E. coli strains producing Shiga toxin in cattle carcasses at abattoir level

Saad, M. S. [1], Hassan, M. A. [1], Abou El-Roos-Nahla, A. [2] and Gaafar, M. H. [2]

[1]Department of food Hygiene, Fac. Vet. Med, Benha University, [2] Food Hygiene Dept., Animal Health Research Institute Shebin El- Kom branch.

A B S T R A C T

A total of one hundred random swab samples were collected from the cattle carcasses slaughtered at four different abattoirs located in Menufia governorate namely A, B, C and D (25 of each). The sampling site was randomly taken from each carcass inside the abattoir. The collected samples were examined for presence of *E. coli* and their virulence genes using PCR technique. Incidence of *E. coli* isolated from the examined cattle swabs samples taken from abattoir A was *E. coli* (O55: H7, O111: H2, O126 and O114: H21) 4 % for each, *E. coli* (O111: H2) was 4 % and *E. coli* (O126) 4%. While in case of abattoir B *E. coli* (O86, O124 and O126) 4 % for all and *E. coli (*O119**:** H4 and O26: H11) 8% for both. On the other hand, results of abattoir C was *E. coli* (O111: H2 8% and O26: H11 12%), *E. coli* (O86, O114: H21, O124 and O142) 4% of each. But in case of abattoir D was *E. coli* O26: H11 16%, *E. coli* (O111: H2 O55: H7 and O126) 8% for each, *E. coli* (O44: H18 and O125: H21) 4% for both**.** We found also that Shiga- toxin 1 gene ***(stx1)*** virulence gene was present in O26: H11, O44: H18, O55: H7, O111: H2, O119: H4, O126 and O128: H2. On the other hand, Shiga- toxin 2 gene ***(stx2)*** virulence gene was present in O26: H11, O44: H18, O86, O111: H2, O114: H21, O119: H4, O125: H21 and O142. While intimin gene ***(eae)*** virulence gene was present in **O**26: H11 and O111: H2. Also haemolysin gene ***(hylA)*** gene was present in O26: H11, O55: H7 and O111: H2.

Key words: Cattle carcasses**,** *E. coli***,** Virulence genes**,** Shiga- toxin

(http://www.bvmj.bu.edu.eg) **(BVMJ-32(1): 89 -93, 2017)**

1. INTRODUCTION

Animal meat supplies human with good quality protein that could be polluted with several kinds of microorganisms resulting in numerous serious food borne diseases (Komba et al., 2012). Extensive microbial contamination of beef carcass surfaces possesses a serious effect to workers during carcass preparation and hazardous effects on health of human eating that contaminated meat or its products (Heiman et al., 2015). There is a direct relationship between carcass meat microbial load and good manufacture practices done inside abattoir during carcass processing. There are several factors resulted in diversity in types of microbial contamination between different abattoirs as soil of abattoir, air, water, hide of animal, animal fecal matter, workers (and their tools used in carcass processing) and the mean of transporting carcass to meat retailers (International commission of Microbiological Specification for Foods "ICMSF", 1996). *E. coli* causes several human foodborne diseases; these diseases have different forms. The clinical signs of those diseases ranging from gastrointestinal symptoms (diarrhea to bloody diarrhea and dysentery), urinary tract complication (hemolytic uremic syndrome (HUS), pneumonia and meningitis (Johnson et al., 2006). Therefore, the current study aimed to detect *E. coli* serotypes contaminate cattle carcass at abattoir level and detect their different virulence genes by using PCR that possess hazardous effects on human health consuming this meat.

2. MATERIAL AND METHODS

2.1. Collection of samples

One hundred random swab samples were collected from surfaces of cattle carcasses slaughtered at four different abattoirs located in Menufia governorate namely A, B, C and D (25 of each). The sampling site was randomly taken from each carcass inside the abattoir; each swab sample was kept in an isolated sterile plastic bag and kept in an ice box then transported to the research facility under entire aseptic conditions without impediment for bacteriological examination. The sterile cotton swab was drawn from screw capped

plastic tube, wetted in rinsing fluid solution (buffered peptone water 0.1%), then rolled over the limited area inside the template, rolled in one direction and perpendicular to this direction to represent all the examined area. Finally, the cotton swabs were aseptically retained into the rinsing fluid screw capped tubes containing ten milliliter buffered peptone water (0.1%).

