j.Egypt.net.med.Assoc 77, no 1. 103 – 125 (2017)

DETECTION OF VIRULENT GENES OF SOME FOODBORNE BACTERIAL PATHOGENS TRANSMITTED BY READY-TO-EAT SHAWARMA FROM RESTAURANTS USING REAL-TIME PCR

By

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ABSTRACT

Greater numbers of people take their meals in restaurants, food chains and by street food vendors. Unhygienic preparation of ready-to-eat food provides generous opportunities for contamination, growth or survival of foodborne bacterial pathogens. The present study was carried out to detect some foodborne bacterial pathogens in ready-to-eat shawarma from restaurants using traditional methods and real-time PCR. A total of 80 samples of shawarma, (40 each of chicken-shawarma and beef-shawarma sandwiches) were randomly collected from different restaurants in various districts of Damietta, Egypt. The obtained results using real-time PCR indicate that, the detection prevalence of pathogenic *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella* spp., were 62.5%, 40%; 5%, 25% and 10%, 7.5% in chicken-shawarma and beef-shawarma respectively. Moreover, the incidence of *Campylobacter jejuni* was 15% in chicken-shawarma only. While, *Listeria monocytogenes*, was not detected in all examined shawarma sandwiches samples. Serotyping of isolated *Escherichia coli* and *Salmonella* revealed that *Escherichia coli* O119:H4, O126:H27, O127:H6 and O111:H4 serovars while, *Salmonella* Enteritidis, *Salmonella* Typhimurium and *Salmonella* Dublin serovars were identified in ready-to-eat shawarma with varying percentages.The results of this investigation indicate that foodborne pathogens were present in shawarma constitute a potential public health hazard.

Keywords:

Shawarma/shawarma - RTE fast food - pathogenic *Escherichia coli* - *Staphylococcus aureus* - *Salmonella* spp. - *Listeria monocytogenes* - *Campylobacter jejuni* - real-time PCR.

INTRODUCTION

Good health starts with good nutrition, which can protect against diseases later in life. So, food safety remains a major challenge to food producers and to legislators endeavoring to

adequate consumer protection. Both man and animals live under a certain degree of "biological hazard" that occur in food and foodstuffs **(WHO, 1999)**. Although governments throughout the world are attempting to improve the safety of the food/food supply, the occurrences of foodborne diseases remain a significant health concern in both developed and developing countries **(WHO, 2011)**. Recently there is an increase in the consumption of ready-to-eat (RTE) fast food because of a change in social patterns characterized by increased mobility, large numbers of circuit workers and less family centered activities. Thus, good manufacturing practices of food taken outside the home such as good sanitation or sanitary measures and proper food handling have been transferred from individuals/families to the food vendor who rarely enforces such practice **(Musa and Akande, 2002)**. Different terms can be used to describe RTE food. These include convenient, ready, instant and fast food. RTE food can be described as the status of food being prepared and served very quickly and ready for immediate consumption at the point of scale or consumption at a later time, it could be raw or cooked and sold in a restaurants and served to the customer in a packaged form for take-out/take-away and can be consumed without further processing or preparation. Shawarma is considered as one of the most highly demanded and delicious products due to their high biological value, reasonable price and agreeable taste and easily served at fast food restaurant chains **(Tsang, 2002 and von Holy and Makhoane, 2006)**. RTE foods are usually not effectively protected from dust and flies which may harbor foodborne pathogens also safe food storage temperatures are difficult to be maintained. Thus, there is potential health risks associated with initial contamination of raw foods with pathogenic bacteria as well as subsequent contamination by vendors during preparation and through post-cooking handling and cross contamination as well as during the preparation of fillings and sandwiches **(WHO, 1989; Soriano *et al*., 2001; Harakeh *et al*., 2005 and McCown and Grzeszak, 2010)**. RTE food is manipulated extensively during processing and therefore has a potential for high bacterial contamination levels on the surface of the meat type as beef and chicken, as well as the inside. As a result, there is an increased risk of pathogens surviving and transferring not only by cross-contamination, but also by undercooking as observed in shawarma manufacturing **(Soriano *et al*., 2001 and Food Protection Services and BC Centre for Disease Control, 2012)**. Consumption of fast food has been reported to be associated with serious international health problems and important cause of reduced economic growth **(FDA, 2000; WHO, 2002, Pelczar *et al*., 2006)**. Foodborne diseases are widespread and are of great public health

