Animal Health Research Journal Vol. 7, No. 1, March 2019 pp. 216-226

ISSN: 2356-7767

Detection of Shiga-Like Toxin Producing *Escherichia coli* in Food of Animal Origin by Street Vendors at Luxor City

***Afaf, A.M. Ahmed; **Samah, Eid; *Shimaa, El-Nagar and **Soad, A. Nasef**

* Reference Laboratory for Veterinary Quality Control on Poultry Production – Luxor Branch, Animal Health Research Institute.

**Reference Laboratory for Veterinary Quality Control on Poultry Production, Animal Health Research Institute.

Received in 9/1/2019
Accepted in 12/2/2019

Abstract

A total of 100 samples of ready-to-eat (RTE) meat sandwiches (50 cooked liver and, 50 chicken shawarma sandwiches) were randomly collected from street vendor carts at Luxor city. Samples were examined for shiga-like toxin producing *Escherichia coli* (STEC). The detected prevalence rates of *Escherichia coli* (*E. coli*) were (16/50) 32% and (2/50) 4% from cooked liver sandwiches and, chicken shawarma sandwiches, respectively. Serotyping of *E. coli* isolated from cooked liver sandwiches revealed the isolation of the following strains; O_{17}:H_{18}, O_{26}:H_{11}, O_{86}, O_{91}:H_{21}, O_{111}:H_2 O_{113}:H_4, O_{119}:H_6, O_{121}:H_7, O_{128}:H_2, O_{146}:H_{21}. On the other hand, serotyping of isolates from chicken shawarma sandwiches revealed the presence of only 2 *E. coli* serotypes (O_{91}:H_{21} and O_{121}:H_7). Investigating the virulence genetic attributes for STEC by PCR for isolates from cooked liver sandwiches revealed that 12/16, 11/16 and 4/16 of *E. coli* isolates were positive for *stx*1 *gene*, *stx*2 *gene*, and *eae*A gene, respectively. While, the two *E. coli* isolates from chicken shawarma sandwiches revealed that one of them was carried for both *stx*1 and *stx*2 *genes* and the other was carriers for *stx*2 *genes*. On the other hand, only 4/16 of *E. coli isolates* (O_{26}:H_{11} and O_{111}:H_2) were carried for *stx*1, *stx*2 and *eae*A genes. It could be concluded that the cooked liver sandwiches and shawarma chicken sandwiches prepared in street vendor carts were subjects of inadequate hygienic measures during preparation, and processing that may lead to contamination by shiga-toxin producing *E. coli* strains consequently may impose a potential public health risk.

Keywords: *Shiga, Like Toxin producing Escherichia coli, Food, Animal Origin, Street Vendors, Cooked liver, chicken, Shawarma.*

Introduction

In Egypt, Ready-to-eat foods are commonly sold in restaurants however a great part of these foods are vended by street vendors where foods are not effectively protected from dust and flies. Moreover, the safe food storage temperatures are difficult to maintain. Liver is considered one of the highly nutritive edible offal which contains vitamin A, vitamin B, vitamin C, vitamin D, Iron, Zinc and copper and mostly eaten as takeaway sandwiches **(Salma *et al.*, 2015).** Shawarma is a Middle Eastern Arabic style sandwich usually composed of shaved lamb, goat, chicken, turkey, beef or a mixture of meats **(Odu and Akano, 2012).** Basically, it is a wrap of shredded meat (beef, lamb, or marinated chicken) prepared by alternately stacking strips of fat and pieces of seasoned meat on a rotating vertical skewer. The meat is roasted from the outside, while most of the inside remains raw.

There is an increase in the consumption of ready-to-eat fast food because of a change in social patterns characterized by increased mobility, large numbers of itinerary workers and less family centered activities.

Vendors were often of poor education level, unlicensed, untrained in food hygiene, technology and worked under crude unsanitary conditions as they often used stands and carts of crude and inefficient construction, running water was not easily accessible **(Abdalla *et al.*, 2008)**. The hands are the most important vehicles for the transfer of organisms from feces, nose, skin, or other sites to food **(WHO, 1989).** Waste water was usually discarded in streets and garbage was discarded nearby providing harborage for insects and rodents **(Muinde and Kuria, 2005).**

Microbiological quality problems of ready-to-eat cooked liver and shawarma sandwiches depend greatly on low initial quality of raw liver and meat type and other ingredients, inefficient cooking process and improper sanitary practices for personnel and for cooking/processing utensils **(Kayaardi *et al.*, 2006)**. Cross-contamination during preparation has been traced back to the use of uncooked vegetables and unhygienic handling **(Reij and Aantrekker, 2004).**