2.2. Preparation of swabs (American Public Health Association "APHA", 2001)

The collected swabs were mixed in 225ml of sterile buffered peptone water (0.1%) to give 1/10 dilution. 1ml from the original dilution was transferred with sterile pipette to another sterile test tube containing nine milliliter of buffered peptone water and mixed well to make the next dilution, from which further decimal serial dilutions were prepared. The prepared samples were subjected to the following examinations:

2.3. Screening for Enteropathogenic E. coli:

2.3.1. Pre-enrichment (International commission of Microbiological Specification for Foods "ICMSF", 1996):

Using MacConkey broth tubes supplemented with inverted Durham's tubes. Inoculated tubes were incubated at 37°C for twenty four hours.

2.3.2. Enrichment broth:

1ml from positive MacConkey tube was inoculated into another MacConkey broth tubes and incubated at 44°C for twenty four hours.

2.3.3. Plating media:

Loopfuls from positive MacConkey broth tubes were separately streaked onto Eosin Methylene Blue agar medium (E.M.B.), which was then incubated at 37°C for twenty four hours. Suspected colonies give metallic green in color. Suspected colonies were purified and inoculated into slope nutrient agar tubes for morphologically and biochemically identification.

2.4. Serotyping of E. coli.

The isolates were serologically identified according to Kok et al. (1996) by using rapid diagnostic *E. coli* antisera sets (DENKA SEIKEN Co., Japan) for diagnosis of the Enteropathogenic types.

2.5. Application of PCR

2.5.1. Primer sequences of E. coli used for PCR identification system by using primers (Pharmacia Biotech) as shown in table 1.

2.5.2. DNA Extraction using QIA amp kit (Shah et al., 2009).

2.5.3. DNA amplification: Amplification reaction of E. coli (Sipos et al., 2007).

3. RESULTS.

Results recorded in table (2) revealed that incidence of *E. coli* isolated from the examined cattle swabs samples taken from abattoir A was Enteropathogenic *E. coli* (O55: H7 and O114: H21) 4 % for each, Enterohaemorrhagic *E. coli* (O111: H2) was 4 % and Enterotoxigenic *E. coli* (O126) 4%. While in case of abattoir B Enteropathogenic *E. coli* (O86 (4 %) and O119: H4 (8%)), Enterohaemorrhagic *E. coli* (O26: H11) 8%, Enteroinvasive *E. coli* (O124) 4 % and Enterotoxigenic *E. coli* (O126) 4 %. On the other hand, results of abattoir C was Enterohaemorrhagic *E. coli* (O111: H2 8% and O26: H11 12%), Enteropathogenic *E. coli* (O86, O114: H21 and O142) 4% of each and Enteroinvasive *E. coli* (O124) 4%. But in case of abattoir D was Enterohaemorrhagic *E. coli* (O26: H11 16% and O111: H2 8%), Enteropathogenic *E. coli* (O44: H18 4% and O55: H7 8%) and Enterotoxigenic *E. coli* (O125: H21 4% and O126 8%).

Table 1: Primer sequences of *E. coli* used for PCR identification system by using primers (Pharmacia Biotech)

Primer	Oligonucleotide sequence (5′ → 3′)	Product size (bp)	References
stx1 (F)	5′ ACACTGGATGATCTCAGTGG ′3		Dhanashree and Mallya (2008)
Stx1 (R)	5′ CTGAATCCCCCTCCATTATG ′3	614	
Stx2 (F)	5′ CCATGACAACGGACAGCAGTT ′3		Dhanashree and Mallya (2008)
Stx2 (R)	5′ CCTGTCAACTGAGCAGCACTTTG ′3	779	
eaeA (F)	5′ GTGGCGAATACTGGCGAGACT ′3		Mazaheri et al. (2014)
eaeA (R)	5′ CCCCATTCTTTTTCACCGTCG ′3	890	
hylA (F)	5′ ACGATGTGGTTTATTCTGGA ′3		Fratamico et al. (1995)
hylA (R)	5′ CTTCACGTGACCATACATAT ′3	165	

Table (2): Incidence of *E. coli* isolated from the examined cattle carcass swab samples at the four tested Menufia abattoirs (n=25).