concerns of the modern world. In developing countries, the greater population is largely affected by foodborne infections **(Akbar, Anal, 2013)**. RTE foods are becoming increasingly popular in the world and could be easily contaminated with various pathogens **(Xing *et al*., 2014)**. Bacteria have accounted for more than 70% of deaths associated with foodborne transmission **(Hughes *et al*., 2007)**. Foodborne illnesses associated with *Listeria monocytogenes*, *Staphylococcus aureus*, *Bacillus* cereus, *Campylobacter* spp., *Escherichia coli* O157:H7, *Clostridium* spp., and *Salmonella* enteritis's, existing a major public health concern throughout the world **(Esteban *et al*., 2008; Isara *et al*., 2010**, **McCown, Grzeszak, 2010)**. The presence of these microorganisms can lead to many foodborne outbreaks **(Borch and Arinder, 2002, McCown, Grzeszak, 2010)**. Microbiological quality of RTE shawarma largely reflects its condition depending on quality of raw beef meat, chicken meat and/or other ingredients, cooking process, sanitary practices for workers and cooking processing utensils **(ICMSF, 2002 and Vazgecer *et al*., 2004)**. Although, a little of information about the incidence of foodborne diseases related to shawarma, in addition to the absence of microbiological standards for this RTE food product **(Nemati *et al*., 2008, Opeolu *et al*., 2010)**. Classical microbiological methods for detection of foodborne bacteria involved the use of pre-enrichment and/or specific enrichment, followed by the isolation of bacteria in solid media and a final confirmation by serological and/or molecular tests **(Malorny *et al*., 2003; Rodriguez-Lazaro *et al*., 2007, Prasad and Ambarish, 2009)**. Recently, international standards have been agreed the use of PCR-based detection of foodborne pathogens and legislations are implementing new types of analyses as the accepted official methods. For example, European regulation EC 2073/2005 allows the use of alternative detection methods based on certified analyses of international standards **(EC, 2005)**. Polymerase chain reaction (PCR) is a technique used for the rapid, specific and highly sensitive detection of food pathogens in a realistic period of time **(Iyer, Kumosani, 2010; Somer**, **Kashi, 2003 and Law *et al*., 2015)**. Real-time PCR has greatly increased the speed and sensitivity of PCR-based detection methods, where the use of fluorogenic probes in real-time PCR assays facilitates detection of only specific gene products thus eliminating detection of products that could result from non-specific amplification and can discover the false positive and false negative possible results **(Jinneman *et al*., 2003; Hanna *et al*., 2005; Sharma, 2006 and Jasson *et al*., 2010)**. Real-time PCR assays were developed to detect the foodborne pathogens, but it is not routinely used because of requirement of expensive specialized instruments,

reagents and trained personnel **(O'Grady *et al*., 2009; Molinos *et al*., 2010 and Jadhav *et al*., 2012)**. The present study was therefore, carried out to detect some foodborne bacterial pathogens in RTE shawarma sold in public restaurants in Damietta city using real-time PCR technique and to declare the potential public health risks for isolated pathogens.

MATERIAL AND METHODS

I. Samples collection:

A total of 80 samples of ready-to-eat shawarma (40 each of chicken-shawarma sandwiches and beef-shawarma sandwiches), were randomly purchased from different public restaurants in various districts of Damietta city. The samples were hygienically collected with their original forms, kept in a separate well labeled, clean, polyethylene bags and preserved in an insulated ice-box, then immediately transferred without delay to the Damietta sea port-food inspection laboratory for bacteriological evaluation.

II. Bacteriological analysis:

1. **Preparation of the samples (ISO 1999).**
2. **Isolation of *Escherichia coli* (FDA 2002).**
3. **Isolation of *Staphylococcus aureus* (FDA 2001).**
4. **Isolation of *Salmonella* species (ISO: 6579: 2002).**
5. **Isolation of *Listeria monocytogenes* (FDA 2011).**
6. **Isolation of *Campylobacter jejuni (*ISO: 10272-1, 2006).**

III. Serological confirmation of serotypable positive isolates:

a) Serological confirmation of *Escherichia coli* positive isolates:

Suspected *E. coli* isolates were serotyped in regional Co-Lab, Ministry of Health according to **(Kok*et al.*,1996)**, by using rapid diagnostic *E. coli* antisera sets (DENKASEIKEN Co., Japan).

b) Serological confirmation of *Salmonella species* positive isolates:

Suspected *Salmonella* isolates were serologically identified in regional Co-Lab, Ministry of Health according to **(Grimont and Weill, 2007)**.The definition of the serotypes is based on the antigenic combination present and is given inthe“Kauffmann-Whiteclassification scheme”.

