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Hygienic evaluation of Liver sandwiches retailed at restaurants and street vendors

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Abstract

Fast food, as liver sandwiches, retailed by small liver restaurants and street vendors. In Egypt; they may be contaminated with several types of microorganisms that may affect their safety and quality. A total of one hundred liver sandwiches (Kebda) samples were collected from different small restaurants and street vendors in Alexandria governorate. Collected samples were examined to determine their bacterial profiles. The results indicated that 6(12%) and 19(38%)of small restaurants and street vendors liver sandwiches samples, respectively has APC equal or exceed 10^6(cfu/g) meanwhile 3 (8.3%) and 21(44.8%) of samples has coliform counts exceed 10^3(cfu/g), respectively. These percentages of examined samples are considered unsatisfactory microbiological quality according to the limits stated by the published **microbiological guidelines for ready-to-eat Food of Center of Food Safety (CFS, 2014).** *E. coli* could be isolated from 49 (49%) of all examined samples from which 18 out of 50 (36.7%) and 31 out of 50 (63.3%) of small restaurants and street vendors livermeals samples, respectively. The incidence of *E. coli* serotypes isolated from examined samples were O_{125}, O_{146}, O_{18}, O_{157}, O_{158}, O_{86a}, O_{63} and O_{44} in different values. The serotype O_{157} was isolated from samples of street vendors. Further molecular identification of *E. coli* serotypes, to investigate the presence of shigatoxin producing *E. coli* (STEC), by conventional PCR could detect eaeA and Stx2 virulence genes in 7 and 3 of the identified serotype isolates, respectively, while all isolates were free from other virulence genes (Stx1 and hly genes). It could be concluded that the retailed liver sandwiches samples, especially from street vendors, were contaminated with high load of microorganisms and may contain shigatoxin-producing *E. coli* and constitute public health hazard. Recommendations for strict hygienic practices to provide safe food such as liver sandwiches were discussed.

***Key words**: Liver sandwiches, Coliform, E. coli $O_{157}H_7$, Shigatoxin, PCR.*

Introduction

Cooked liver was one of the most popular fast food in Egypt, furthermore; liver sandwiches do not undergo any further treatments to assure their safety **(Cabedo *et al.,* 2008).**

Street foods are perceived to be a major public health risk due to lack of basic infrastructure and services, difficulty in controlling the large numbers of street food vending operations because of their diversity, mobility and temporary nature **(Ghosh *et al.,* 2007 and de Sousa, 2008).**

Food borne diseases are main problems, particularly in developing countries and cause the majority of illnesses and death around the world. Food is the most important vehicle that transmits the microorganisms to human **(Varnam, 1991).**

Escherichia coli is a member of the genus *Escherichia* with the family *Enterobacteriaceae;* it is a facultative anaerobe non-sporing. Members are widely distributed in the environment and contaminated food and water are the major sources on which the bacteria are spread,

selected strains can cause a wide variety of infections in hospitals and community settings **(Donnerberg *et al.,* 2005).**

The majority of *E. coli* strains are commensal that cause a wide spectrum of intestinal and extra intestinal infections, such as diarrhea, urinary tract infection, meningitis, and septicemia (**Nataro, and Kaper, 1998).** *E. coli* O_{157}:H_7, constitute the majority of food-borne illness outbreaks **(Park *et al,* 2007 and Wang *et al.,* 2011).**

All enterohemorrhagic *Escherichia coli* (EHEC) strains cause serious disease in humans and possess at least one Shiga-like toxin (*stx*1 or *stx*2) gene. The detection of Shiga-like toxins is very useful for the identification of EHEC and Non-EHEC strains which were negative for both stx1 and stx2 **(Paton & Paton 2002).** Shiga toxin (Stx)-producing *Escherichia coli* (STEC) were first recognized as a human pathogen in 1982, in the USA, when strains of the serotype O_{157}: H_7 caused two outbreaks of hemorrhagic colitis **(Riley *et al.,* 1983).**

Food Safety and Inspection Service (USDA FSIS) declared the top six non-O_{157} STEC as adulterants in raw, non-intact beef products **(Anonymous, 2011),** and regulatory testing for these STEC serogroups began in June, 2012. Thus, there is a need for rapid methods that can be used for regulatory testing and for detection of STEC serogroups. There have been numerous reports on PCR-based methods for detection of food-borne pathogens, including STEC. Several methods have been explored for the bacteria determination, including the culture and colony counting method, polymerase chain reaction (PCR), and immunology-based method **(de Boer and Beumer, 1999).** In developing countries like Egypt, where effective food safety controls by concerned regulatory agencies are yet to berealized evaluation of food microbial hazards and their indicators would help providing criteria for setting functional microbial guideline values **(Ekanem *et al.,* 1998).** In Egypt, street vended food as liver sandwiches could represent a hazard due to the bad conditions at which they are produced as using raw materials of poor quality, in adequate personnel hygiene of vendors and holding for long period lead to contamination of food with pathogenic microorganism. Such contamination might render the product to be unfit for human consumption **(Gundogan *et al.,* 2005)**. In under developed countries where contaminated worker supplies were common, refrigeration was rare, foodborne illness might cause billion illness and 406 million deaths each year **(CDC, 2011)**. Therefore, the present study was conducted to evaluate hygienically Liver sandwiches retailed in different small restaurants and street vendors, and to highlight the public health implication of consuming heavily contaminated cooked liver.

Materials and Methods

1- Collection of samples:

A total of one hundred liver sandwiches samples were collected from different districts (small liver restaurants and street vendors) in Alexandria governorate. Each sample was kept in a separated sterile plastic bag and put in an insulated ice box, then the samples were transferred under aseptic condition to the laboratory where they were prepared and subjected to the following examinations:

2- Bacteriological examination:

2-1. Determination of Aerobic Plate Count: according to **(APHA, 2001).**

2-2. Determination of *Coliform* count: according to **(FDA, 2002).**

2-3. Isolation and identification of *Escherichia coli*: according to **(APHA, 1992)**.