True food poisoning or food intoxication caused by eating food that contains a toxin due to bacterial growth in food while food infection is the second type of food-borne illness.
Enteric *E. coli* are part of the natural flora of many animals. Human infections occur through consumption of contaminated food products, drinking water contaminated with animal or human waste, or through direct person-to-person spread from poor hygiene **(Berger *et al.*, 2010)**. *E. coli* considered as reflection of environmental contamination during slaughter processing and product handling (**Kanpelancher, 1981)**. Enteric *E. coli* infections are traditionally divided into 6 pathotypes based on their pathogenicity profiles (virulence factors, clinical disease and phylogenetic profile): Enteropathogenic *E. coli* (EPEC), Enterohamerrhagic *E. coli* (EHEC), Enteroinvasive *E. coli* (EIEC, including Shigella spp), Enteroaggregative *E. coli* (EAEC), Enterotoxigenic *E. coli* (ETEC) and Diffusely Adherent *E. coli* (DAEC) **(Kaper *et al.*, 2004).**
Shiga toxin-producing Escherichia coli (STEC) or verotoxin-producing Escherichia coli remains a major cause of foodborne-related gastrointestinal diseases in humans **(Wani *et al.*, 2004),** particularly since these infections may result in life-threatening sequel such as hemorrhagic colitis (HC), hemolytic uremic syndrome (HUS), and thrombotic thrombocytopenia purpura (TTP). Enterotoxigenic *E. coli* (ETEC) is reported to be the most commonly isolated bacterial enteropathogenic in children under 5 year of age in developing countries, accounting for approximately 20% of cases, equivalent to several hundred million cases of diarrhea and several tens of thousands of deaths each year **(Qadri *et al.*, 2005).** ETEC is also the most common cause of travelers' diarrhea accounting for 10–60% of infections depending on the region visited **(Gascón *et al.*, 1998)**.

The pathogenicity of these bacteria is mainly mediated by shiga toxins (*Stx*1, *Stx*2 and their variants) encoded by stx1 and stx2 genes. Within human disease-associated strains, those producing Shiga toxin type 2 appear to be more commonly responsible for serious complications such as HUS than those producing only Shiga toxin type 1. In addition, a subset of STEC strains considered to be highly virulent for humans has the capacity to produce attaching and effacing lesions on intestinal mucosa, a property encoded on a pathogenicity island termed the locus for enterocyte effacement (LEE). LEE encodes a type III secretion system and *E. coli* secreted proteins, which deliver effector molecules to the host cell and disrupt the host cytoskeleton. LEE also carries *eaeA*, which encodes an outer membrane protein (intimin) required for intimate attachment to epithelial cells. The *eaeA* gene has been used as a convenient diagnostic marker for LEE-positive STECs **(Wani *et al.*, 2004; Best *et al.*, 2005; Paton and Paton, 2005; Dhanashree and Mallya, 2008; Feng, 2013 & Mohammadi *et al.*, 2013).** The real-time multiplex PCR is robust and effective for the rapid and reliable detection of the seven predominant STEC serogroups of major public health (O_{26}, O_{45}, O_{103}, O_{111}, O_{121}, O_{145}, and O_{157}) and the detection of their virulence genes **(Anklam *et al.*, 2012 and Díaz-Sánchez *et al.*, 2012**).
Therefore, the present study was conducted to evaluate the prevalence of Shiga like-toxin producing *E. coli* in cooked liver sandwiches

and Shawarma chicken sandwiches sold in Luxor city and their significance as public health hazards.

Materials and Methods

Samples Collection: A total of 100 ready-to-eat meat sandwiches (50 samples of cooked liver sandwiches and 50 samples shawarma sandwiches) were purchased from different street vendor carts in Luxor City. All collected samples were immediately transferred in ice-box containers, aseptically handled and moved promptly to Reference Laboratory for veterinary Quality control on Poultry Production, Luxor Branch / Animal Health Research Institute for bacteriological examination, isolation and identification of shiga-like toxin producing *E. coli*.

Preparation of samples: Twenty-five grams (±0.5) of each sample were weighed and transferred into a sterile stomacher bag containing 225 ml 0.1% (W\V) sterile buffered peptone water were homogenized using a lab blender (Seward stomacher lab system 400 R \UK) for 2 minutes to obtain the original homogenate fluid of a dilution rate 10^{-1}. The preparation is carried out according to **(ISO 6887-2: 2017)**

Isolation of *E. coli*: was carried out according to the method described by **Lee and Arp (1998).** All isolates were detected in RLQP-Luxor branch/Animal Health Research Institute.

Identification of Enteropathogenic *E. coli*: Suspected isolates of *E. coli* were identified according to **MacFaddin (2000).**

Serological identification of *E. coli* isolates: 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.