IV. Molecular confirmation of positive isolates:

a) DNA extraction:

DNA was extracted from each isolate confirmed by biochemical tests, using bacterial DNA extraction kit (Magnetic particles-proteinase K protocol) (PrepseqTM nucleic acid extraction kit, Applied Biosystems) according to the manufacturer guidelines.

b) PCR primers and probe.

Species-specific primers and TaqMan probe sets targeting Intimin encoding gene (eae gene) specific for *Escherichia coli* were used according to (Sure Fast® *E. coli* eae gene PLUS detection kit, CONGEN). Simply for one reaction (25 µl) the master mix consists of 19.9 µl (reaction mix), and 0.1 µl (Taq polymerase), with a total volume 20 µl. The master mix includes an internal amplification control. Mix the solution by gently pipetting up and down, then cap the tube. Pipette 20 µl of master mix solution into each well to be used, gently pipetting at the bottom of the well. Close the tube or well of the negative control. Transfer 5 µL of unknown sample DNA into each sample well, gently pipetting up and down to mix the solution. Transfer 5 µL of positive control into the positive control well, gently pipetting up and down to mix the solution. Close tubes or apply an optical cover to the plate. Make sure reagents are in the bottom of the wells. If available, use a centrifuge with a plate adapter to briefly centrifuge the plate. Load the reaction plate into the real-time PCR system (Applied Biosystems 7500 fast). For *Staphylococcus aureus* species-specific primers and TaqMan probe sets targeting egc gene specific for *Staphylococcus aureus* were used according to (TaqMan® *Staphylococcus aureus* detection kit, Applied Biosystems). Genus-specific primers and TaqMan probe sets targeting invasion gene (invA gene) specific for *Salmonella* spp. were used according to (TaqMan® *Salmonella* spp. detection kit, Applied Biosystems).For *Campylobacter jejuni* species-specific primers and TaqMan probe sets targeting hipO gene specific for *C. jejuni* were used according to (TaqMan® *Campylobacter jujeni* detection kit, Applied Biosystems). The same real-time PCR protocol was used for detection of *Staphylococcus aureus*, *Salmonella* spp. and *Campylobacter jejuni* simply the reaction mix consists of 2X Environmental Master Mix (EMM) and 10X Target Assay Mix (TAM). For one reaction (30 µl) the reaction mix consists of 15 µl (EMM) and 3 µl (TAM) with a total volume 18 µl. Mix the solution by gently pipetting up and down, then cap the tube. Transfer 18 µl of premix solution into each well to be used, gently pipetting at the bottom of the well. Transfer 12 µL of unknown sample into each sample well, gently pipetting up and down to mix the solution. Transfer 12 µL of negative control into each negative control well, gently pipetting up and down to mix the solution. Complete the technique as above-mentioned. The sequences of the primers and TaqMan probe are showing in (Table A).

c) Thermo-cycling conditions.

Thermo-cycling conditions used for amplification of genes are showing in (Table B).

Table (A): Oligonucleotide primers for the detected foodborne bacteria in this study.

Primer	Target gene	Primer sequence (5`-3`)	Fragment size (bp)	Reference
eae-1	eae (Intimin encoding) genefor *Escherichia coli*	GGAACGGCAGAGGTTAATCTGCAG	775-bp	Blanco *et al.*, 2004
eae-2		GGCGCTCATCATAGTCTTTC	775-bp	Blanco *et al.*, 2004
egcAUf	egc gene for *Staphylococcus aureus*	CTTCATATGTGTTAAGTCTTGCAGCTT	82-bp	Fusco *et al*, 2011
egcAUr		TTCACTCGCTTTATTCAATTGTTCTG	82-bp	Fusco *et al.*, 2011
Sal-F	invA gene for *Salmonella* spp.	GTG AAA TTA TCG CCA CGT TCG GGC AA	139-bp	Malorny *et al.*, 2003
Sal-R		TCA TCG CAC CGT CAA AGG AAC C	141-bp	Malorny *et al.*, 2003
hipO-F	Hippuricase (hipO) gene for *Campylobacter jejuni*	AATGCACAAATTTGCCTTATAAAAGC	123-bp	Toplak *et al.*, 2012
hipO-R		TNCCATTAAAATTCTGACTTGCTAAATA	123-bp	Toplak *et al.*, 2012

Table (B): Thermo-cycling conditions for the primers used in this study.

