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STUDY ON SOME ENTEROPATHOGENS OF STREET VENDED MEAT MEALS

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ABSTRACT

Sixty random samples of street vended meat meals including 20 samples each of Shawerma, Liver sandwich and Hawawshi were randomly collected from different street vendors at Damanhur city Elbehaira Governorate for chemical and microbiological evaluation. The results revealed that, the mean values of total volatile nitrogen (TVB-N), Thiobarbituric acid (TBA) and Aerobic Plate count (APC) were 27.8±0.48, 25.8±0.17, 27.6±0.41 mg/100gm; 0.71±0.02, 4.2±0.33, 0.73±0.01mgMD/kg and $7.5x10^4$±$0.85x10^4$, $4.8x10^4$±$0.50x10^4$, $4.5x10^4$±0.48 $x10^4$cfu/g in the examined samples of Shawerma, liver sandwich and Hawawshi, respectively. Also, enterococci, enteropathogenic *E.coli*, Salmonella and Shigella were isolated with an incidence of 25, 20, 25 %; 30, 20, 25 %; 10, 15, 0 % and 15, 10,10% from the examined samples of Shawerma, liver sandwich and Hawawshi, respectively. Serotyping of the obtained isolates of enteropathogenic *E. coli* revealed the detection of *O157: H7, O128: K67, O86: K61, O26: K60* and *O26: K72* strains at varying rates, Stereotyping Salmonella isolates revealed the detection of *S. Enteritidis, S. Typhi* and *S. Paratyphi*. In addition to, biochemical identification of the obtained Shigella isolates revealed the detection of *S. dysenteriae, S. flexneri* and *S. sonnei*. Two strain of isolated *E.coliO157:H7* and one salmonella isolates were investigated by using PCR to detect presence of virulent genes stx1 (614 bp) and stx2 (779 bp) in *E.coli* O157:H7 and stn. Gene (617 bp) in Salmonella. From previously mentioned results, enteric pathogens still constitute common contaminants of street vended meat meals and their presence was very important due to its public health significance therefore strict hygienic measures must be applied to obtain safe and wholesome street vended meat meals.

Key words: Meat meals, TVB-N, TBA, *E.coli, O157:H7 Salmonella, Shigella*.

INTRODUCTION

Street-vended meat meals are ready-to-eat (RTE) foods, excellent source of high quality protein, mineral and vitamins, prepared and sold by vendors on streets and public places. They provide a source of readily available, inexpensive and nutritional meals, while providing a source of income for the vendors (Swanepoel *et al.,* 1998). Despite the economic and nutritional benefits of street foods, the consumption of these meat meals increase the risk of food borne diseases as these meals are readily contaminated from different sources (Tambekar *et al.,* 2008).

In Egypt, the most ready-to-eat sandwiches sold in street vendors and fast food restaurants are Shawerma, liver sandwich and Hawawshi. Poor quality of raw materials, inadequate personnel hygiene of street vendor's and holding for long period lead to contamination of street vended foods with pathogenic microorganism. Such contamination may render the product of inferior quality or unfit forhuman consumption (Gundogan *et al.,* 2005).

TVB-N value was more useful for assessing the degree of meat deteri0ration than for evaluating the changes occurring during storage. TBA is agood indicator of the quality of meat products Its value used as indicator for degree of lipid oxidation in meat (Elshafay, 2014).

Unhygienic sanitary conditions in which street vended meat meals are prepared, stored and served raise the microbial load. High bacterial counts and a high incidence of foodborne pathogens in such foods have been reported many Researchers in different countries; (Khaitsa *et al.,* 2007).

Salmonella is found worldwide and universally recognized as zoonotic agent. Many foods particularly of animal origin and those subjected to sewage pollution had been identified and must be taken into considerations as a vehicle for transmitting such pathogen to human being (ICMSF, 2006).

Most Pathogenic bacteria such as *Escherichia Coli*, salmonella species and Shigella have been implicated in a number of food borne illnesses and cause a

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variety of zoonotic infection in human and animals (Nouichi and Hamdi, 2009). *E. coli* has become recognized as a serious food borne pathogen and has been associated with numerous outbreaks of disease in the UK, Japan and USA (Scotter *et al.,* 2000).

Escherichia coli O157:H7 is considered one of the most serious of known foodborne pathogens (Blanco *et al*., 2003). *Escherichia coli O157:H7* strain produces shiga toxin 1 (stx-1) and shiga toxin 2 (stx-2) which are also referred as verotoxins. *Escherichia coli O157:H7* strains carrying stx genes along with enterohaemolysin (hlyA) and intimin (eae) genes are potentially dangerous to human health (Manna *et al.,* 2006).

