Detection of Shiga toxin produced by *Escherichia coli* in poultry and meat in Luxor city using multiplex PCR

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A B S T R A C T

Shiga toxins were widely spread in the meat especially in minced meat. These toxins have an important significance to human health because it is a major cause of food poisoning. About 150 meat samples purchased from a number of supermarkets and butcher shops in Luxor city were examined for presence of *E. coli* (50 raw meat samples - 50 minced meat samples - 50 sample of chicken meat). 62 samples were positive for *E.coli* spp. 26 isolates were confirmed serologically using O &H specific antisera as *E.coli*. Incidence of *E. coli* was in chicken meat 6/50 (12%) and raw meat 11/50 (22%) and minced meat 9/50 (18%). 26 *E. coli* isolates tested serology using special antisera (O & H) recognize that there are 12 genetic groups They are O26: H11, O114: H21, O119: H4, O2: H6, O125: H21 in chicken meat. O111: H2, O55: H7, O125: H21, O128: H2, O26: H11, O124 in raw meat. In minced meat O128: H2, O119: H4, O44: H18, O26: H11, O111: H2, O78, O55: H7. *E.coli* O111,O26 and O119 the most prevalent serotype. Polymerase chain reaction was used to detect virulence genes in isolated strains *stx*1, *stx*2, *eae*A.

Keywords: *E. coli*, *stx*1, *stx*2, *eae*A.

1. INTRODUCTION

Escherichia coli is a normal inhabitant of the intestinal tract of humans and warm-blooded animals. Its presence in raw foods is considered an indication of direct or indirect fecal contamination. Thus, it is used as an indicator organism for possible presence of enteric pathogens in food and water (Cohen et al., 2007). It is an important organism in the food microbiology; besides being involved in food borne gastroenteritis, it is considered a good indicator of possible faecal contamination as this species normally live in the intestines of humans and animals (ICMSF, 1982). *E. coli* may contaminate foods in a variety ways, including bowel rupture during evisceration, indirect contamination with sewage and polluted water, and handling and packaging of finished products (Schroeder et al., 2004). Meats are a common source of *E. coli* contamination, which may be acquired during slaughter through fecal contact (Cohen et al., 2007). *E. coli* cause intestinal infections such as diarrhoea or haemorrhagic colitis, or cause extra-intestinal infections such as neonatal meningitis, nosocomial septicaemia, haemolytic uremic syndrome, urinary tract and surgical site infections (Falagas and Gorbach, 1995). Several classes of *E. coli* were recognized specifically enteroinvasive *E.coli* (EIEC), entero toxigenic *E.coli* (ETEC), Shiga like toxin-producing (STEC) or entero hemorrhagic *E.coli* (EHEC) or verotoxin producing *E.coli* (VTEC), enteroaggregative *E.coli* (EAggEC) (Nataro and Kaper, 1998).

STEC has a confirmed zoonotic origin among different groups of pathogenic *E.coli* with ruminants, especially cattle, as the major reservoir for human infections. STEC are the most devastating and a major public health concern for its association with large foodborne outbreaks and life-threatening hemolytic uremic syndrome (HUS). More than 400 different serotypes of VTEC have been isolated from humans but only few are associated with the majority of human EHEC cases (Scheutz and Strockbine, 2005). Virulence factors for non-O157 STEC include production of the shiga-like toxins 1 and/or 2 (*stx*1, *stx*2) and intimin (*eaeA*). Cattle and other ruminants appear to be the main reservoir of non-O157 STEC, as well as the O157:H7 serotype.

With carriage rates of non-O157 STEC in cattle being a public health concern, a method was devised to detect and isolate the six major non-O157 STEC serogroups (O26, O45, O103, O111, O121 and O145) in ground beef and beef trim (Arthur et al., 2002).

In the sight of these facts, the aim of this study was to record the incidence of STEC in meat samples collected from Luxor city

2. MATERIAL AND METHOD

2.1. Collection of samples:

A total of 150 samples of (50 chicken meat -50 raw meat 50 minced meat) were collected during the period from November 2014 to April 2015 from butchers, meat retailers and supermarkets in Luxor Governorate.

2.2. Isolation of E. coli

It was done according to Quinn et al. (1994).

2.3. Serotyping of E. coli Isolates

E. coli isolates were sero-grouped according to Kok et al. (1996) using rapid diagnostic E. coli antisera sets (DIFCO Laboratories, Detroit Michigan 48232-7058, USA) at Food Analysis Center, Faculty of Veterinary Medicine, Benha University, Egypt.

The serologically identified E. coli isolates were analyzed for the presence of stx1 and stx2 genes. Genomic DNA was extracted from each E. coli isolate using Bacterial DNA extraction kit (Spin-column) (BioTeke Corporation, Catalogue, DP2001) according to the manufacturer's instructions.