2-4. Isolation and identification of *Escherichia coli* O_{157}: H_7: according to **(Lee and Choi, 2006)** by using Modified *E. coli* broth (with novobiocin) and incubated for 24 hours at 37^0C for isolation of *E. coli* O_{157}: H_7, Broth culture were plated onto Sorbitol MacConkey (SMAC) Agar medium (Oxoid, England) with cefixime for 24 hours at 37 °C. After incubation, Typical *E. coli* O_{157}: H_7 were kept for further biochemical and serological tests.

2-5. Serological typing of *E. coli:* according to **(Varnam and Evans,1991),** was done at serology unit, Animal Health Research Institute (AHRI).

3- Polymerase chain reaction (PCR): Detection and identification of Shigatoxin (Stx)-producing *Escherichia coli* (STEC) according to **(Jasson *et al.,* 2010),** was done at biotechnology unit of reference laboratory for poultry production control.

3-1. DNA extraction. DNA extraction from samples was performed using the QIAamp DNA Mini kit (Qiagen, Germany, GmbH) with modifications from the manufacturer's recommendations. Briefly, 200 µl of the sample suspension was incubated with 10 µl of proteinase K and 200 µl of lysis buffer at 56°C for 10 min. After incubation, 200 µl of 100% ethanol was added to the lysate**.** The sample was then washed and centrifuged following the manufacturer's recommendations. Nucleic acid was eluted with 100 µl of elution buffer provided in the kit.

3-2. Oligonucleotide Primer. Primers used were supplied from **Metabion (Germany)** are listed in table (1).

3-3. PCR amplification for uniplex PCR (for 8 *E. coli* serotypes): Primers were utilized in a 25- µl reaction containing 12.5 µl of Emerald Amp Max PCR Master Mix **(Takara, Japan)**, 1 µl of each primer of 20 pmol concentration, 4.5 µl of water, and 6 µl of DNA template. The reaction was performed in an applied biosystem 2720 thermal cycler.

For stx1, stx2 duplex PCR, primers were utilized in a 50- µl reaction containing 25 µl of Emerald Amp Max PCR Master Mix **(Takara, Japan)**, 1 µl of each primer of 20 pmol concentration, 13 µl of water, and 8 µl of DNA template. The reaction was performed in an applied biosystem 2720 thermal cycler.

3-4. Analysis of the PCR Products. The products of PCR were separated by electrophoresis on 1.5% agarose gel (Applichem, Germany, GmbH) in 1x TBE buffer at room temperature using gradients of 5V/cm. For gel analysis, 20 µl of each uniplex PCR product and 40 µl of each multiplex PCR product were loaded in each gel slot. A gelpilot 100 bp DNA ladder and a 100 bp plus DNA Ladders (Qiagen, Germany, GmbH) and generuler 100 bp DNA ladder (Fermentas, Thermo) were used to determine the fragment sizes. The gel was photographed by a gel documentation system (Alpha Innotech, Biometra) and the data was analyzed through computer software.

Table (A). Primers sequences, target genes, amplicon sizes and cycling conditions.

Target gene	Primers sequences	Amplified segment (bp)	Primary denaturation	Amplification (35 cycles)			Final extension	Reference
				Secondary denaturation	Annealing	Extension		
hly	AACAAGGATAAGCACTGTTCTGGCT	1177	94˚C 5 min.	94˚C 30 sec.	60˚C 50 sec.	72˚C 1 min.	72˚C 10 min.	**Piva *et al.,* (2003)**
	ACCATATAAGCGGTCATTCCCGTCA							
eaeA	ATGCTTAGTGCTGGTTTAGG	248	94˚C 5 min.	94˚C 30 sec.	51˚C 30 sec.	72˚C 30 sec.	72˚C 7 min.	**Bisi-Johnson *et al.,* (2011)**
	GCCTTCATCATTTCGCTTTC							
Stx1	ACACTGGATGATCTCAGTGG	614	94˚C 5 min.	94˚C 30 sec.	58˚C 40 sec.	72˚C 45 sec.	72˚C 10 min.	**Dipineto *et al.,* (2006)**
	CTGAATCCCCCTCCATTATG							
Stx2	CCATGACAACGGACAGCAGTT	779						
	CCTGTCAACTGAGCAGCACTTTG							

Statistical analysis

Comparison of the mean test was done by LSD and Duncan's multiple range test **(Duncan, 1955).**

Results

Table (1). Acceptability of the examined liver sandwiches samples according to the microbiological guidelines **(CFS, 2014)** for APC (n= 50 of each).

Categories	Range cfu/g	Liver sandwiches			
		small restaurants		Street vendors	
		No.	%	No.	%
Satisfactory	**$< 10^5$**	14	28%	10	20%
Acceptable	**$10^5<0^6$**	30	60%	21	42%
Unsatisfactory	**$\geq 10^6$**	6	12%	19	38%

Table (2). Acceptability of the examined liver sandwiches samples according to the microbiological guidelines **(CFS, 2014)** for *Coliform* Counts cfu/g (n=50 of each).

Categories	Range cfu/g	Liver sandwiches			
		Small restaurants		Street vendors	
		No.	%	No.	%
Satisfactory	**< 10**	23	46	9	18
Acceptable	**$10\text{-}10^3$**	24	48	20	40
Unsatisfactory	**$>10^3$**	3	6	21	42

Table (3). Incidence of *E. coli* recovered from examined liver sandwiches samples.

Samples	No. of samples	Positive samples of *E. coli*	
		No	%
Small restaurants	50	18	36.7
Street vendors	50	31	63.3
Total	100	49	49 %

Table (4). Results of serological identification of *E. coli* isolates from liver sandwiches.

E. coli serogroups	Small restaurants		Street vendors	
	No.	%	No.	%
O_{125}	4	22.2 %	2	6.4%
O_{146}	1	5.6 %	6	19.4 %
O_{18}	3	16.6 %	9	29 %
O_{157}	0	0	1	3.2 %
O_{158}	2	11.1 %	8	25.8 %
O_{86a}	5	27.8 %	1	3.2 %
O_{63}	2	11.1 %	0	0
O_{44}	1	5.6 %	4	13 %
Total	18	36.7%	31	63.3%

Table (5). Results of PCR molecular identification of eaeA, Stx1, Stx2 and hly virulence genes in the *E. coli* isolates serotypes (n=8).