Target gene	Settings	Stage 1 Enzyme activation	Stage 2 PCR	
	Number of cycles	1 (Hold)	45 cycles	
			Denature	Anneal /extend
eae gene	Temperature	95°C	95°C	60°C
	Time	5 min.	15 sec.	30 sec.
egc gene	Temperature	95°C	95°C	60°C
	Time	10 min.	15 sec.	45 sec.
invA gene	Temperature	95°C	95°C	60°C
	Time	2 min.	3 sec.	30 sec.
hipO gene	Temperature	95°C	95°C	58°C
	Time	10 min.	15 sec.	1 min.

RESULTS

Table (1): Incidence of *Escherichia coli* in shawarma samples.

Type of shawarma	No. of examined samples	Positive samples for *Escherichia coli*			
		Conventional method		Real-time PCR	
		No.	%	No.	%
Chicken-shawarma	40	27	67.5	25	62.5
Beef-shawarma	40	16	40	16	40

Table (2): Incidence of *Staphylococcus aureus* in shawarma samples.

Type of shawarma	No. of examined samples	Positive samples for *Staphylococcus aureus*			
		Conventional method		Real-time PCR	
		No.	%	No.	%
Chicken-shawarma	40	2	5	2	5
Beef-shawarma	40	10	25	10	25

Table (3): Incidence of *Salmonella* species in shawarma samples.

Type of shawarma	No. of examined samples	Positive samples for *Salmonella* species			
		Conventional method		Real-time PCR	
		No.	%	No.	%
Chicken-shawarma	40	5	12.5	4	10
Beef-shawarma	40	3	7.5	3	7.5

Table (4): Incidence of *Campylobacter jejuni* in chicken shawarma samples.

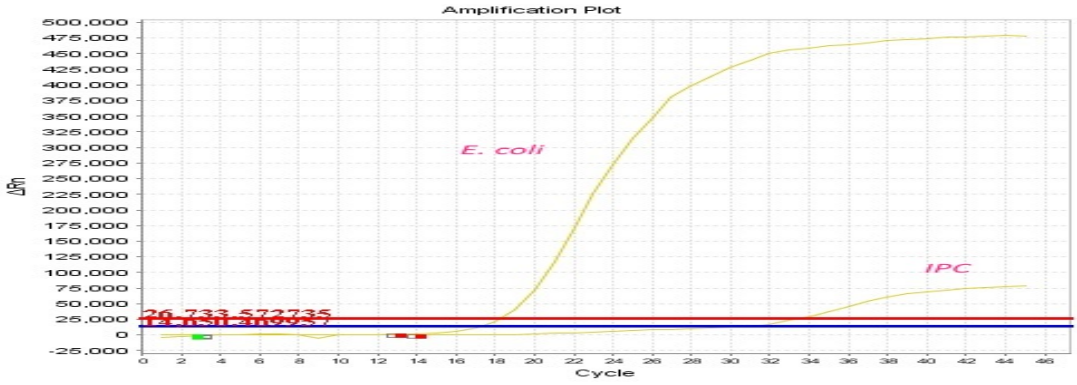
Type of shawarma	No. of examined samples	Positive samples for *Campylobacter jejuni*			
		Conventional method		Real-time PCR	
		No.	%	No.	%
Chicken-shawarma	40	7	17.5	6	15

Table (5): Serotyping of *Escherichia coli* isolated from shawarma samples.

Type of shawarma	Positive samples of *Escherichia coli* by Conventional method	Identified serovars	No. of serovars	% of serovars
Chicken-shawarma	27	O119:H4	12	44.44
		O126:H27	7	25.93
		O127:H6	5	18.52
		untypable	3	11.11
Beef-shawarma	16	O127:H6	9	56.25
		O111:H4	6	37.50
		untypable	1	6.25