2.4. Polymerase chain reaction (PCR)

The confirmation of isolated strains and detection of shiga toxin1 (stx1 gene) and shiga toxin2 (stx2 gene) and eae A gene were done according to EL-Jakee et al. (2009).

Table (1) The primers used in PCR

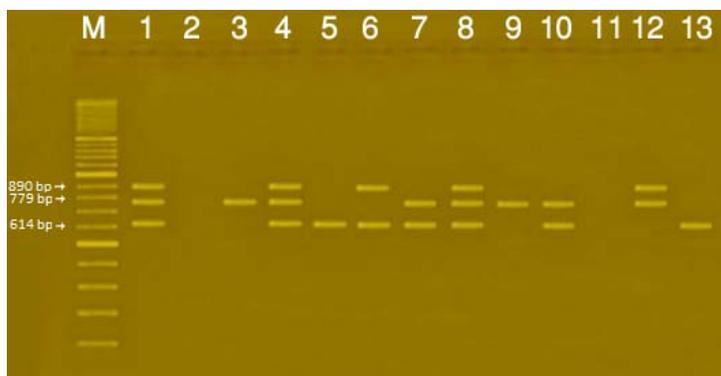| Gene	Primer Sequence 5'-3'	Amplified size (pb)	References
eaeA	GACCCGGCACAAGCATAAGC	384 bp	(EL-Jakee et al., 2009)
	CCACCTGCAGCAACAAGAGG		
stx1	ACACTGGATGATCTCAGTGG	614 bp	
	CTGAATCCCCCTCCATTATG		(Chassagne et al., 2009)
Stx2	CCATGACAACGGACAGCAGTT	779 bp	
	CCTGTCAACTGAGCAGCACTTTG		

Fig (1): Agarose gel electrophoresis of multiplex PCR of stx1 (614 bp), stx2 (779 bp) and eaeA (890 bp) genes for characterization of Enteropathogenic E.coli. Lane M: 100 bp ladder as molecular size DNA marker. Lane 1: Control positive for stx1, stx2 and eaeA genes. Lane 2: Control negative for stx1, stx2 and eaeA genes. Lanes 3 & 9 (E.coli O2 & O114): Positive strains for stx2 gene. Lanes 4 & 8 (E.coli O26 & O111): Positive strains for stx1, stx2 and eaeA genes. Lanes 5 & 12 (E.coli O44 & O128): Positive strains for stx1 gene. Lane 6 (E.coli O55): Positive strain for stx1 and eaeA genes. Lane 7 & 10 (E.coli O78 & O119): Positive strains for stx1 and stx2 genes. Lane 11 (E.coli O124): Negative strain for stx1, stx2 and eaeA genes. Lane 12 (E.coli O125): Positive strain for stx2 and eaeA genes.

3. RESULTS

E. coli is one of the most important food poisoning bacteria and has an important indication in food hygiene. *E. coli* spp. could be isolated in 62 samples form a total 150 collected samples. 26 isolates were confirmed serologically using O &H specific antisera as *E.coli* serovars. Incidence of *E. coli* was in chicken meat 6/50 (12%) and raw meat 11/50 (22%) and minced meat 9/50 (18%).

26 *E. coli* isolates tested serology using special antibodies (O & H) recognize that there are 12 genetic groups followed these 26 isolates using these antibodies. Found O26: H11, O114: H21, O119: H4, O2: H6, O125: H21 in chicken meat. O111: H2, O55: H7, O125: H21, O128: H2, O26: H11, O124 in raw meat. O128: H2, O119: H4, O44: H18, O26: H11, O111: H2, O78, O55: H7 in minced meat. serotypes O111, O26 and O119 the most prevalent serovars.

4. DISCUSSION

Six out of the 50 chicken samples investigated for the presence of *E. coli* by percentage 12%. The prevalence of *E. coli* in chicken samples 12% was nearly similar with that was reported by Momtaz et al. (2012) and Zende et al. (2013) who reported that the incidence of *E.coli* in chicken meat was 11% and 16.67 %, respectively. On the other hand, the present results were lower than those reported by Hyun-jung et al. (2015) and Nguyen et al. (2016) who reported *E.coli* presented in chicken meat with percentage 75.9% and 92.7%, respectively. These differences could be attributed to the hygienic measures proceeded in different localities under investigation and health condition of the meat handlers.

The incidence of *E.coli* in the examined meat samples was 22% (11 out of 50 examined samples). Concerning to previous work, the prevalence of the isolated *E.coli* was reported in meat samples examined by Momtaz et al. (2012) and Farhan et al. (2014) was 29%, 30%, respectively. Higher prevalence was reported by Patricia et al. (2014) and Hyun-jung et al. (2015) who isolated *E.coli* with percentage 36.1%, 42.3%, respectively. On the other hand, lower recovery rates were recorded of 12.5% by Sethulekshmi et al. (2016). The presence of *E.coli* as intestinal commensal organism in human and animal resulting from faecal contamination or contamination during food animal slaughter it is often found in soil, water and

foods (Riley et al., 1983) and this responsible for the highest result in this study.