Shiga toxins and intimin are key virulence factors for the pathogenesis of Escherichia coli O157 and other Enteropathogenic *E.coli* (EPEC) strains (Gyles, 2007). Shiga toxin–producing *Escherichia coli (STEC)* have emerged as important enteric food born zoonotic pathogens of considerable public health significance in Egypt (Ahmed and Shimamoto, 2015) and worldwide (Brooks *et al.,* 2005). STEC comprise a diverse group that elaborate one or both Shiga toxins (stx1 and stx2) and can cause diarrhea, hemorrhagic colitis, and hemolytic uremic syndrome in human beings (Grant *et al.,* 2011). Both STEC O157 and non-O157 are bacteria that cause serious human disease outbreaks through the consumption of contaminated food products (Gyles, 2007), but most of these STEC infections are caused by *E. coli O157:H7* (20–70%) throughout the world are attributed to *E. coli* of non-O157 (Brooks *et al.,* 2005).

Presence of specific microorganisms such as *E.coli*, *Salmonella* and *Shigella* in foods served by the street vendors is an indicative of a degree of ignorance on the part of the handlers towards proper hygienic practices (Lues *et al.,* 2006). Also the major sources contributing to microbial contamination are the place of preparation, utensils for cooking and serving, raw materials, time and temperature abuse of cooked foods and the personal hygiene of vendors (Rane- Sharmila, 2011).

The virulence of *Salmonella* is linked to a combination of chromosomal and plasmid factors. Different genes such as inv, spv, fimA and stn have been identified as major virulence genes responsible for salmonellosis (Darwin and Miller, 1999). The enterotoxin production gene is mediated by the stn thus it plays a significant role in causing gastroenteritis by producing enterotoxin (Chopra *et al.,* 1987).

In Egypt, there is lack of information about the incidence of food-borne diseases and problems related to consumption of street vended meat meals. Therefore, the aim of the present study was to evaluated street vended meat meals (Shawerma, Liver sandwich and Hawawshi) chemically through determination of Total Volatile Nitrogen (TVB-N) and Thiobarbituric acid (TBA), bacteriologically through determination the incidence of enterococci, Enteropathogenic *Escherichia coli* and Salmonella and Shigella species in these meals and serotyping of isolated *E.coli*, salmonella and Shigella strains. Also, using PCR technique for detection of virulence gene in Salmonella isolates (Stn gene) and Stx2 gene in *E.coli* isolates.

MATERIALS AND METHODS

2.1. Collection of samples:

A total of 60 random samples of street vended meat and liver meals including; Shawerma, Hawawshi, and Liver sandwich (20 samples of each) were collected from different street vendors at Damanhour city. Samples were kept in a separate sterile plastic bag and transferred in an ice box as soon as to the laboratory of the Food Control Department, inanimal health research institute under possible aseptic conditions. The samples were subjected to the following examinations:

2.2. Determination of chemical parameters:

- Determination of Total Volatile Nitrogen (TVB-N) (mg %): The technique of Conway's test was applied "FAO" (1980)
- Determination of Thiobarbituric acid number (TBA) (mg malonaldehyde /kg sample). The applied technique was recommended by (Kirk and Sawyers, 1991).

2.3. Preparation of samples for bacteriological examination (ICMSF, 1996):

Twenty-five grams of each sample were aseptically transferred into sterile blender flask containing 225 ml of sterile peptone water 1% and homogenized at 14000 rpm for 2.5 minutes.

2.4. Bacteriological examination:

- Aerobicplate countaccording to (ICMSF, 1996) using plate count agar (Oxoid).
- Enterococci count on Kanamycin Esculin Azide Agar (KAA) (Suzzi *et al.,* 2000).
- Isolation and identification of *E. coli* according to (ICMSF, 1996).
- Serological Identification of *E. coli:* All *E. coli* isolates were subjected to serological typing by slide agglutination test according to Lee *et al.,* (2009) using standard polyvalent and monovalent *E. coli* antisera (Seiken, Japan). Only fresh bacterial culture from 24 hours' age colonies onto nutrient agar media were used.

- Isolation and identification of Salmonellae and Shigella carried out according to the method described by USA/FSIS (2004) with a slit modification in the type of the used selective media.