Serotypes		Virulence genes			
		eaeA	Stx1	Stx2	hly
1	**O_{125}**	-	-	+	-
2	**O_{146}**	+	-	-	-
3	**O_{18}**	+	-	+	-
4	**O_{157}**	+	-	+	-
5	**O_{158}**	+	-	-	-
6	**O_{86a}**	+	-	-	-
7	**O_{63}**	+	-	-	-
8	**O_{44}**	+	-	-	-

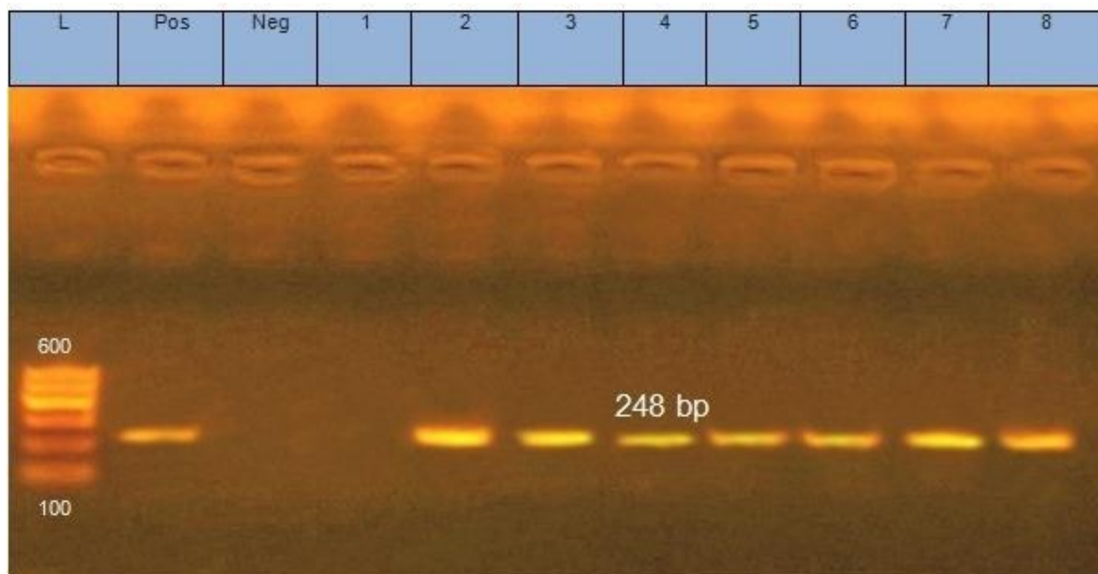

Figure (1). Agarose gel showing PCR amplification of *E. coli* (eaeA) gene product at (248 bp)
Pos.: Positive control, Neg: Negative control, L: DNA Ladder, Lane 1: negative *E. coli* strains and Lane 2 to 8: positive *E. coli* strains.

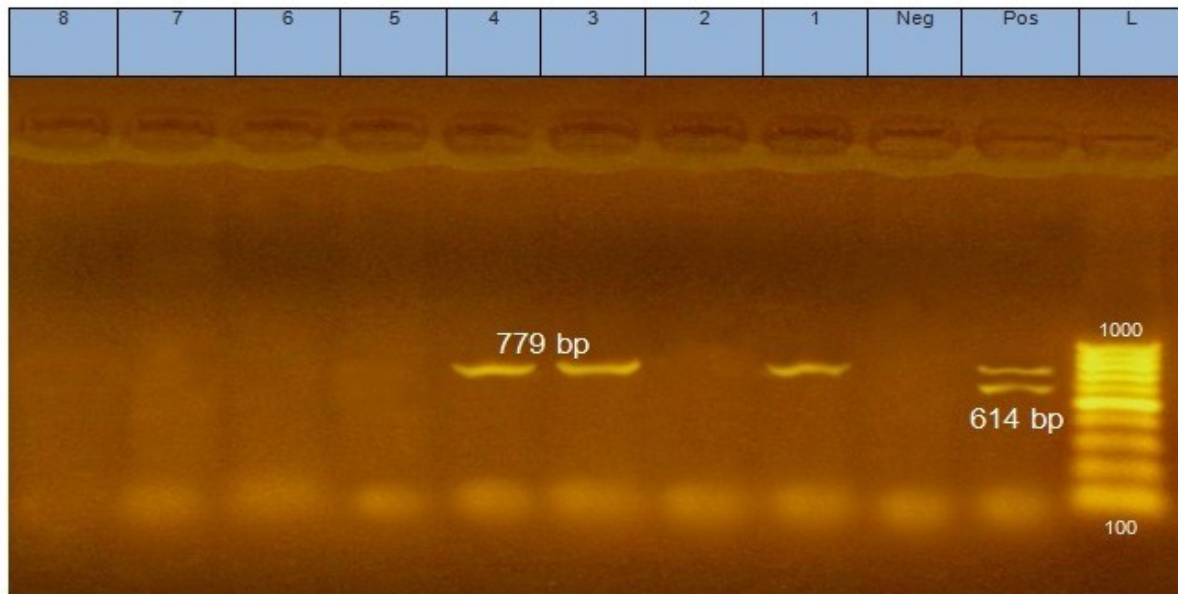

Figure (2). Agarose gel showing PCR amplification of *E. coli* (Stx2) gene products at (779 bp)
Pos.: Positive control, Neg: Negative control, L: DNA Ladder, Lane 1, 3 and 4: positive
E. coli strains and Lane 2,5,6,7 and 8: negative *E. coli* strains.
(Stx1) gene product at (614bp), lane 1 to 8: negative *E. coli* strains