Concerning to minced meat samples, 9 out of 50 samples with the isolation rate of 18% were recorded. It was in close agreement with the previous results of Panahee and Pourtaghi (2016) who isolated *E.coli* with percentages 23.5% respectively. Higher results were reported by Badri et al. (2009) who isolated *E.coli* with percentage 45% and 42.5%, respectively. While, lower result was reported by Wenting et al. (2012) who isolate *E.coli* by percent 5.2% in minced meat.

Serotyping is a common way to characterize STEC strains, and is based on the O antigen (somatic antigen) and H antigen (flagellar antigen) (Gyles, 2007). The most common EHEC serogroup are: O4, O5, O16, O26, O46, O48, O55, O91, O98, O111ab, O113, O117, O118, O119, O125, O126, O128, O145, O157 and O172. Recently, several new EHEC serogroup have been described: O176, O177, O178, O179, O180 and O181 (Scheutz and Strockbine, 2005). The data showed that the isolated serotypes in chicken meat were O26 (2), O114 (1), O119 (1), O2 (1) and O125 (1). O26 reported the highest serotype present. This result was nearly similar to results of Kudakwashe et al. (2013) and agree with Zende et al. (2013) in O_2 serotype only.

On the other hand, the isolated serotypes in raw meat were (3) O111, (3) O55, (2) O125, (1) O128, (1) O26 and (1) O124. Such results were nearly similar to Al-Zogibi et al. (2015) who isolated O111 but other serotypes (O157, O174, O22). While, STEC of serogroup O157, O26, O111 were not found. In addition, Bergey's manual of systematic bacteriology (2005) and Karmali et al. (2003) who reported that there are 300-400 known STEC serotypes, but not all of them have been associated with human illness. STEC can be found in soil, water, and food vehicles.

The isolated serotypes in minced meat were O128 (3), O119 (1), O44 (1), O26 (1), O111 (1), O78 (1) and O55 (1) with the highest percentage is O128 (33%). Related serotypes O55 and O111 recorded with rate 22%, 30%, respectively. While lower recovery rates were recorded 2.6% by Perelle et al. (2007) On the other hand, higher result recorded with rate 46%.

Multiplex PCR was reported to be more sensitive and accurate for determination of Shiga toxin-producing *Escherichia coli* gene in foods. Multiplex PCR reported the presence of *stx*1, *stx*2 and *eae*A genes in chicken meat samples with rate

of 33%, 83% and 33%, respectively with the incidence of Shiga toxin producing *E. coli* of 12%. Lower percent of STEC isolated by Abdul Razzaq et al. (2013) was 2 % of chicken meat. *Stx*2 percent which detected in this study (83%) was higher than that recorded by Panahee and Pourtaghi (2016) and Zende et al. (2013) which was 21%, 27% respectively. The present data showed that STEC results in raw meat 45% *Stx1*; 36% *Stx2* and 36% *eae* A. Patricia et al. (2014) reported that percent of virulence gene 5.3% *Stx*1; 86.0% *Stx*2; 26.3% *eae* A. 50% *Stx*1, 61% *Stx*2 and 9% *eae* A gene were detected in some reports. It is clear that *Stx*2 is higher than *Stx*1 but in the current study, *Stx*1 is higher than *Stx*2. Al-Zogibi et al. (2015) reported highly percent of virulence gene *Stx* of 94.12% in serotype of *E. coli* recovered from meat samples. On the other hand, *eae* A gene was detected in 58.82%. Sethulekshmi et al. (2016) found 57% *Stx*1, 57% *Stx*2 with absence of *eae* A gene. This high result of shiga toxin indicates high level of contamination. Dhanashree and Shrika (2007) isolated 40 *eae* A of 103 meat samples, the heighest percent of *eae* A gene due to decreasing of STEC strains. Hyun-jung et al. (2015) isolated 25 STEC strains from meats, five strains (20%) were positive for the *eae* A gene, 80% *Stx, Stx2*. on the other hand, Abdul Razzaq et al. (2013) isolated STEC in meat with low percent (1%) and confirmed by PCR.

5. CONCLUSION

From achieved results, the highest percentage of *E.coli* was presented in raw meat by percentage 22% and the lowest one was found in chicken 12%. The highest result of shiga-toxin producing *E.coli* was detected in raw minced meat due to exposure of minced meat to several processes. *stx*1 was found in chicken meat by percentage 33%, *stx*2 83% and *eae* A gene 33%. While in raw meat, the results were 45% *stx*1, 36% *stx*2 and 36% *eae* A gene. In minced meat, they were found 77% *stx*1, 44% *stx*2 and 33% *eae* A gene.

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