- Serotyping of Salmonella and Shigella: All salmonella and Shigella isolates were subjected to serological typing by slide agglutination test in animal health research institute according to Grimont and Weill (2007) using standard polyvalent and monovalent salmonella antisera (Seiken, Japan). Only fresh bacterial cultures from 24 hours' age colonies onto nutrient agar media were used.

2.5. PCR techniques:

- **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^{O}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.

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

- **PCR amplification.** 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 pmolconcentration, 4.5 µl of water, and 6 µl of DNA template. The reaction was performed in an Appliedbiosystem 2720 thermal cycler.

- **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 PCR product were loaded in each gel slot. A gene ruler 100 bp DNA Ladder (Fermentas, Germany) 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		
stn	TTG TGT CGC TAT CAC TGG CAA CC ATT CGT AAC CCG CTC TCG TCC	617	94˚C 5 min.	94˚C 30 sec.	58˚C 45 sec.	72˚C 45 sec.	72˚C 10 min.	**Murugkar *et al*., 2003**
Stx1	5′ ACACTGGATGAT CTCAGTGG ′3 5′ CTGAATCCCCCT CCATTATG ′3	614	94˚C 5 min.	94˚C 30 sec.	58˚C 45 sec.	72˚C 45 sec.	72˚C 10 min.	**Dhanashree and Mallya (2008)**
Stx2	5′ CCATGACAACGG ACAGCAGTT ′3 5′ CCTGTCAACTGA GCAGCACTTTG ′3	779	94˚C 5 min.	94˚C 30 sec.	58˚C 45 sec.	72˚C 45 sec.	72˚C 10 min.	**Dhanashree and Mallya (2008)**

RESULTS

Table 1: Statistical analytical results of total volatile nitrogen (TVB-N) mg/100 gm in examined samples of street vended meat meals.

Products	Min	Max	X ±SEM
Shawerma	25.48	30.80	27.8±0.48
Liver Sandwich	24.75	26.60	25.8±0.17
Hawawshi	25.00	30.30	27.6±0.41

Minimum=MinMaximum=MaxMean= XStandarderror =**SEM**

Table 2: Statistical analytical results of thiobarbituric acid (TBA) mgMD/kg in examined samples of street vended meat meals.

Products	Min	Max	X ±SEM
Shawerma	0.616	.827	0.71±0.02
Liver Sandwich	2.62	6.07	4.2±0.33
Hawawshi	0.600	.835	0.73±0.01

Minimum= Min Maximum=Max Mean = X Standard error = **SEM**

Table 3: Statistical analytical results of aerobic plate count in examined samples of street vended meat meals.

Products	Min	Max	X ±SEM
Shawerma	$1.3x10^4$	$1.5x10^5$	$7.5x10^4 \pm 0.85x10^4$
Liver Sandwich	$1.6x10^4$	$9.5x10^4$	$4.8x10^4 \pm 0.50x10^4$
Hawawshi	$1.2x10^4$	$8.0x10^4$	$4.5x10^4 \pm 0.48x10^4$

Minimum = Min Maximum= Max Mean= X Standard error = **SEM**

Table 4: Incidence of isolated enterococci in examined samples of street vended meat meals.

Products	No. of examined samples	Positive samples	
		NO.	Percent (%)
Shawerma	20	5	25
Liver Sandwich	20	4	20
Hawawshi	20	5	25

Table 5: Incidence of enteropathogenic *E.coli* in examined samples of street vended meat meals.

Products	No. of examined samples	Positive samples	
		NO.	Percent (%)
Shawerma	20	6	30
Liver Sandwich	20	4	20
Hawawshi	20	5	25

Table 6: Serotyping of enteropathogenic *E.coli* isolated from examined samples of street vended meatmeals.

Products	Shawerma		Liver Sandwich		Hawawshi	
Strains	**NO**	**%**	**NO**	**%**	**NO**	**%**
O157:H7	1	5	0	0	1	5
O128: K67	2	10	2	10	1	5
O86: K61	0	0	1	5	1	5
O26: K60	1	5	1	5	2	10
O26: K72	2	10	0	0	0	0
Total	6	30	4	20	5	25

Table 7: Incidence of Salmonella in examined samples of street vended meat meals.

Products	No. of examined samples	Positive samples	
		NO.	Percent (%)
Shawerma	20	2	10
Liver Sandwich	20	3	15
Hawawshi	20	0	0

Table 8: Serotyping of Salmonella species isolated from examined samples of street vended meat meals.