Detection of Virulence genes of STEC by Polymerase Chain Reaction (PCR):

Primer sequences of *E. coli* used for PCR identification system: Application of PCR for identification of shiga toxins (*stx*1 & *stx*2) and intimin (*eae*A) genes was performed by using primers (Pharmacia Biotech) as shown in the following table

Target gene	Oligonucleotide sequence (5′ → 3′)	Product size (bp)	References
***stx*1 (F)**	5′ ACACTGGATGATCTCAGTGG ′3	614	**Dhanashree and Mallya (2008)**
***stx*1 (R)**	5′ CTGAATCCCCCTCCATTATG ′3		
***stx*2 (F)**	5′ CCATGACAACGGACAGCAGTT ′ 3	779	
***stx*2 (R)**	5′ CCTGTCAACTGAGCAGCAC-TTTG ′3		
***eae*A (F)**	5′ GTGGCGAATACTGGCGAGACT ′ 3	890	**Mazaheri *et al.*, (2014)**
***eae*A (R)**	5′ CCCCATTCTTTTTCACCGTCG ′3		

DNA Extraction using QIA amp kit (Shah *et al.*, 2009): For genomic DNA extraction, single cloning of the bacteria was picked up from selective agar plate onto nutrient agar plates and incubated at 37 °C for 26 hours. After overnight culture on nutrient agar plates, one or two colonies were suspended in 20 ml of sterile distilled water, and the suspension was then heated at 100ºC for 20 minutes. Accurately, 50-200 µl of the culture were placed in Eppendorf tube and frozen at -40 ºC for further testing.

DNA amplification reaction of *E. coli* (Fagan *et al.*, 1999): The amplification was performed on a Thermal Cycler (Master cycler, Eppendorf, Hamburg, Germany). PCR assays were carried out in 1 ml of nucleic acid template prepared by using reference EHEC isolates (approximately 30 ng of DNA), 10 mM Tris-HCl (pH 8.4), 10 mM KCl, 3 mM MgCl2; 2 mM concentrations of each primer, 0.2 mM concentrations of each 29-deoxynucleoside 59-triphosphate, and 4 U of Ampli Taq DNA polymerase (Perkin-Elmer). The reference strains were *E. coli* O157:H7 Sakai (positive for *stx*1, *stx*2 and *eae*A) and *E. coli* K12DH5α (a non-pathogenic negative control strain) that does not possess any virulence gene. The amplified DNA fragments were analyzed by 2% of agarose gel electrophoresis (Applichem, Germany, GmbH) in 1x TBE buffer stained with ethidium bromide and captured as well as visualized on UV transilluminator. A 100 bp plus DNA Ladder (Qiagen, Germany, GmbH) was used to determine the fragment size.

Cycling conditions of the different primers during PCR in the following table:

Target gene	Primary denaturation	Secondary denaturation	Annealing	Extension	Final extension
stx1	95˚C 3 min.	95˚C 20 sec.	58˚C 20 sec.	72˚C 1.5 min.	72˚C 5 min.
stx2	95˚C 3 min.	95˚C 20 sec.	58˚C 20 sec.	72˚C 1.5 min.	
eaeA	95˚C 3 min.	95˚C 20 sec.	58˚C 20 sec.	72˚C 1.5 min.	

Results and Discussion

E. coli virulence factors are mostly encoded on mobile elements that can move between organisms, and some organisms can have characteristics of more than one pathotype. (**ISU/CFSPH, 2016).** The presence of *E. coli* in food of animal origin is considered as indicator of mis-preparation, mis-handling, mis-storage or unhygienic service **(Tebbut, 1999).** Although, *E. coli* is readily killed by temperature above 55°C, serious incidents occurred in such products, which reflects high level of abuse, cross contamination between raw and cooked foods **(Varnam and Evans, 1991).**

Table (1). Prevalence rate of *E. coli* isolated from the examined ready-to-eat meat sandwiches samples

Ready-to-eat meat sandwiches	No. of Samples	Positive Samples	
		No.	%
Cooked liver	50	16	32
Chicken Shawarma	50	2	4

Results in Table (1) revealed that the prevalence rate of *E. coli* was (32 % and, 4 %) in the examined cooked liver sandwich samples and, chicken shawarma sandwich samples, respectively. The current results of cooked liver samples were nearly similar to those reported by **Doaa (2015)** (30 %) and **El-zekaty *et al.*, (2016)** (32 %); higher prevalence rates were recorded by **Dalia *et al.*, (2013)** (42%) and **Shaltout *et al.*, (2017)** (40%). Furthermore, lower prevalence rates were recorded by **Salma *et al.* (2015)** (10 %) and **El-shenawy *et al.*, (2016)** (20 %). Prevalence rate of *E. coli* in the examined chicken shawarma samples were lower than that recorded by **Eman and Sherifa (2012)** (20 %) and **Hassanin *et al.*, (2014)** (33.3 %).