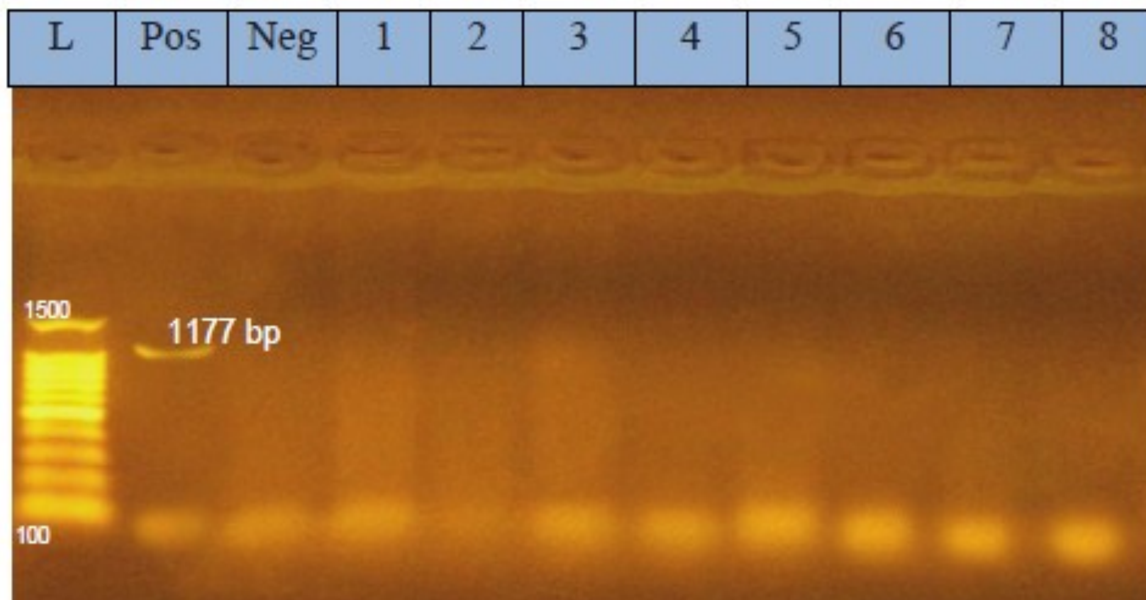

Figure (3). Agarose gel showing PCR amplification of *E. coli* (hly) gene products at (1177bp)
Pos.: Positive control, Neg.: Negative control, L: DNA Ladder, Lane 1 to 8: negative
E. coli strains

Discussion

Risks to human health are associated with initial contamination of raw foods with pathogenic bacteria and subsequent contamination by vendor's practices during preparation through cross-contamination, survival of pathogens during preparation and microbial proliferation during display **(Abou-Zaid *et al.,* 2001 and Soliman *et al.,* 2002).**

Table (1) based on the microbiological guidelines of ready-to-eat food of **Center of Food Safety (CFS, 2014),** the microbiological quality of ready to eat sandwiches has been placed into three categories, where the APC at $<10^5$ is rated as satisfactory, 10^5 to $<10^6$as acceptable, $\geq 10^6$ (log cfu/g) as unsatisfactory. it is cleared that, the level of contaminations was within acceptable microbiological limits for 88%(44) and 62%(31) of liver sandwiches samples and about 12% (6) and 38%(19) were of unsatisfactory microbiological quality of small restaurants and street vendors samples, respectively. Nearly similar results were observed by **Khater *et al.,* (2013)** were 8(80%) acceptable ratio from restaurant and lower results were obtained by **El-Zekaty *et al.,* (2016)** were 24% acceptable ratio from street vendors. The high bacterial count in cooked foods would indicate that they were contaminated during cooking, handling procedures, demonstrating an overall lack of hygiene. Other studies have shown that during the preparation of raw meat and vegetables in kitchens, numerous surfaces can become contaminated, the contaminating microorganisms can survive for considerable periods of time **(Scott and Bloomfield, 1990** and **Gillespie *et al.,* 2000).** In these instances, the cross -contamination may occur. The Fast food become probably the source of most diseases caused by bacteria due to uncovered food, areas infected by flies and others insects also using poor quality water for washing the material and equipment **(Barro *et al.,* 2002).** Microorganisms which contaminate food, comes from natural microflora or are introduced by manufacturing steps ranging from harvesting, processing storage and distribution **(Adams and Moss, 2000). Johnston and Tompkin, (1992)** cited that the combination of drying and frying should have a synergistic effect on the lowering of the total microbial counts.

Results for coliforms count of examined liver sandwiches samples from the data presented in Table (2) revealed that 3(6%) and 21(42%) of liver sandwiches samples obtained from small restaurants and street vendors, respectively, were considered as unsatisfactory. according to **(CFS, 2014),** which stated that coliforms count of ready to eat sandwiches were categorized as satisfactory (< 10), acceptable (from 10 to 10^3) and unsatisfactory (> 10^3cfu/ g.). lower results were obtained by **El-Zekaty *et al.,* (2016)** were 12% acceptable ratio from street vendors. The presence of Coliforms group in vended meals has an epidemiological interest as some of its members are pathogenic and may result in serious infection and food poisoning. Thus, the total Coliforms count may be used aboard base indicating fecal contamination of meat **(ICMSF, 1998)**. They belong to the family *Enterobacteriaceae*; that do not form spores; they also ferment lactose to produce acid and gas **(Jawetz, 2008)**. They are among the most common bacteria that cause disease. The presence of these organisms in ready-to-eat food describes a deplorable state of poor hygiene and sanitary practices employed in the processing and packaging of this food product **(Jay, 2005).**

The incidences of isolated *E. coli* in examined liver sandwiches samples were recorded in Table (3). *E. coli* was detected in 49 (49%) of examined samples, 18 out of 50 (36.7%) and 31 out of 50 (63.3%) from small restaurants and street vendors respectively. Serological identification proved the detection of one O_{157}:H_7, the highly pathogenic serotype; from street vendor's samples while the other *E. coli* isolates were Non-O_{157}:H_7. These results were higher than that obtained by **(Khater *et al.,* 2013)** were (40%) from street vendors liver sandwiches. *E. coli* were the most predominant bacteria isolated from cooked meals **(Abdalla, 2009).** The presence of *E. coli* in RTE foods is