Products	Shawerma		Liver Sandwich		Hawawshi	
Strains	**NO**	**%**	**NO**	**%**	**NO**	**%**
S. enteritidis	1	5	1	5	0	0
S. typhi	1	5	1	5	0	0
S. paratyphi	0	0	1	5	0	0
Total	2	10	3	15	0	0

Table 9: Incidence of Shigella in examined samples of street vended meat meals.

Products	No. of examined samples	Positive samples	
		NO.	Percent (%)
Shawerma	20	3	15
Liver Sandwich	20	2	10
Hawawshi	20	2	10

Table 10: Sero-typing of Shigella species isolated from examined samples of street vended meat meals.

Products	Shawerma		Liver Sandwich		Hawawshi	
Strains	**NO**	**%**	**NO**	**%**	**NO**	**%**
S. dysenteriae	1	5	1	5	0	0
S. Flexneri	1	5	1	5	1	5
S. Sonni	1	5	0	0	1	5
Total	3	15	2	10	2	10

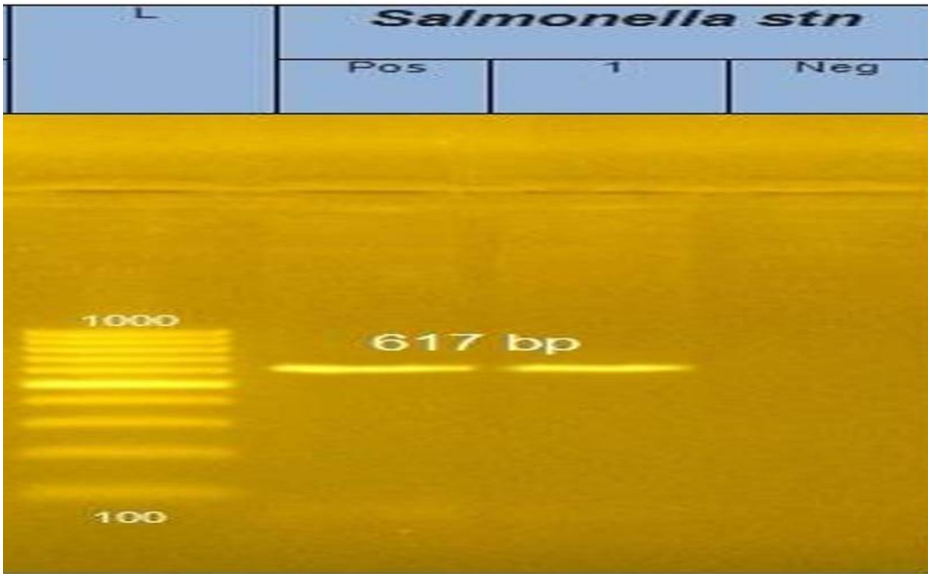

Fig. (1): Agarose gel showing polymerase chain reaction amplification products of stn gene (617 bp) in one Salmonella isolates, L:00 bp ladder as molecular size DNA marker, pos: control positive for shiga toxins 1 (stx1) and shiga toxin 2(stx2), Neg: control negative lane 1: positive strain for stn gene (617 bp).