E. coli are serotyped based on the O (somatic lipopolysaccharide), H (flagellar) and K (capsular) antigens. A number of serotypes are known to contain EHEC. Some well-known organisms involved in human disease include *E. coli* O_{157}:H_7, *E. coli* O_{157}: H- (also known as *E. coli* O_{157}: NM, for "nonmotile"), and members of serogroups O_{26}, O_{55}, O_{91}, O_{103}, O_{111}, O_{121} and O_{145}. Additional serogroups that have been reported in human clinical cases are O_{45}, O_{80}, O_{104}, O_{113}, O_{117}, O_{118}, O_{128} and others **(ISU/CFSPH, 2016).**

Table (2). Serological identification of *E. coli* isolated from examined ready-to-eat meat sandwiches samples (N. = 18):

Serotypes	Prevalence rate of *E. coli in examined sandwiches*			
	Cooked liver		Chicken Shawarma	
	No.	(1) %	No.	(1) %
O_{17}:H_{18}	1	5.6	0	0
O_{26}:H_{11}	4	22.1	0	0
O_{86}	1	5.6	0	0
O_{91}:H_{21}	2	11	1	5.6
O_{111}:H_2	1	5.6	0	0
O_{113}:H_4	1	5.6	0	0
O_{119}:H_6	1	5.6	0	0
O_{121}:H_7	1	5.6	1	5.6
O_{128}:H_2	3	16.5	0	0
O_{146}:H_{21}	1	5.6	0	0
Total (10 serotypes)	16		2	

(1) No. of isolates /No. of examined strains

Results in Table (2) revealed that the identified *E. coli* serotypes were O_{17}:H_{18} (5.6%), O_{26}:H_{11} (22.1%), O_{86} (5.6%), O_{91}:H_{21} (11%), O_{111}:H_2 (5.6%), O_{113}:H_4 (5.6%), O_{119}:H_6 (5.6%), O_{121}:H_7 (5.6%), O_{128}:H_2 (16.5%), O_{146}:H_{21} (5.6%) from the examined cooked liver sandwich samples; while the identified *E. coli* serotypes from the examined chicken shawarma sandwich samples were O_{91}:H_{21} (5.6%), and O_{121}:H_7 (5.6%), respectively.

Similar results for cooked liver sandwiches were reported by many researchers as that reported by **El-zekaty *et al.*, (2016)** who identified O_{26} (12%), O_{86} (4%) and O_{119} (8%). Results were higher in studies by **Salma *et al.*, (2015)** who found that the incidence of E. coli were serotyped to O_{26} (67%) in examined cooked liver sandwiches; and by **Doaa (2015)** who reported that the incidence of E. coli O_{111} in cooked liver sandwiches was 10 % (3/30).

On the other hand, **Morshdy et al. (2018)** reported that incidence of E. coli O_{26}, O_{111}, O_{113}, O_{119} and O_{127} in examined chicken shawarma sandwiches were 3(6%), 3(6%), 2(4%), 4(8%) and 3(6%), respectively.

Table (3). Occurrence of virulence genes of Shiga toxin-producing *E. coli* isolated from the examined ready to eat sandwiches:

Type of RTE samples	Serotypes	No. of isolates	Detection rate of Shiga-toxin virulence genes		
			* *stx*1	***stx*2	****eae*A
			No.	No.	No.
Cooked Liver (50)	O_{17}:H_{18}	1	0	1	0
	O_{26}:H_{11}	4	4	3	3
	O_{86}	1	0	1	0
	O_{91}:H_{21}	2	2	2	0
	O_{111}:H_2	1	1	1	1
	O_{113}:H_4	1	1	0	0
	O_{119}:H_6	1	1	1	0
	O_{121}:H_7	1	0	1	0
	O_{128}:H_2	3	3	0	0
	O_{146}:H_{21}	1	0	1	0
Total		16	12	11	4
Chicken Shawarma (50)	O_{91}:H_{21}	1	1	1	0
	O_{121}:H_7	1	0	1	0
Total		2	1	2	0

*** *stx1*:** Shiga- toxin 1 gene, **** *stx2*:** Shiga- toxin 2 gene, ***** *eaeA*:** intimin gene

Results in Table (3) and Figure (1) revealed that most of the studied isolates produced shiga toxins (shiga-toxin 1 (*stx*1) and shiga-toxin 2 (*stx*2) and only two serotypes were produced intimin gene (eaeA). From cooked liver sandwich samples, out of 12/16 of *E. coli* serotypes (O_{26}:H_{11}, O_{91}:H_{21}, O_{111}:H_2, O_{113}:H_4, O_{119}:H_6, O_{121}:H_7 and O_{128}:H_2) carried *stx*1; out of 11/16 of *E.coli* serotypes (O_{17}:H_{18}, O_{26}:H_{11}, O_{86}, O_{91}:H_{21}, O_{111}:H_2, O_{119}:H_6, O_{121}:H_7, and O_{146}:H_{21}) carried *stx*2, while ; out of 4/16 of *E. coli* serotypes (O_{26}:H_{11} and O_{111}:H_2) carried *eaeA* gene. While, from chicken shawarma sandwich samples, were isolated two E. coli serotypes; O_{91}:H_{21} which carried *stx1* and *stx2* genes and O_{121}:H_7 that had *stx2* gene only. On the other hand, only 4/16 of *E. coli isolates* (O_{26}:H_{11} and O_{111}:H_2) were carriers *stx*1, *stx*2 and *eae*A genes.