undesirable because it indicates that the food has been prepared under poor hygienic conditions. *E. coli* considered as reflection of environmental contamination during slaughter processing and product handling and its count is an index of sanitary quality of examined samples, high number can easily give rise to public health hazards **(Mercuri & Cox, 1976).** There are many factors affecting the differences in prevalence rates among studies; these differences are mainly related to samples and sampling (type, source/location and initial bacterial load), environmental and seasonal factors and the used detection methodology **(Temelli *et al.,* 2012).** *E. coli* $O_{157}:H_7$ is now one of the causes of bloody diarrhea worldwide when grows in the intestines **(Tortora *et al.,* 2013).** The non-O_{157} STEC serogroups has also resulted in outbreaks of human illness, including O_{111} **(Boudailliez *et al.,* 1997** and **Caprioli *et al.,* 1994),** and O_{26}: H_{11} **(Miyajima *et al.,* 2007),** The list of non-O_{157} STEC associated with human illnesses consists of over 100 different serotypes **(Eklund *et al.,* 2001)**. While *E. coli* O_{157} is the principal STEC strain isolated from implicated food and clinical isolates in the US, non-O_{157} STEC predominate in other countries including Australia, Brazil, Canada, Germany and the UK, **(Pradel *et al.,* 2000; Baffone *et al.,* 2001 and Guth *et al.,* 2003)**. Recently, it has become evident that non-O_{157} Shiga toxin-producing *E. coli* (STEC), particularly STEC serogroups O_{26}, O_{45}, O_{103}, O_{111}, O_{121}, and O_{145} (referred to as the top six non-O_{157} STEC) cause illnesses similar to those caused by *E. coli* $O_{157}:H_7$ **(Mathusa *et al.,* 2010** and **Gould *et al.,* 2013).** Pathogenic elements of ETEC strains, are intestinal *E. coli* and cause diarrhea in infected individuals, also can cause urinary hemolytic syndrome which often happens after an intestinal infection **(Johnson *et al.,* 2002).**

It is evident from the present results recorded in Table (4) that the *E. coli* serotypes 'strains identified from examined samples of liver sandwiches, were O_{125}, O_{146}, O_{18}, O_{157}, O_{158}, O_{86a}, O_{63} and O_{44} in different values. Samples from small restaurants, the most predominant serotype were O_{86a} in a ratio of 27.8% followed by O_{125} (22.2 %). While, samples from street vendors observed the most predominant serotype were O_{18} (29%) followed byO_{158} (25.8 %), and O_{157} (3.2%) serotype was detected also from the same samples. Many studies have been conducted around the world to determine the presence of *E. Coli* $O_{157}H_7$ in various food **(Hussein and Sakuma, (2005) and Fedio *et al.,* 2011).** Nearly similar results were recorded by **Jamshidi *et al.,* 2012 and Sheikh *et al.,* 2013)** which they recorded the incidence of *E. coli* $O_{157}H_7$ was 4% and 1% from examined samples, respectively. **Brooks *et al.,* 2005; Johnson *et al.,* 2006; Bettelheim, 2007 and Mellmann *et al.,* 2009)** they reported that *E. coli* $O_{157}:H_7$ and the top six non-O_{157} STEC (O_{26}, O_{45}, O_{103}, O_{111}, O_{121}, and O_{145}) cause the majority of the food-borne illnesses. According to the frequency of hemolytic uremic syndrome (HUS), the European Food Safety Authority reported the major serotypes or serogroups of *E. coli* concern are $O_{157}:H_7$, O_{26}, O_{103}, O_{145}, O_{111}, and O_{91} **(EFSA, 2009).** However other emerging STEC serogroups including O_{113} and O_{91} have caused outbreaks and serious illness in the US and in other countries **(Bettelheim, 2007; Mellmann *et al.,* 2009)**. Cattle are a major reservoir for STEC in the U.S., and outbreaks due to non-O_{157} STEC, including serogroups O_{26} and O_{111} have been associated with beef or contact with cattle **(Paton *et al.,* 1996, Ethelberg *et al.,* 2009 and Kaspar *et al.,* 2010).** Further molecular identification of *E. coli* serotypes, to investigate the presence of shigatoxin-producing *E. coli* (STEC), by conventional PCR could detect eaeA and Stx2 virulence genes in 7 and 3 of the examined serotype isolates, respectively, while all isolates were free from other virulence genes (Stx1 and hly genes).

Results of agarose gel electrophoresis using 16S rRNA gene for the eight tested *E. coli* strains in Table (5) and figure (1) indicated that; 7 tested strains were *E. coli* (eaeA) gene product positive with molecular weight 248bp and one negative result assays were developed with specific primers for the detection of dif-

ferent virulence genes of *E. coli* specially (eaeA) gene, which considered a significant in food safety threat. Different serotypes of STEC have been reported as recovered from humans with hemorrhagic colitis or hemolytic uremic syndrome (HUS). However, a subset of STEC strains (e.g., those of serogroups O_{26}, O_{103}, O_{111}, O_{113}, and O_{157}), often referred to as enterohemorrhagic *E. Coli* (EHEC), are more commonly associated with such serious disorder and often possess associated virulence factors such as plasmid-encoded (EaeA, Tir, and Esp) by the locus of enterocyte effacement (LEE) **(Gyles *et al.*, 1998).**

Results for detection of Stx1 and Stx2 genes of the eight serotyping strain isolates were recorded in Table (5) and figure (2, 3). Moreover, the results of molecular identification of these isolates by conventional PCR revealed that the three identified isolates were positive for Stx2 virulence genes with molecular weight (779 bp), while all isolates were negative for Stx1with molecular weight (614bp) and Hemolysin (hly) with molecular weight (1177bp) virulence gene. The detection of Shiga-like toxins is very useful for the identification of EHEC, while Non-EHEC strains were negative for both Stx1 and Stx2 **(Paton and Paton 2002 and Wang *et al.*, 2002).** Shiga toxin-producing *E. coli* (STEC) are enteric pathogens that have been linked to outbreaks from food-borne and waterborne sources. STEC causes human gastro-intestinal illnesses with diverse clinical spectra, ranging from watery and bloody diarrhea to hemorrhagic colitis **(Gyles, 2007).** Shiga toxin-producing strains of *E. coli* (STEC) are now recognized as an important human pathogen of public health concern. Whereas STEC isolates belong to many different serotypes, *E. coli* O_{157}:H_7 and an occasional nonmotile variant O_{157}: H_7 are the most common serotypes associated with human illness. Isolates of this pathogen are a major cause of hemorrhagic colitis and mild diarrheal illness and are the major etiological agent of hemolyticuremic syndrome (HUS). HUS is characterized by a prodromal of gastroenteritis, frequently including bloody diarrhea, followed by acute hemolytic anemia, thrombocytopenia **(Griffin and Tauxe, 1991).**

Shigatoxin-producing *E. coli* excrete potent Shiga toxins that are encoded by the *Stx*1 and *Stx*2 genes **(Hussein and Sakuma, 2005).** The STEC isolates predominantly carried the *Stx*2 gene. Epidemiological data suggest that *Stx*2 is more important than *Stx*1 in the development of hemolytic uremic syndrome, a life-threatening illness associated with STEC infection in children **(Nataro and Kaper, 1998).**

Conclusion

These findings demonstrate that:

1- Liver sandwiches (Kebda) samples collected from different small liver restaurants and street vendors in Alexandria governorate may constitute a potential hazard to human health.