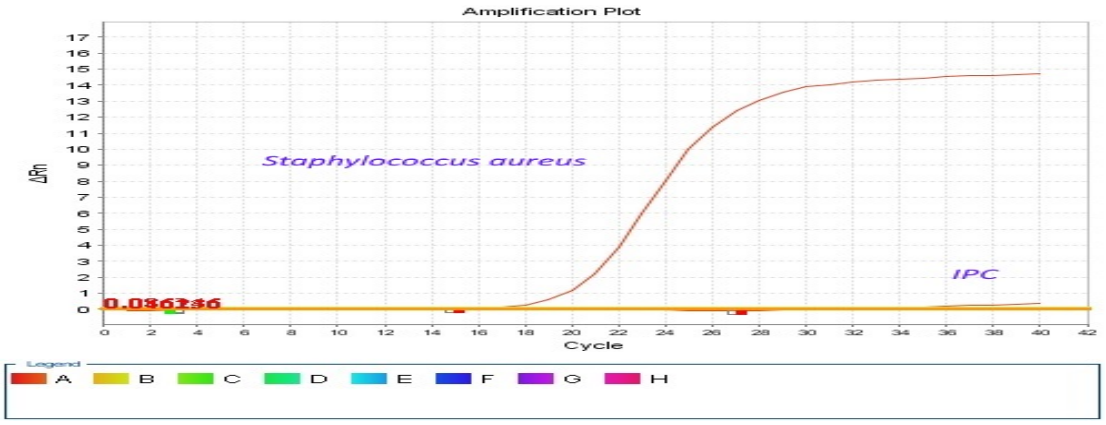
Table (6): Serotyping of *Salmonella* species isolated from shawarma samples.

Type of shawarma	Positive samples of *Salmonella* spp. by Conventional method	Identified serovars	No. of serovars	% of serovars
Chicken-shawarma	5	*Salmonella* Enteritidis	3	60.00
		Salmonella Typhimurium	1	20.00
		untypable	1	20.00
Beef-shawarma	3	*Salmonella* Typhimurium	2	66.67
		Salmonella Dublin	1	33.33



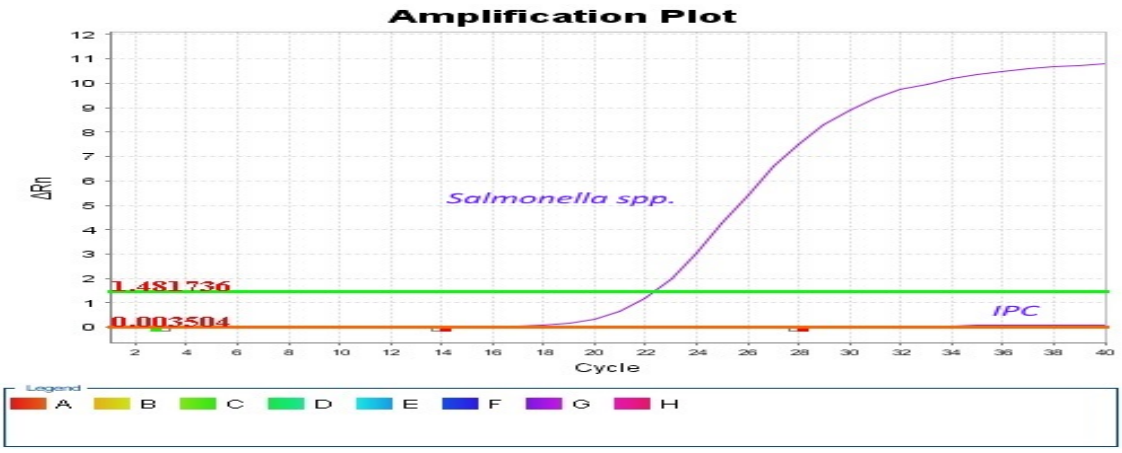
IPC: Internal Positive Control

Fig. (1): Amplification plot of positive shawarma for *Escherichia coli*.



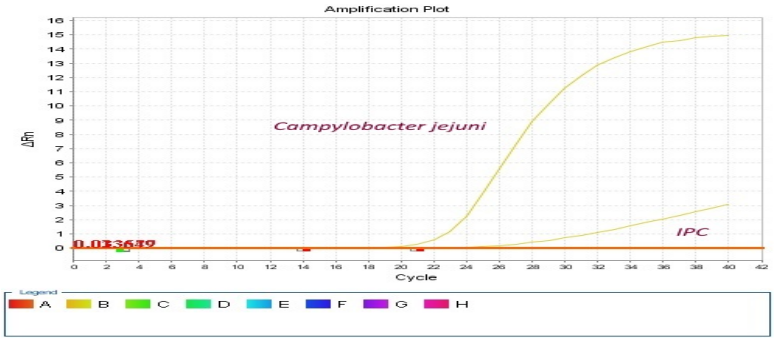
IPC: Internal Positive Control

Fig. (2): Amplification plot of positive shawarma for *Staphylococcus aureus*.



IPC: Internal Positive Control

Fig. (3): Amplification plot of positive shawarma for *Salmonella* species.