Abattoir / *E. coli* Strains	A		B		C		D		Strain characteristics
	No.	%	No.	%	No.	%	No.	%	
O26 : H11	-	-	2	8	3	12	4	16	EHEC
O44 : H18	-	-	-	-	-	-	1	4	EPEC
O55 : H7	1	4	-	-	-	-	2	8	EPEC
O86	-	-	1	4	1	4	-	-	EPEC
O111 : H2	1	4	-	-	2	8	2	8	EHEC
O114 : H21	1	4	-	-	1	4	-	-	EPEC
O119 : H4	-	-	2	8	-	-	-	-	EPEC
O124	-	-	1	4	1	4	-	-	EIEC
O125 : H21	-	-	-	-	-	-	1	4	ETEC
O126	1	4	1	4	-	-	2	8	ETEC
O142	-	-	-	-	1	4	-	-	EPEC
Total	4	16	7	28	9	36	12	48	

EPEC = Enteropathogenic *E. coli.* EIEC = Enteroinvasive *E. coli.* ETEC = Enterotoxigenic *E. coli*
EHEC = Enterohaemorrhagic *E. coli*

Table (10): Occurrence of virulence genes of Shiga toxin-producing *E. coli* isolated from the examined cattle carcass swab samples at the four tested Menufia abattoirs

E. coli Serovars	*stx1*	*stx2*	*eae*	*hylA*
O26 : H11	+	+	+	+
O44 : H18	+	+	-	-
O55 : H7	+	-	-	+
O86	-	+	-	-
O111 : H2	+	+	+	+
O114 : H21	-	+	-	-
O119 : H4	+	+	-	-
O124	-	-	-	-
O125 : H21	-	+	-	-
O126	+	-	-	-
O128 : H2	+	-	-	-
O142	-	+	-	-

***Stx1*:** Shiga- toxin 1 gene. ***Stx2*:** Shiga- toxin 2 gene. ***Eae*:** intimin gene. ***hylA*:** haemolysin gene

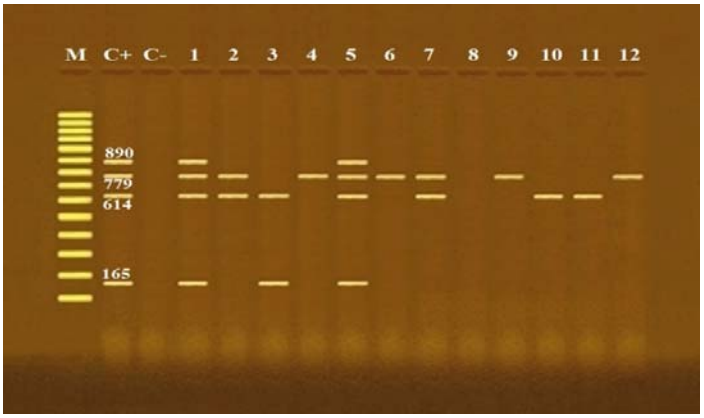

Photo (1): Agarose gel electrophoresis of multiplex PCR of *stx1*(614 bp), *stx2* (779 bp), *eaeA* (890 bp) and *hlyA* (165 bp) genes for characterization of Enteropathogenic *E. coli.* Lane M: 100 bp ladder as molecular size DNA marker. Lane C+: Control positive *E. coli* for *stx1*, *stx2*, *eaeA* and *hlyA* genes. Lane C-: Control negative. Lanes 1 (O26) & 5 (O111): Positive *E. coli* for *stx1*, *stx2*, *eaeA* and *hlyA* genes. Lanes 2 (O44) & 7 (O119): Positive *E. coli* strain for *stx1* and *stx2* genes. Lane 3 (O55): Positive *E. coli* strains for *stx1* and *hlyA* genes. Lanes 4 (O86), 6 (O114), 9 (O125) & 12 (O142): Positive *E. coli* for *stx2* gene. Lanes 10 (O126) & 11 (O128): Positive *E. coli* strains for *stx1* gene. Lanes 8 (O124): Negative *E. coli* for *stx1*, *stx2*, *eaeA* and *hlyA* genes.