Morshody *et al.* (2018) reported that shiga toxins genes were detected in chicken shawarma sandwiches samples. *Stx1* was detected in 3 *E. coli* isolates including O_{26}:H_{11}, O_{119}:H_6 and O_{127}:H_6 while only one isolate was positive for *Stx2* (O_{119}:H_6). In addition, four isolates including O_{26}:H_{11}, O_{127}:H_6, O_{113}:H_4, and O_{111}:H_2 were all positive for *eaeA* gene.

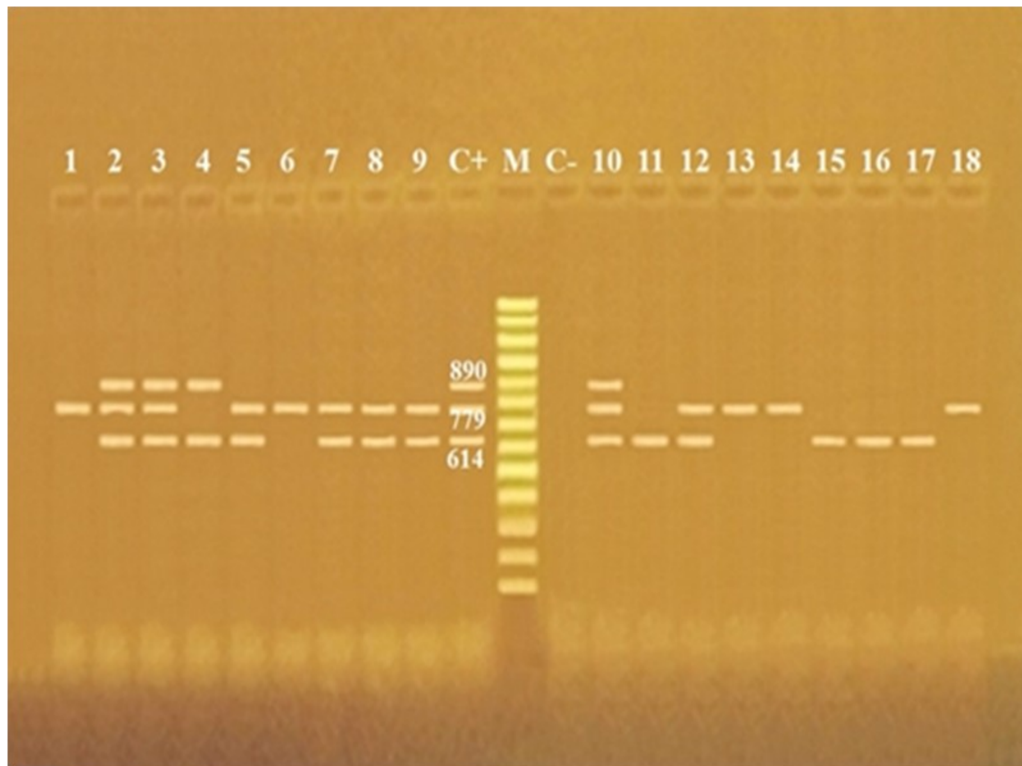

Figure (1): Agarose gel electrophoresis of multiplex PCR for *stx*1 (614 bp), *stx*2 (779 bp) and *eae*A (890 bp) virulence genes for characterization of Enteropathogenic *E. coli* (n =18)

Lane M: 100 bp ladder. **Lane C+**: Control positive *E. coli* for *stx1*, *stx2* and *eaeA* genes. **Lane C-:** Control negative. **Lanes 1** (O17), **6** (O86), **13, 14** (O121) & **18** (O146): Positive *E. coli* for *stx2* gene. **Lanes 2, 3** (O26) & **10** (O111): Positive *E. coli* for *stx1*, *stx2* and *eaeA* genes. **Lane 4** (O26): Positive *E. coli* for *stx1* and *eaeA* genes. **Lanes 5** (O26), **7, 8, 9** (O91) & **12** (O119): Positive *E. coli* for *stx1* and *stx2* genes. **Lanes 11** (O113) & **15, 16, 17** (O128): Positive *E. coli* for *stx1* gene.

Note: lanes 9 & 14 were from chicken shawarma sandwiches and other lanes were from cooked liver sandwiches.