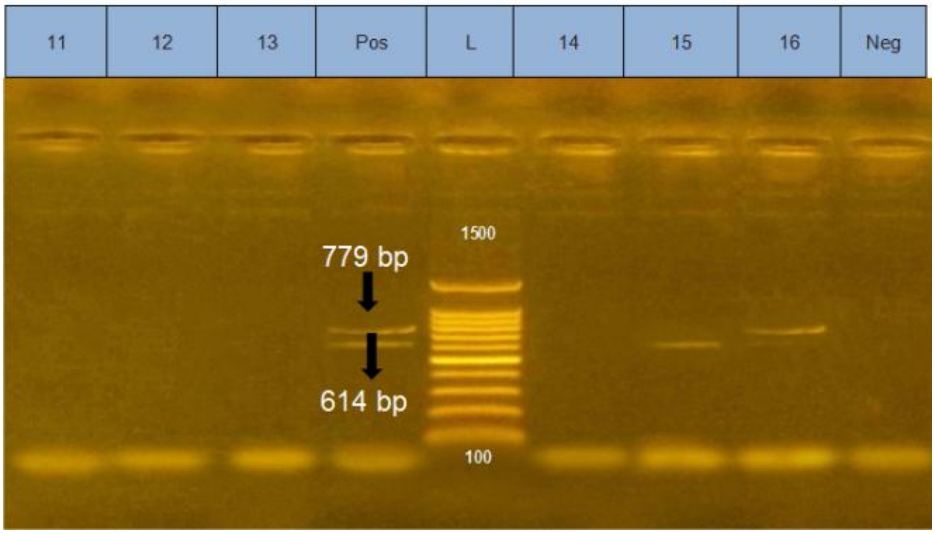

Fig. (2): Agarose gel showing polymerase chain reaction amplification products of stx1 (614 bp) and stx2gene (779 bp) in two *E.coli* O157:H7 strain, L:00 bp ladder as molecular size DNA marker, pos: control positive for shiga toxins 1 (stx1) and shiga toxin 2(stx2), Neg: control negative for stx1 and stx2, lane 15: positive strain for stx1, lane 16: positive strain for stx2.

DISCUSSION

TVB-N value was more useful for assessing the degree of meat deterioration than for evaluating the changes occurring during the first storage stages (El-Marrakchi *et al.,* 1990).

Presented data in Table (1) revealed that TVB-Nmean values in the examined Shawerma, liver sandwich and Hawawshi were 27.8±0.48, 25.8±0.17 and 27.6±0.41, respectively. The present data in Table (1) showed that, all examined Shawerma, and Hawawshi were higher than the permissible limit according to EOS, (2005) for TVN in red meat, should not exceed (20 mg/ 100 gm). Examined liver samples were within permissible limits according to EOS, (2005) for TVN in edible offal (should not exceed 30 mg / 100 gm).

Ibrahim *et al.* (2013) they reported that mean values of TVB-N of 30 liver samples collected from two traditional abattoirs of Elbehira province was 13.06 ± 0.04.

Metabolization of meat s low molecular substances, such as sugar and free amino acids and the release of undesirable volatile metabolites. As soon as the glucose present in aqueous phase has been exhausted other substrates are consequently utilized by metabolizing microorganisms to produce odoriferous

nitrogenous compounds, the most predominant of which is ammonia (Stanbridge and Davis, 1998). Microbial loads from 10 ^{7}cfu /cm-2 have been associated with occurrence of off-odors when the loads increased to as high as 10^{9}cfu/cm-2 when the meat becomes putrid (Jay, 2000).

TBA is a good indicator of the quality of meat. TBA value is a widely used indicator for the assessment of degree of lipid oxidation (Raharjo and Sofos, 1993). Moreover, Kautsoumanis *et al.* (2008) Stated that lipid oxidation is a complex process whereby, unsaturated fatty acid react with molecular oxygen leading to degradation of lipids and development of oxidative rancidity

Results achieved in Table (2) revealed that (TBA mg malonaldehyde/kg of sample) mean values in examined Shawerma, liver sandwich and Hawawshi were 0.71±0.02, 4.2±0.33 and 0.73±0.01, respectively. These results showed that, all examined samples Shawerma, and Hawawshi were within the permissible limit according to EOS, (2005) which stated that the maximum permissible limit for (TBA) in meat and edible offal should not exceed 0.9 mg malonaldehyde / kg of sample. The examined samples of liver were higher than the permissible limit for TBA according to EOS, (2005) that could be attributed to the use rancid oil in preparation of liver for sandwich.

Ibrahim *et al.* (2013) they reported that mean values of TBA of 30 liver samples collected from two traditional abattoirs of Elbehira province was 0.16 ± 0.01.

Microbiological quality problems of street vended meat meals depend greatly on low initial quality of raw materials and other ingredients, insufficient cooking process and improper sanitary practices for personal and for cooking/processing utensils (Kayaardi *et al.,* 2006). Even though their ingredients reach a temperature that is ideal to ensure that the food is cooked thoroughly, cross contamination during preparation of these meals could be traced back to the use of uncooked green vegetables and unhygienic handling (Reij and Aantrekker, 2004).

The obtained results presented in Table (3) revealed that aerobic plate count of Shawerma, liver sandwich and Hawawshi ranged from 1.3×10^{4} to 1.5×10^{5}, 1.6×10^{4} to 9.5×10^{4} and 1.2×10^{4} to 8.0×10^{4} with an average count $7.5x10^{4}\pm0.85x10^{4}$, $4.8x10^{4}\pm0.50x10^{4}$ and $4.5x10^{4}\pm0.48x10^{4}$cfu/g, respectively. APC is tended to indicate the level of microorganisms in products (FDA, 2001).