From the results in table (3) and photograph (1) obtained by using Agarose gel electrophoresis of multiplex PCR of *stx1* (614 bp), *stx2* (779 bp), *eaeA* (890 bp) and *hlyA* (165 bp) genes for characterization of virulence genes of Shiga toxin-producing *E. coli* isolated from the examined cattle carcass swab samples showing that Shiga- toxin 1 gene *(stx1)* virulence gene was present in O26: H11, O44: H18, O55: H7, O111: H2, O119: H4, O126 and O128: H2. On the other hand, Shiga-toxin 2 gene *(stx2)* virulence gene was present in O26: H11, O44: H18, O86, O111: H2, O114: H21, O119: H4, O125: H21 and O142. While intimin gene *(eae)* virulence gene was present in O26: H11 and O111: H2. Also haemolysin gene *(hylA)* gene was present in O26: H11, O55: H7 and O111: H2.

4. DISCUSSION

E. coli presence in carcass meat gives an indication of unclean slaughtering processes starting from slaughtering animal until meat transporting to butcher shops (International Commission and Microbiological Specification for Foods "ICMSF", 1996 b). Intestinal pathogenic *E. coli* can be categorized into the following pathotypes: Enterotoxigenic *E. coli* (ETEC), Entero-pathogenic *E. coli (*EPEC), *Entero-hemorrhagic E. coli* (EHEC), *Enteroinvasive E. coli* (EIEC) (Croxen et al., 2013) . The obtained results agree with those reported by Moustafa (1993); Nashid - Heba (1993); Saad - Asmaa (2012) and Haileselassie et al. (2013). While lower results were obtained by Abdallah et al. (2009b); El-Dally (1994) and Saad et al. (2011). But higher results were obtained by Adwan et al. (2015) and Mathew et al. (2016).

Serovars O111, O26, O128, O86 and O119 are considered shiga toxin-producing *E. coli* (STEC) and All of the STEC isolates produced 1, 2, 3 or 4 virulence factors (i.e. *Stx1, Stx2, Stx1+ Stx2* or *eae*) (Hornitzky et al., 2002). Shiga toxin producing *E. coli* has many virulence factors including Shiga toxin type 1 (*Stx1*), Shiga toxin type 2 (*Stx2*), intimin (*eaeA*) and *hlyA* Enterohaemolysin produced by Enterohaemorrhagic E. coli (EHEC) (Paton and Paton, 1998).

So we summarized that using Agarose gel electrophoresis of multiplex PCR was greatly valuable for detection of *E. coli* virulence genes (shiga toxins (*stx1 & stx2*), intimin (*eaeA*) and *hylA)* that considered very hazardous to human consuming meat contaminated with *E. coli* serotypes.

5. REFERENCES

Abdallah, M.A., Suliman, S.E., Ahmed, D.E., Bakhiet, A.O., 2009b. Estimation of bacterial contamination of indigenous bovine carcasses in Khartoum (Sudan). Afri. J. Microbio. Res. 3, 882-886.

Adwan, G.M., Alqarem, B.R., Adwan, K.M., 2015. Prevalence of foodborne pathogens in meat samples in Palestine. Inter. Food Res. J. 22, 1806-1812.

American Public Health Association "APHA", 2001. Compendium of methods for microbiological examination of foods. 4th Edition 365-366-800. 1st, NW Washington DC 2000 1-3710.

Croxen, M., Law, R., Scholz, R., Keeney, K., Wlodarska, M., B., F., 2013. Recent advances in understanding enteric pathogenic *E. coli*. . Clin. Microbiol. Rev. 26, 822–880.

Dhanashree, B., Mallya, P.S., 2008. Detection of shiga-toxigenic E. coli (STEC) in diarrhoeagenic stool and meat samples in Mangalore, India. Ind. J. Med. Res. 128, 271-277.

El-Dally, K. 1994. Correlation between parasitism and microbial load and meat quality of the Egyptian food animalsPhD, Moshtohor, Zagzig Univ.

Fratamico, P., Sackitey, S., Wiedmann, M., Deng, M., 1995. Detection of E.coli O157:H7 by multiplex PCR. J. Clin. Microbiol. 33, 2188–2191.