2- The presence of *E. coli* indicated fecal contamination, thereby suggesting possible risk of infection involved in the consumption of such food.

3- Issuing of healthy certificates to food handlers and vendors must be restricted.

4- Effective awareness and training on food safety requirements to food handlers will lead to improvement in their hygienic practices and can provide safe and high quality RTE foods and sandwiches.

5- While there is still no microbial guideline value for APCs of Egyptian RTE foods, the adoption of the generally accepted APC guideline value of $<10^6$cfu/g of RTE food sample may be appropriately used and is recommended until more comprehensive APC guideline values for Egyptian RTE foods are to be established.

References

Abdalla, M.A.; Suliman, S.E. and Bakhiet, A.O. (2009). Food safety knowledge and practices of street food vendors in Atbara City (Naher Elneel State Sudan). African Journal of Biotech. 8(24): 6967-6971.

Abou-Zaid, M.A.S.; Enshrah, K.I. Mira; Hashim, M.F. and Azza, H. Ahmed, (2001). Bacteriological status and safety of ready to eat street vended food in Giza Gov-

ernorate. J. Egypt. Vet. Med. Ass. 61(6A): 79-88.

Adams, M.R. and Moss, M.O. (2000). The Scope of Food Microbiology 2nd Ed. The Royal Society of Chemistry Cambridge. p. 15.

Anonymous (2011). Proposed rules: Shiga toxin-producing *E. coli* in certain raw beef products. Fed. Reg. 76, 58157–58165.

APHA (American Public Health Association) (1992). Standard Methods for the Examination of Dairy Products,16th Ed., APHA, Washington, D.C.

APHA (American public Health Association) (2001). "Compendium of methods for the microbiological examination of foods.", 2nd ed. Speck, H.L. (ed.) AP HA. Washington D.C.

Baffone, W.; Ciaschini, G.; Pianetti, G.; Casaroli, A. and Bruscolini, F. (2001). Detection of *E. coli* O_{157}:H_7 and other intestinal pathogens in patients with diarrheal disease. European Journal of Epidemiology 17: 97-99.

Barro, N.; Outtara, C.A.; Nikiema, P.A.; Ouattara, A.S. and Traore, A.S. (2002). Microbial quality assessment of some food widely consumed in Ouagadougou, Burkina Faso. Sante, 12(4): 369–374.

Bettelheim, K.A. (2007). The non-O_{157} Shiga-toxigenic (verocytotoxigenic) *Escherichia coli*; under-rated pathogens. Crit. Rev. Microbiol.33, 67–87.

Bisi-Johnson, M.A.; Obi, C.L.; Vasaikar, S.D.; Baba, K.A. and Hattori, T. (2011). Molecular basis of virulence in clinical isolates of *E. coli* and *Salmonella* species from a tertiary hospital in the Eastern Cape, South Africa. Gut Pathogens 2011, 3:9.

Boudailliez, B.; Berquin, P.; Mariani-Kurkdjian, P.; Ilef, D.; Cuvelier, B.; Capek, I.; Tribout, B.; Bingen, E. and Pi-ussan, C. (1997). Possible person-to-person transmission of *E. coli* O_{111} – associated hemolytic uremic syndrome. Pediatric Nephrol.,11, 36.

Brooks, J.T.; Sowers, E.G.; Wells, J.G.; Greene, K.D.; Griffin, P.M. and Hoekstra, R.M. (2005). Non-O157 Shiga toxin-producing *Escherichia coli* infections in the United States, 1983–2002. J. Infect. Dis. 192, 1422–1429.

Cabedo, L.; L.P.I. Barrot and A.T.I. Canelles (2008). Prevalence of *Listeria monocytogenes* and *Salmonella* in ready-to-eat food in Catalonia, Spain. J. of Food Protection, 71: 855-859.

Caprioli, A.; Luzzi, I.; Rosmini, F.; Resti, C.; Edefonti, A.; Perfumo, F.; Farina, C.; Goglio, A.; Gianviti, A. and Rizzoni, G. (1994). Community-wide outbreak of hemolytic-uremic syndrome associated with non-O_{157}verocytotoxin-producing *E. coli*. J. Infect. Dis 169, 208.

"CDC" Center for Disease Control and Prevention. (2011). Investigation of a multi-state outbreak of human *Salmonella* 14, 5, 12 from November, 2010, through 9 February, 2011.

CFS (Center for Food Safety). (2014). Microbiological guidelines for food (for ready-to-eat food in general and specific food items). Risk Assessment Section, Food and Envir. Hygiene Department 43/F, Queensway Government Offices, 66 Queensway, Hong Kong.

de Boer, E. and Beumer, R.R. (1999). Methodology for detection and typing of food borne microorganisms. Int. J. Food Microbiol., 1999, 50, 119-130.

de Sousa, C.P. (2008). The impact of food manufacturing practices on food borne diseases. Brazilian Archives of Biology and Technology Journal, 51(4): 815 823.

Dipineto, L.; Santaniello, A.; Fontanella, M.; Lagos, K.; Fioretti, A. and Menna, L. F. (2006). Presence of Shiga toxin-producing *E. coli* O_{157}:H_7 in living layer hens. Letters in Appl. MicrobioL., 43 (2006) 293–295.

Donnerberg, M.S.; G.L. Mendel; J.E. John and D. Mandel (2005). *Enterobacteriaceae* Principles and Practice of Infectious Dis-

ease.6th Ed. Elsevier Churchill Living Stone Philadelphia. pp. 267 – 286.

Duncan, D.R. (1955). Multiple range and multiple F tests. Biometrics, 11: 31-42.