The present results nearby results obtained by (Mohammed *et al.,* 2004) they reported that mean values for aerobic plate count of Shawerma, liver, and El-Hawawshi sandwiches were $29x10^{3}\pm4.9x10^{3}$, $27x10^{3}\pm1.8x10^{3}$, and $20x10^{4}\pm2.6x10^{4}$cfu/g, respectively. Also, similar to El Zekaty *et al.* (2016) they reported that mean values of APC of Hawawshi, liver sandwiches and Shawerma were $8.9\times10^{5}\pm 1.1\times10^{5}$, $5.2\times10^{5}\pm1.3\times10^{5}$ and $5.4\times10^{5}\pm1.2\times10^{5}$cfu/g, respectively.

Higher finding of APC were recorded by (Abou, 1995 and Mohamed, 2001) in Alexandria and Assiutcity where microbial investigation of 30 samples of roasted liver sandwich revealed that the average counts was 8×10^{6}cfu/g in Alexandria city and 4×10^{7}cfu/gin a related study in Assiut city. On the other hand, Mohamed *et al.* (2004) and El-Mossalami *et al.* (2008) found lower incidence of APC of liver sandwiches in another research in Assiut and Alexandria city.

Dalia *et al.* (2013) they reported that the log count of aerobic plate count in all liver sandwich samples ranged from 3.73 to 5.99.

The data presented in Table (4) showed that the incidence of enterococci in the examined samples of Shawerma, Liver sandwich and Hawawshi was 25, 20 and 25 %, respectively.

The recorded data in Table (5) illustrated that the incidence of enteropathogenic *E.coli* in the examined samples of Shawerma, Liver sandwich and Hawawshi was 30, 20 and 25 %, respectively.

Our finding agreed with (Emara *et al.*, 2016) they reported that the incidence of *E.coli* in examined Shawerma, Liver sandwich, Hawawshi (50 samples of each) was 30, 20 and 26 %. higher result recorded by Hiko A *et al.* (2008), Rahimi *et al.* (2012) and Jacob *et al.* (2013) while, lower finding recorded by Ahmad *et al.* (2013).

Heating food at high temperature 51ºC decrease the survival of E. coli cells after 10 min. and no colonies were observed after heating for 180 minute, viable E. coli were not found after at 53 ºC for 60 min (Nakano *et al.,* 2012).

The recorded data in Table (6) illustrated that serotyping of the obtained isolates of enteropathogenic *E. coli* revealed the detection of O_{157}: H_7 strain at the rate of 5, 0 and 0%, O_{128}: k_{67} strain at the rate of 10, 10 and 5%, O_{86}: K_{61} strain at the rate of 0, 5 and 5%, O_{26}: K_{60} strain at the rate of 5, 5 and 10%, O_{26}: K_{72} strain at the rate of 10, 0 and 0% from the examined samples of Shawerma, Liver sandwich and Hawawshi, respectively.

Our findings agree with Emara *et al.* (2016) they could isolate enteropathogenic *E. coli* and serotyped, they found that the detection rate of O_{26}: K_{60} (B6) strain were 4, 6 and 2 %; O_{128}: K_{67} (B12) strain at the rate of 4, 2 and 4 %; O_{86}: K_{61} (B7) strain at the rate of

4, 4 and 6 % and O_{157}: H_7 strain at the rate of 4, 4 and 6 % from the examined samples of Shawerma, Liver sandwich and Hawawshi, respectively.

E. coli O_{157} more serious strain of *E.coli* isolates, so detection of *E. coli* O_{157}:H_7 and other Shiga toxin-producing *E. coli* (STEC) in ready-to-eat food possesses a public health hazards (EFSA, 2014).

As shown in Fig (2) both Shiga toxin stx1 and stx 2 genotypes occurred among two STEC O157 strains isolated from Shawerma and Hawawshi.

Detection of *E. coli* O_{157} and other verocytotoxin producing *E. coli* (VTEC) in different meat sources considered a high microbiological Risk, potentially injurious to health and / or unfit for human consumption (European Commission, 2002).

Shiga toxin (stx) producing *E. coli* (STEC) contamination in food is one of the most recognized concerns and a major financial burden in human hygiene control worldwide. Rapid and highly reliable methods of detecting and identifying STEC causing gastroenteric illnesses are crucial to prevent food borne outbreaks. In order to screen pathogenic STEC without relying on O:H serotyping, we developed a rapid detection and genotyping assay for STEC virulence genes using a PCR for detection of major virulence genes, Shiga toxin 1 and 2 (stx1 and stx2), intimin (eae) (Goji *et al.*, 2015).