The production of Shiga toxins (*stx*) is the unifying feature of all non-O157 STEC and O157 STEC. Various types of *stx* are produced, but they fall into two main types: Shiga toxin 1 (*stx*1) and Shiga toxin 2 (*stx*2) **(Paton and Paton, 1998).** Production of *stx* alone is usually insufficient for STEC strains to cause disease. Studies have shown that accessory virulence factors such as intimin, which are encoded by *eaeA* gene, are usually needed for STEC to be pathogenic **(Paton *et al.,* 2004).** The majority of *stx* genes are bacteriophage encoded, thus allowing a level of interstrain and possibly interspecies mobility. The presence of *stx* is thought to be the primary factor responsible for intestinal manifestations (bloody diarrhoea) and systemic complications (HUS). The toxin uptake mechanisms in the intestine have yet to be fully elucidated for all STEC. **(WHO, 1998)** Shiga toxin–producing *E. coli* (STEC), also known as verotoxin-producing *E. coli* (VTEC), infections are public health concerns because of the severe illnesses they cause, such as hemorrhagic colitis and hemolytic uremic syndrome (HUS). STEC constitute a heterogeneous group of bacteria abundant in the reservoir and in the environment. (**Smith *et al.,* 2014).**

According to **EFSA (2013)** Serotypes responsible for haemorrhagic colitis (HC) and haemo-

lytic uraemic syndrome (HUS), O_{157}:H_7 and O_{157}:NM, were assigned to seropathotype A. Seropathotype B strains (O_{26}:H_{11}, O_{103}:H_2, O_{111}:NM, O_{121}:H_{19} and O_{145}:NM) have a strong association with outbreaks and HUS but less commonly than those caused by seropathotype A. Seropathotype C serotypes (O_{91}:H_{21}, O_{104}:H_{21}, O_{113}:H_{21}, O_5:NM, O_{121}:NM and O_{165}:H_{25}) are associated with sporadic HUS cases but not with epidemics. **Rehamand Mohamed (2018)** found *E. coli* serovars O_{111}:H_2 that are carriers for *stx*1, *stx*2 and *eae*A genes, O_{119}:H_4 that are carriers of *stx*1 and *stx*2 genes and O_{128}:H_2 that are also carriers of *stx1* gene from stool samples of children with diarrheal symptoms in hospitals.

Food was recognized as a major vehicle of transmission of O157 STEC and was likely to play the same role for non-O157 STEC, but there is limited information on the occurrence of non-O157 STEC in the food supply. Meat and meat products, dairy products and any foods cross-contaminated by animal products during preparation may be contaminated (**WHO, 1998).** On the other hand, complete elimination of pathogens from raw materials **(Eisel *et al.,* 1997)** and food processing environment **(Tompkin, 2012)** is difficult, particularly when many food pathogens were known to be able to attach on food contact surfaces **(Jessen and Lammert, 2009).**

Conclusion

From the results it could be concluded that the hygienic conditions of some processed RTE food as cooked liver and chicken shawarma sandwiches offered by some street vendors in Luxor city demonstrated unsatisfactory microbiological quality with reference to the relevant Egyptian standards in a way that may impose a high risk for consumers.

Recommendations

Hygienic awareness should be applied for personnel who involved in handling of food at street vendor shops

Routine microbiological examination should be adopted in meat product, butcher's shops, groceries and other outlets that offer food of animal origin.

One health approach shall be apply for official regular monitor campaigns on food and food products of animal origins and street vendors.

References

Abdalla, M.A.; Siham, E.S.; Alian, Y.Y.H.A. and Amel, O.B. (2008). Microbial content of the domestic refrigerators in Khartoum area (Khartoum North). Sud. J. Vet. Sci. Anim. Husb. 47(1&2): 15-23.

Anklam, K.S.1.; Kanankege, K.S.; Gonzales, T.K.; Kaspar, C.W. and Döpfer, D. (2012). Rapid and reliable detection of Shiga toxin-producing Escherichia coli by real-time multiplex PCR. J Food Prot. Apr;75(4): 643-50.

Berger, C.N.; Sodha, S.V.; Shaw. R.K.; Griffin, P.M., Pink, D.; Hand, P. and Frankel, G. (2010). Fresh fruit and vegetables as vehicles for the transmission of human pathogens. Environ. Microbiol., 12: 2385-97.

Best, A.; La Ragione, R.M.; Sayers, A.R. and Woodward, M.J. (2005). Role for flagella but not intimin in the persistent infection of the gastrointestinal tissues of specific pathogen free chicks by shiga toxin-negative Escherichia coli O157:H7. Infect Immun. 73: 1836-1846.

Dalia, F.K.; Gamal, E.H.; Amal, A.S. and Fatma, I.E. (2013). The microbiological assessment of ready to eat food (liver and kofta sandwiches) in Tanta City, Egypt. Benha veterinary medical journal, 25(2): 187-197.