Haileselassie, M., Taddele, H., Adhana, K., Kalayou, S., 2013. Food safety knowledge and practices of abattoir and butchery shops and the microbial profile of meat in Mekelle City, Ethiopia. Asian Pac. J. Trop. Biomed. 3, 407-412.

Heiman, K.E., Mody, R.K., Johnson, S.D., Griffin, P.M., Gould, L.H., 2015. *E. coli* O157 Outbreaks in the United States, 2003-2012. Emerg. Infect. Dis. 21, 1293-1301.

Hornitzky, M.A., Vanselow, B.A., Walker, K., Bettelheim, K.A., Corney, B., Gill, P., Baily, G., Djordjeevic, S.P., 2002. Virulence properties and serotypes of shiga toxin-producing E. coli from healthy Australian cattle. Appl. Environm. Microbiol. 68, 6439-6445.

International Commission and Microbiological Specification for Foods "ICMSF", 1996 b. Salmonellae. In: Roberts, T. A., Baird-

Parker, A. C., and Tompkin, R. B. eds. Microorganisms in foods 5: Microbiological specifications of food pathogens. Blackie Academic & Professional, London, UK, pp. 217-264.

International commission of Microbiological Specification for Foods "ICMSF", 1996. Microorganisms in Food. Their Significance and methods of enumeration. 3rd Ed. Univ. of Toronto, Canada.

Johnson, J., Kuskowki, M., Menard, M., Gajewski, A., Xercavins, M., Garau, J., 2006. Similarity between human and chicken E. coli isolates in related to ciprofloxins resistance status. Infect. Dis. 194, 71- 78.

Kok, T., Worswich, D., Gowans, E., 1996. Some serological techniques for microbial and viral infections. In Practical Medical Microbiology (Collee, J.; Fraser, A.; Marmion, B. and Simmons, A., eds.). Edinburgh, Churchill Livingstone, UK.

Komba, E., Komba, E.V., Mkupasi, E.M., Mbyuzi, A.O., Mshamu, S., Luwumba, D., Busagwe, Z., Mzula, A., 2012. Sanitary practices and occurrence of zoonotic conditions in cattle at slaughter in Morogoro Municipality, Tanzania: implications for public health. Tanzania J. of Health Res. 14, 1-12.

Mathew, B., Nanu, E., Sunil, B., 2016. Isolation of bacteria of public health significance from market beef. Int. J. Adv. Res. Biol. Sci. 3, 160-164.

Mazaheri, S., Ahrabi, S., Aslani, M., 2014. Shiga Toxin-Producing E.coli Isolated From Lettuce Samples in Tehran, Iran. Jundishapur J. Microbiol. 7, 1- 6.

Moustafa, M. 1993. Bacteriological studies on certain Grame negative food born pathogensPhD.

Nashid - Heba. 1993. Salmonella and Enteropathogenic E.coli serotype in meat and meat productsM.V.Sc., Moshtohor, Zagazig Uni.

Paton, A., Paton, J.C., 1998. Detection and characterization of shiga toxigenic E.coli by using multiplex PCR assays for stx1, stx2, eaeA, enterohemorrhagic E.coli hlyA, rfbO111, and rfbO157. J Clin. Microbiol. 36, 598–602.

Saad - Asmaa. 2012. Prevalence of E. coli in meat and meat products in Dakahlia provinceM.V.Sc., Mansoura University.

Saad, S.M., Edris, A.M., Hassan, M.A., Sabike, I.I.A., 2011. Enterobacteriacae in slaughtered animals with particular reference to pathogenic strains. B.V.M.J. 1, 146-152.

Shah, D., Shringi, S., Besser, T., Call, D., 2009. Molecular detection of foodborne pathogens, Boca Raton:. CRC Press, In Liu, D. (Ed). Taylor & Francis group, Florida, USA.

Sipos, R., Anna, J., Marton, P., Sara, R., Karoly, M., Marcell, N., 2007. Effect of primer mismatch, annealing temperature and PCR cycle number on16S rRNA gene-targetting bacterial community analysis. FEMS Microbial Ecology 60, 341-350.