Shiga toxin-producing *Escherichia coli* (STEC), encompassing E. coli O157 and non-O157 STEC, are a significant cause of food-borne illnesses and deaths in the United States and worldwide. Shiga toxins (encoded by stx1 and 2) are important virulence factors for STEC strains linked to severe human illnesses (Fei *et al.,* 2012). These groups are important for determining potential pathogens, the presence of virulence attributes, such as stx1 and stx2 are important parameters for pathogenicity of the strains (Johnson *et al.*, 1996).

Salmonella is worldwide foodborne illness transmitted to human through food of animal origin, sodetection of Salmonella species in different street vended meat meals was considered a high microbiological risk, potentially injurious to health and/ or unfit for human consumption (European Commission, 2002). Salmonella must be absent in relevant products when placed on the market, during their shelf-life (European Commission, 2005).

The recorded data in Table (7) illustrated that the incidence of Salmonella in the examined samples of Shawerma and Liver sandwich was 10 and15 %, while, Salmonella cloud not be detected from Hawawshi. Presence of street vended meat meals objectionable and indicated high microbiological risk, so Salmonella must be absent in these food (European Commission, 2005). In addition, the obtained results in Table (8) showed that the serotyping of Salmonella isolates revealed detection of Salmonella enteritidis at rate of 5,5 and 0 %, and Salmonella typhi at rate of 5, 5 and 5%.

The results of present study nearby the results obtained by Emara *et al.* (2016) they could isolate *S. Enteritidis* strain at the incidence rate of 2 and 2 % from the examined samples of Shawerma and Liver sandwich, respectively; detect S. Typhi strain at the incidence rate of 2 and 4 % from the examined samples of Shawerma and Liver sandwich, respectively and *S. Paratyphi* failed to be detected in Hawawshi samples.

The present data revealed that incidence of Salmonella in liver sandwich was 15 %, these finding agree with Abd El-Malek (2014) while, disagree with Büyükyörük *et al.* (2014) and Salma *et al.* (2015) where *Salmonella* failed to be isolated from cooked liver sandwiches, while disagreed with *Salmonella* causing food poisoning infection, most common symptoms of Salmonellosis were diarrhea, abdominal cramps and fever within 8 to 72 hours. Other symptoms including fever, headache, nausea and vomiting that can last up to 7 days (FSIS, 2008).

As shown in Fig (1) showing that one salmonella isolate produced 617 bp DNA fragment specific for stn gene which responsible for virulence. Thus, this Salmonella isolate was found highly invasive and enterotoxigenic.

The recorded data in Table (9) illustrated that the overall incidence of Shigella in the examined samples of street vended meat meals in the current study was 35 % and the incidence of Shigella in the examined samples of Shawerma, Liver sandwich and Hawawshi was 10, 10 and 15 %, respectively. The obtained results recorded in examined samples were higher than that recorded by Ali *et al.* (2010). On contrary, Dabassa and Bacha, (2012) detected no Shigella on examined meat samples. Detection of Shigella spp. in ready-to-eat food possesses apublic health hazards (EFSA, 2014).

Detection of Shigella was considered a high microbiological risk. Potentially injurious to health and /or unfit for human consumption were recorded (European Commission, 2002).

Moreover, the recorded data in Table (10) clarified that biochemical identification of the obtained isolates of Shigella species from the examined samples of street vended meat meals revealed detection of *S. dysenteriae* at the rate of 5, 5 and 0%; *S. flexneri* at the rate of 5, 5 and 5% and *S. sonnei* at the rate of 5, 0 and 5 % from the examined samples of Shawerma, Liver sandwich and Hawawshi, respectively.

Our finding agrees with Emara *et al.* (2016) they could have isolated Shigella from examined street vended meat meals at incidence rate 10%, also, they found after biochemical identification of isolated Shigella strains that *S. dysenteriae* isolated at incidence rate of 4, 6 and 4 %; *S. flexneri* at incidence rate of 4, 2 and 2 % and *S. sonnei* at incidence rate of 2, 4 and 2% from the examined samples of Shawerma, Liver sandwich, and Hawawshi, respectively.

Results of the present study revealed that incidence of Shigella in liver sandwich was 10 %, lower incidence of Shigella obtained by Salma *et al.* (2015) demonstrate that incidence of *Shigella* was 1(3%) in cooked liver sandwiches. While, higher incidence of *Shigella* obtained by Abd El-Malek (2014) who could detect *Shigella* in 23% of liver sandwich (kebda) samples.