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

Díaz-Sánchez, S.; Sánchez, S.; Sánchez, M.; Herrera-León, S.; Hanning, I. and Vidal, D. (2012). Detection and characterization of Shiga toxin-producing Escherichia coli in game meat and ready-to-eat meat products. International Journal of Food Microbiology**.** 160(2):179-182

Doaa M. Abd El-Aziz (2015). Prevalence of E. coli with special concern to shiga-

toxigenic E.coli O157 and O111 in street-vended sandwiches. Annals. Food Science and Technology, 16(1):257-261. Available on-line at www.afst.valahia.ro

Eisel, W.G.; Linton, R.H.; and Muriana. P.M. (1997). A survey of microbial levels for incoming raw beef, environmental sources, and ground beef in a red meat processing plant. Food Microbiol. 14:273-282.

El-Shenawy, M.A.; Zaghloul, R.A.; Abbass, I.H.; Amira I.E. and Fouad M.T. (2016). Incidence of some epidemiologically relevant food-borne pathogens in street-vended sandwiches. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 7(2):468-474.

El-zekaty, A.; Nossair, M.A.; Samaha, I.A. and Eman, K. (2016). Microbial Evaluation of Selected Ready to Eat Street Vended Meat Products Sandwiches with Emphasis on Public Health Importance of E. coli Isolates. Alexandria Journal of Veterinary Sciences. 51 (2):27-32.

Eman, M.S. and Sherifa M.S. (2012). Microbiological Loads for Some Types of Cooked Chicken Meat Products at Al-Taif Governorate, KSA. World Applied Sciences Journal. 17(5): 593-597.

EFSA, European Food Safety Authority. (2013). Scientific opinion on VTEC-seropathotype and scientific criteria regarding pathogenicity assessment. EFSA J. 2013; 11: 3138.

Fagan, P.; Hornitzky, M.; Bettelheim, K. and Djordjevic, S. (1999). Detection of Shiga-Like Toxin (stx1 and stx2), Intimin (eaeA), and Enterohemorrhagic Escherichia coli (EHEC) Hemolysin (EHEC hlyA) Genes in Animal Feces by Multiplex PCR. Appl. Environ. Microbiol. 65 (2): 868–872.

Farhan, R.; Abdalla, S.; Abdelrahaman, H.A.; Fahmy, N. and Salama, E. (2014). Prevalence of Escherichia Coli in Some Selected Foods and Children Stools with Special Reference to Molecular Characterization of Enterohemorrhagic Strain. American Journal of Animal and Veterinary Sciences. 9 (4): 245-251.

Feng, P. (2013). Escherichia coli (ETEC, EPEC, EHEC, EIEC). In: Bad Bug Book. Hand book of Foodborne Pathogenic Microorganisms and Natural Toxins. (3rd ed.) Food and Drug Administration. USA.

Gascón, J.; Vargas, M.; Quintó, L.; Corachán, M.; Jimenez de Anta, M.T. and Vila, J. (1998). Enteroaggregative Escherichia coli strains as a cause of traveler's diarrhea: a case-control study. J. Infect. Dis. 177:1409-12

Hassanin, F.S.; Reham, A. Amin; Shawky, N.A. and Gomaa, W.M. (2014). Incidence of Escherichia coli and Salmonella in Ready to eat Foods. Benha veterinary medical journal. 27(1): 84-91.

International organization for standardization (ISO 6887 part 2, 2017). Microbiology of the food chain -- Preparation of test samples, initial suspension and decimal dilutions for microbiological examination -- Part 2: Specific rules for the preparation of meat and meat products.

ISU/CFSPH; Iowa State University/Center for Food Security and Public Health. (2016). Enterohemorrhagic Escherichia coli and Other E. coli Causing Hemolytic Uremic Syndrome. Center for Food Security and Public Health Technical Factsheets. 61. http://lib.dr.iastate.edu/cfsph_factsheets/61

Jessen, B. and Lammert, L. (2009). Biofilm and disinfection in meat processing plants. Int. Biodeterior. Biodegradation. 51: 265-269.

Kanpelancher, K.H. (1981). Lab for food microbial and hygiene. Agriculture University wagninen, Nether land. Zentablatt for, Bacteriologic IB, 172(4-5): 390.

Kaper, J.B.; Nataro, J.P. and Mobley, H.L.T. (2004). Pathogenic Escherichia coli. Nat. Rev. Microbiol., 2: 123-140.

Kayaardi, S.; Kayacier, Q.A. and Gok, V. (2006). Sensory and Chemical Analysis of Doner Kebab Made from Turkey Meat. J. Muscle Food.17: 165 -173.

Kok, T.; Worswich, D. and 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.), 14th ed., Edinburgh, Churchill Livingstone, UK.

Lee, M.D. and Arp, L.H. (1998). A laboratory manual for the isolation and identification of avian pathogen Daviv E. Swayne, Chairman, John R. Glisson, Mark W. Jackwood, James E. Pearson, Willie M. Reed. Editorial Board for the American Association of Avian Pathologists, 4th ed., Chapter 3, Colibacillosis. 14-16.

MacFaddin, J.F. (2000). Biochemical tests for identification medical bacteria. Warery Press Inc, Baltimore, Md. 21202 USA.