EFSA (2009). Technical specifications for the monitoring and reporting of verotoxigenic *Escherichia coli* (VTEC) on animals and food (VTEC surveys on animals and food), EFSA (European Food Safety Authority); 2009. Contract no.: 1366.

Ekanem, E.O. (1998). The street food trade in Africa: safety and socio-environmental issues. Food Cont. 1998, 9: 211-215.

Eklund, M.; Scheutz, F. and Siitonen, A. (2001). Clinical isolates of non-O_{157}Shiga toxin-producing *Escherichia coli*: serotypes, virulence characteristics, and molecular profiles of strains of the same serotype. J. Clin. Microbiol. 2001 Aug; 39(8): 2829-34.

El-zekaty, A.; Mohammad, A. Nossair; Ibrahim, A. Samaha and Eman, Khalifa (2016). Microbial evaluation of selected ready to eat sreet vended meat products sandwiches with emphasis on public health importance of *E. coli* isolates. AJVS., 2016, Nov. 51(2): 27-32.

Ethelberg, S.; Smith, B.; Torpdahl, M.; Lisby, M.; Boel, J. and Jensen, T. (2009). Outbreak of non-O_{157} Shiga toxin-producing *Escherichia coli* infection from consumption of beef sausage. Clin. Infect. Dis. 48, e78–e81.

FDA (Food and Drug Administration) (2002). Enumeration of *E. coli* and the *Coliform* Bacteria.8th Edition, Chapter 4 Enumeration of *Escherichia coli* and the *Coliform* Bacteria.

Fedio, W.M.; Jinneman, K.C.; Yoshitomi, K.J.; Zapata, R.; Wendakoon, C.N.; Browning, R. and Weagant, S.D. (2011). Detection of *E. coli* $O_{157}H_7$ in raw ground beef by pathatrix (TM) immuno-magnetic separation, real time PCR and Cultural methods. Int. J. Food Microbiol. 148(2): 87-92.

Ghosh, M.; Wahi, S.; Kumar, M. and Ganguli, A. (2007). Prevalence of enterotoxigenic *Staphylococcus aureus* and *Shigella* species. In some raw street vended Indian foods. International Journal of Environmental Health Research, 17(2): 151–157.

Gillespie, I.; Little, C. and Mitchel, R. (2000). Microbiological examination of cold ready-to-eat sliced meats from catering establishments in the United Kingdom. J. Appl. Microbiol. 88:467-474.

Gould, L.H.; Mody, R.K.; Ong, K.L.; Clogher, P.; Cronquist, A.B. and Garman, K.N. (2013). Increased coli infections in the United States during 2000-2010: epidemiologic features and comparison with *E. coli* O_{157} infections. Foodborne Pathog. Dis. 10, 453–460.

Griffin, P.M. and Tauxe, R.V. (1991). The epidemiology of infections was caused by *E. coli* and the associated hemolytic uremic syndrome. Epidemiological Reviews 13, 60-98.

Guth, B.E.; Chinen, I.; Miliwebsky, E.; Cerqueira, A.M.; Chillemi, G.; Andrade J.R.; Baschkier, A. and Rivas, M. (2003). Serotypes and Shiga toxin genotypes among *Escherichia coli* isolated from animals and food in Argentina and Brazil. 29; 92(4): 335 -49.

Gundogan, N.; Citak, S.; Yucel, N.; Olkkonen, L. and Deveren, A. (2005). A note on the incidence and antibiotic resistance of *staphylococcus aureus* isolated from meat and chicken samples. J. Meat Sci., 69: 807-810.

Gyles, C.; Johnson, R.; Gao, A.; Ziebell, K.; Pierard, D.; Aleksic, S. and Boerlin, P. (1998). Association of enterohemorrhagic *E. coli* hemolysin with serotypes of shiga-like-toxin-producing *E. coli* of human and bovine origins. Appl. Environ. Microbiol. 1998 Nov; 64(11): 4134-41.

Gyles, C.L. (2007). Shiga toxin-producing *E. coli*: an overview. J Anim. Sci 2007; 85: E45 – 62.

Hussein, H.S. and Sakuma, T. (2005). Prevalence of Shiga toxin-producing *Escherichia coli* in dairy cattle and their products.J. Dairy Sci. 2005 Feb; 88 (2): 450-65.

ICMSF (International commission on microbiological specification for food) (1998). Microorganisms in foods. Intestinally pathogenic *Escherichia coli*. Blackie Academic and Professional. London.

Jamishidi, A.B.; Bassami, M.R.; Khanzadi, S. and Soltaninejad, V. (2012). Using multiplex PCR assay in identification of *E. coli.* $O_{157}H_7$ isolated from hamburger samples in Mashhad, Iran. J. Nutr. Sci. Food Technol. 35: 101-106.

Jasson, V.; Jacxsens, L.; Luning, P.; Rajkovic, A. and Uyttendaele, M. (2010). Alternative microbial methods: An overview and selection criteria. Food Microbiol. 2010 Sep; 27(6): 710-30.

Jawetz, Melnick and Adelberg's Medical Microbiology (2008). In: Medical Microbiology, 24th edition, Geo. F. Brooks, Karen C. Carroll, Janet S. Butel, Stephen A. Morse (edited). McGraw-Hill Medical; 24 editions; pp. 832.

Jay, M.J. (2005). Modern Food Microbiology 4th Ed. Chapman and Hall, New York. p. 187.

Johnson, J.R.; Jerome, C.; Boster, D.R.; Stapleton, A.E. and Tarr, P.I. (2002). Analysis of Urinary *E. coli* isolates for ability to produce shigatoxin. J. Clin. Microbiol., 40(6): 2247-2248.

Johnson, K.E.; Thorpe, C.M. and Sears, C.L. (2006). The emerging clinical importance of non-O_{157} Shiga toxin-producing *Escherichia coli*. Clin. Infect. Dis. 43, 1587–1595.

Johnston, R.W. and Tompkin, R.B. (1992). Meat and poultry products. In Compendium of Methods for the Microbiological Examination of Foods, 3rd Ed. (C. Vanderzant and D.F. Splittstoesser, eds.) pp, American Public Health Association, Virginia.

