81.

19- Ghanbarpour, R., Salehi, M. and Oswald, E. (2010). Virulance genotyping of E. coli isolates from avian cellulitis in relation to phylogeny. Com. Clin. Pathol., 19: 147-153.

20-Law, D. (2000). Virulence factors of *Escherichia coli* O157 and other Shiga toxin- producing *E. coli*. J. Appl. Microbiol. 88:729–745.

21-Schmidt, H., L. Beutin, and H. Karch. (1995). Molecular analysis of the plasmid-encoded hemolysin of *Escherichia coli* O157:H7 strain EDL 933. Infect. Immun. 63:1055–1061.