Mazaheri, S.; Ahrabi, S. and Aslani, M. (2014). Shiga Toxin-Producing Escherichia Coli Isolated From Lettuce Samples in Tehran, Iran. Jundishapur J. Microbiol. 7(11):1-6.

Mohammadi, P.; Abiri, R.; Rezaei, M. and Salman-zadeh, A.S. (2013). Isolation of Shiga toxin-pro-ducing Escherichia coli from raw milk in Ker-manshah, Iran. Iran J Microbiol. 5: 233-238.

Muinde, O.K. and Kuria, E. (2005): Hygienic and Sanitary Practices of Vendors of Street Foods in Nairobi, Kenya. African Journal of Food Agriculture and Nutritional Development. 5:1-15.

Odu, N.N. and Akano, U.M. (2012). The Microbiological Assessment of Ready-To-Eat-Food (Shawarma) In Port Harcourt City, Nigeria. Nature and Science.10(8):1-8.

Paton, J.C. and Paton, A.W. (1998). Pathogenesis and diagnosis of Shiga toxin-producing Escherichia coli infections. Clin. Microbiol. Rev. 11: 450–79.

Paton, A.W. and Paton, J.C. (2005). Multiplex PCR for direct detection of shiga toxigenic Escherichia coli strains producing the novel subtilase cytotoxin. J Clin Microbiol. 43: 2944–2947.

Paton, A.W.; Srimanote, P.; Talbot, U.M.; Wang, H. and Paton, J.C. (2004). A new family of potent AB (5) cytotoxins produced by Shiga toxigenic Escherichia coli. J. Exp. Med. 200: 35–46.

Qadri, F.; Svennerholm, A.M.; Faruque, A.S. and Sack, R.B. (2005). Enterotoxigenic Escherichia coli in developing countries: epidemiology, microbiology, clinical features, treatment, and prevention. Clin. Microbiol. Rev. 18:465-83.

Reham, Y.A. Mohamed. (2018). PCR detection of shiga toxin producing Escherichia coli in chicken meat and red meat in Luxor city. A thesis submitted for degree of M.V.Scs. in meat Hygiene and control. Faculty of Veterinary Medicine. South Vally University.

Reij, M.W. and Aantrekker, E.D.D. (2004). Recontamination as a source of pathogens in processed foods. International Journal of Food Microbiology. 91:1-11.

Salma J.I.M.; Ahmed, A.M. and Ismail, T.H. (2015). Prevalence of Some Food-borne Pathogens in Cooked Liver Sandwich. 2nd Conference of Food Safety, Suez Canal University, Faculty of Veterinary Medicine. 1: 82-89.

Shaltout, F.A.; Farouk, M.; Ibrahim, H. A.A. and Afifi, M.E.M. (2017). Incidence of E. coli and Salmonellae in ready to eat fast foods. Benha Veterinary Medical Journal. 32 (1): 18 – 22.

Smith, J.L.; Fratamico, P.M. and Gunther, N.W. (2014). Shiga toxin-producing Escherichia coli. In Sima S., Geoffrey M.G. eds. Advances in Applied Microbiology. Burlington, US: Academic Press pp. 145–97.

Shah, D.; Shringi, S.; Besser, T. and Call, D. (2009). Molecular detection of foodborne

pathogens, Boca Raton: CRC Press, In Liu, D. (Ed). Taylor & Francis group, Florida, USA, Pp. 369-389.

Tebbut, G.M. (1999). Comparative study of visual inspections and microbiological sampling in premises of manufacturing and selling high risk foods. Epidemiol. Infect. 103: 775-786.

Tompkin, R.B. (2002). Control of Listeria monocytogenes in the Food-Processing Environment. Journal of Food Protection. 65(4): 709-725.

Varnam, A.H. and Evans, M.G. (1991). Food borne pathogens. An illustrated text chapter 13, pp. 267 England. Wolfe publishing Ltd. ISBN 07234, 1521, 8.

Wani, S.A.; Samanta, I.; Bhat, M.A. and Nishika-wa, Y. (2004). Investigation of shiga toxin-producing Escherichia coli in avian species in In-dia. Lett Appl Microbiol. 39: 389-394.

WHO (World Health Organization). (1989). Health surveillance and management procedures for food handling personnel. WHO technical report series, 785. Geneva. p. 52.

WHO (World Health Organization). (1998). Zoonotic non-O157 shiga Toxin-producing Escherichia coli (STEC). Report of a WHO scientific working group meeting. WHO/ CSR/APH/98.8.

Morshdy, A.M.A.; Hussein, M.A.; Tharwat, A.E.; Moustafa, N.A. and Hussein, O.K. (2018). Prevalence of shiga toxigenic and multi drug resistant *Escherichia coli* in ready to eat chicken products' sandwiches. Slov. Vet. Res. 55(20): 349–56.