Kaspar, C.; Doyle, M.E. and Archer J. (2010). White Paper on Non-O_{157}:H_7 Shiga Toxin-Producing *E. coli* from Meat and Non-Meat Sources.

Khater, F.D.; Gamal, E. Heikal; Amal, A. Shehata and Fatma I. El-Hofy (2013). The Microbiological Assessment of Ready-To-Eat-Food (Liver and Kofta sandwiches) in Tanta City, Egypt Banha Vet., Med. J., 25 (2): 187-197, December 2013.

Lee, J.H. and Choi, S.J. (2006). Isolation and characteristics of sorbitol fermenting *E. coli* O_{157} strains from cattle. Microbes Infect. 8:2021-26.

Mathusa, E.C.; Chen, Y.; Enache, E. and Hontz, L. (2010). Non-O_{157} Shiga toxin-producing *Escherichia coli* in foods. J Food Prot 2010; 73: 1721-36.

Mellmann, A.; Fruth, A.; Friedrich, A.W.; Wieler, L.H.; Harmsen, D. and Werber, D. (2009). Phylogeny and disease association of Shiga toxin–producing *Escherichia coli* O_{91}. Emerg, Infect. Dis. 15, 1474–1477.

Mercuri, A.J. and Cox, N.A. (1976). Coliforms and *enterobacteriaceae* isolated from selected food. Journal of Food Protection, 9 (3): 712-714.

Miyajima, Y.; Takahashi, M.; Eguchi, H.; Honma, M.; Tanahashi, S.; Matui, Y.; Kobayashi, G.; Tanaka, M.; Higuchi, T. and Takeuchi, Y. (2007). Outbreak of enterohemorrhagic *Escherichia coli* O_{26} in Niigata City, Japan. Jpn J. Infect. Dis., 60, 238–239.

Nataro, J.P. and Kaper, J.B. (1998). "Diarrheagenic *Escherichia coli*, Clinical Microbiology Reviews, vol.11, no.1, pp. 142–201, 1998.

Park, Y.; Juarez, M.; Ramos, M. and Haenlein, G. (2007). Physico-chemical characteristicsof goat and sheep milk. Small Ruminant Res. 68: 88113.

Paton, A.W.; Ratcliff, R.M.; Doyle, R.M.; Seymour-Murray, J.; Davos, D. and Lanser, J.A. (1996). Molecular microbiological investigation of an outbreak of hemolytic-uremic syndrome caused by dry fermented sausage contaminated with Shiga

like toxin producing *Escherichia coli*. J. Clin. Microbiol. 34, 1622–1627.

Paton, A.W. and Paton, J.C. (2002). Direct detection and characterization of Shiga toxigenic *E. coli* by multiplex PCR for stx1, stx2, eae, ehxA, and saa. J. Clin. Microbiol. 40(1): 271-274.

Piva, I.C.; Pereira, A.L.; Ferraz, L.R.; Silva, R.S.N.; Vieira, A.C.; Blanco, J.E.; Blanco, M.; Blanco, J. and Giugliano, L.G. (2003). Virulence Markers of Enteroaggregative *E. Coli* isolated from Children and Adults with Diarrhea in Brasília, Brazil. J. of Clin. Microbiol., May 2003, p. 1827–1832.

Pradel, N.; Livrelli, V.; De Champs, C.; Palcoux, J.B.; Reynaud, A.; Scheutz, F.; Sirot, J.; Joly, B. and Forestier, C. (2000). Prevalence and characterization of Shiga toxin-producing *E. coli* isolated from cattle, food, and children during a one-year prospective study in France. J. of Clinical Microbiol., 38(3): 1023-31.

Riley L.W., Remis R. S., Helgerson S. D., McGee H.B., Wells J. G., Davis B. R., Hebert R. J., Olcott E. S., Johnson L. M., Hargrett N. T., Blake P. A., Chen M. L. (1983). Hemorrhagic colitis associated with a rare *Escherichia coli* serotype. N. Engl. J. Med., 308: 681-685.

Rompré, A., Servais, P., Baudart, J., de-Roubin ‹M. and Laurent, P. (2002). Detection and enumeration of coliforms in drinking water: current methods and emerging approaches. J. Microbiol. Meth. 49:31-54.

Scott, E. and Bloomfield, S.F. (1990). The survival and transfer of microbial contamination via cloths, hand and utensils. J. Appl. Bacteriol. 68: 271-278.

Sheikh, F.A.; Rostami, S.; Amin, M.; Armaghan, A.; Hamed, G. and Mohamed, H. (2013). Isolation and identification of *E. coli* $O_{157}H_7$ from ground beef hamburger in Khuzestan Province, Iran. 7(5): 413-417. 29 January 2013.

Soliman, M.R.; Abd El-Monem, K.H.M. and Saad, S.M. (2002). Microbiological quality of ready-to-eat meat products and fishes in Urban and Rural areas. J. Egypt. Vet. Med. Asso. Vol. 62, No. 6A: 39-51.

Temelli, S.; A. Eyigor and S. Anar (2012). Prevalence of *E. coli* O_{157}in red meat and meat products determined by VIDASECPT and light cycler PCR. Turk J. Vet. Anim. Sci., 36: 305-310.

Tortora, G.; Funke, B. and Case, C. (2013). Microbiology, An Introduction. 11^{th}Ed. Library of Congress Cataloging -in-Publication Data. Pearson, 19.

Varnam, A.H. (1991). Foodborne pathogens. 1st. Ed n. Wolfe Publication Ltd., PP: 71-76.

Varnam, A.H. and Evans, M.G. (1991). Food -borne pathogens. An illustrated text chapter 13, pp267 England, wolfe Publishing Ltd. ISBN 07234, 1521,8.

Wang, G.; Clark, C.G. and Rodgers, F.G. (2002). Detection in *Escherichia coli* of the genes encoding the major virulence factors, the genes defining the O_{157}:H_7 serotype, and components of the type 2 shiga toxin family by multiplex PCR, J. Clin. Microbiol. 40: 3613-3619.

Wang, Y.X.; Ye, Z.Z.; Si, C.Y. and Ying, Y.B. (2011). Subtractive inhibition assay for the detection of *E. coli*O_{157}:H_7 using surface plasmon resonance. Sensors 2011, 11, 2728-2739.