Presence of *Shigella* in street vended meat meals indicate a human fecal contamination due to Shigella transmission by the fecal-oral route, including direct person-to-person contact and may be indirect through ingestion of contaminated food or water (Jomezadeh, 2014), so it is most likely to occur in children and those who neglect to clean hands thoroughly.

CONCLUSION AND RECOMMENDATION

From the results it be concluded that street vended meat meals in Damanhur city constitutes a likely potential hazard to human health. The presence of enterococci, enteropathogenic *E. coli, salmonella and Shigella* in these mealsindicates fecal contamination, so consumption of these meals cause possible risk of infection. Using of high quality raw materials, efficient heat treatment, adequate cleaning and sanitization of utensils may assist reducing this contamination, training of all food handlers and implement a hazard analysis may lead to an improvement in hygienic, finally the policy makers should address legislations for street-vendors to assure their personal hygiene and sanitation.

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دراسة عن بعض الممرضات المعوية لوجبات اللحوم من الأسواق

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تم تجميع ٦٠ عينة من الشاورما وساندوتشات الكبدة والحواوشى (٢٠ عينة من كل نوع) جمعت بطريقة عشوائية من مدينة دمنهور بمحافظة البحيرة. وكشفت النتائج أن متوسط النيتروجين الكلى المتصاعد فى عينات الشاورما، ساندوتشات الكبدة والحواوشى كان كالتالى (٠.٤٨ ± ٢٧.٨)، (٠.١٧ ± ٢٥.٨) و(٠.٤١ ± ٢٧.٦) وكان متوسط حمض الثيوباربتيورك فى عينات الشاورما، ساندوتشات الكبدة والحواوشى كالتالى (٠.٠٢ ± ٠.٧١)، (٠.٣٣ ± ٤.٢) و (.٠٧٣ ± 0.01). أظهرت النتائج أن متوسط العد الكلى للبكتريا كان ($7.5x10^4$±$0.85x10^4$)، ($4.8x10^4$±$0.50x10^4$)و ($4.5x10^4$±$0.48x10^4$) فى عينات الشاورما، ساندوتشات الكبدة والحواوشى على التوالى وأظهرت النتائج أن نسبة الاصابة بميكروب المكورالمعوى والبكتربا القولونية الضارة وميكروب السالمونيلا وميكروب الشيجلا ٢٥,٢٠ ،٢٥ ٪ و٣٠,٢٠، ٢٥ % و ١٠، ١٥، ٠ % و ١٥,١٠، ١٠ % في عينات الشاورما، ساندوتشات الكبدة والحواوشى على التوالى. وأوضح التصنيف السيرولوجى لميكروب الاشريكية القولونية الضارة عزل *O157:H7* , *O128:K67* *O86:K61*, *O26:K60*, *O25:K72* بنسب ٥، ١٠، ٠، ٥، ١٠ % و ٠, ١٠, ٥ ,٥, ٠% و ٥, ٥, ٥, ٠,١٠% فى عينات لشاورما، ساندوتشات الكبدة والحواوشى على التوالى، وتم تصنيف السالمونيلا سيرولوجيا ووجد أن السالمونيلا أنتريتيدس، السالمونيلا تيفاى والسالمونيلا بارتيفاى موجودة بنسبة ٥,٥,٠ % فى الشاورما و ٥,٥,٥% فى ساندوتشات الكبدة ولم يتم عزل السالمونيلا من الحواوشى أما بالنسبة للتصنيف السيرولوجى لميكروب الشيجلا كان كالتالى شيجلا الزحار ، شيجلا الفلكسنرى وشيجلا سونى بنسب ٥، ٥ ،٥ % و٥، ٥، ٠ % و ٠ ،٥، ٥ % فى عينات الشاورما، ساندوتشات الكبدة والحواوشى على التوالى. وقد تم أستخدام تفاعل البلمرة المتسلسل لإكتشاف جين stn فى معزولة السالمونيلا عند وزن (٦١٧ bp) وجينstx1 عند وزن (٦١٤ bp) وجين stx2 عند وزن (٧٧٩ bp) فى معزولتين للايشيريكيا القولونية *O157:H7* من الحواوشى والشاورما. هذا وقد تم دراسة ومناقشة الاهمية الصحية للميكروبات المعزولة وكذلك الشروط الصحية الواجب توافرها أثناء إعداد وتقديم هذه الساندوتشات لتجنب خطر هذه الميكروبات الضارة. مع اهمية استخدام لحوم وكبد جيدة المواصفات مع المعاملات الحرارية اللازمة وضرورة تشديد الرقابة على محلات البيع.