IPC: Internal Positive Control

Fig. (4): Amplification plot of positive chicken-shawarma for *Campylobacter jejuni*.

DISCUSSION

In the current study, real-time PCR based method was used for detection of pathogenic *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* spp., *L. monocytogenes* and *Campylobacter jujeni* in ready-to-eat shawarma. The sensitivity and specificity of this real-time PCR assay were explained by various investigators (**O'Grady *et al*., 2009; Jadhav *et al*., 2012 and Law *et al*., 2015)**. A few published researches using real-time PCR to detect that mentioned bacterial pathogens in shawarma were carried out. Real-time quantitative PCR has also been applied to detect and quantify the foodborne pathogens. From the perspective of public health importance, Foodborne diseases are an increasingly recognized problem involving a wide spectrum of illnesses. Although, the most hospitalizations and deaths related to foodborne infections are due to bacterial agents **(Teplitski *et al*., 2009)**. The incidence of foodborne illness is increasing worldwide in modern society **(Mead *et al.*, 1999 and Nguz, 2007)**. More than 250 different diseases have been described that can be foodborne and can cause food poisoning **(CDC, 2012)**. Recorded data in Table, Fig. (1), indicated that out of each 40 chicken-shawarma and beef-shawarma samples, 27 samples (67.5%) and 16 samples (40%) were positive for *Escherichia coli* respectively, using conventional culture method, while by real-time PCR technique, 25 samples (62.5%) and 16 samples (40%) were positive for *Escherichia coli* respectively. The results in (Table 5) revealed that *E. coli* isolates from Chicken-shawarma was serologically identified as enteropathogenic O119:H4 (44.44%), O126:H27 (25.93%), O127:H6 (18.52%) and untypable serovars (11.11%). While, from beef-shawarma were O127:H6 (56.25%) and O111:H4 (37.50%) and untypable serovars (06.25%). Lower prevalence was mentioned by **(Odu and Akano, 2012; Sharaf and Sabra, 2012; Abu-Zaid *et al*., 2013; El-Dosoky *et al*., 2013 and Nimri *et al*., 2014)** for chicken-shawarma and by **(Mohamed, 2001; Abd El-Rahman *et al.*, 2011; Al-Mutairi, 2011;Alhaddad*et al*.,2013;Nimri *et al*.,2014 and Hassanien *et al*.,2015)** for beef-shawarma at fast food restaurants in various localities, using conventional microbiological method. Moreover, higher result was obtained for beef-shawarma traded in restaurants where all samples were positive for *Escherichia coli* by **(Abu-Zaid, *et al*., 2013)**, elsewhere, lower finding 30% was obtained by **(Nimri *et al*., 2014)**, from chicken-shawarma and beef-shawarma sandwiches through using PCR assay. *Escherichia coli* and faecal coliform is major pathogens has emerged as being of significant importance in terms of human health and

disease. Pathogenic strains of *E. coli* is one of the most significant foodborne pathogens that have gained increased attention in recent years to be responsible for a wide range of illnesses in humans, including hemorrhagic colitis and hemolytic uremic syndrome **(Jasson *et al*., 2010 and Mathusa *et al*., 2010)**. *E. coli* on the other hand is common, usually harmless, bacteria of the human intestinal flora. However, five groups of *E. coli*-causing diarrhea in humans have been identified **(Wasteson, 2001)**. Outbreaks of infection with those bacteria have emerged due to the consumption of contaminated animal-derived food products including RTE shawarma **(Belongia *et al*., 1991)**. Regarding to the results in Table, Fig. (2), indicated that out of each 40 chicken-shawarma and beef-shawarma samples, 2 samples (5%) and 10 samples (25%) were positive for *Staphylococcus aureus* respectively, using conventional culture method, while by real-time PCR technique,2 samples (5%) and 10 samples (25%) were positive for *Staphylococcus aureus* respectively. Using conventional microbiological method nearly similar findings were declared by **(Ayaz *et al*., 1985; Nimri *et al*., 2014 and Ahmed *et al*., 2015)**, for beef-shawarma from food restaurants in different localities. Higher result wasrecorded by **(Oduand Akano,2012 and Morshdy *et al*.,2016)**,for chicke-shawarma. Moreover, higher data recorded by **(Abd El Rahman *et al*., 2011; Ali and Abd-El-aziz, 2011; Alhaddad *et al*., 2013; El-Dosoky *et al*., 2013 and Morshdy *et al*., 2016)**, for beef-shawarma and considerably higher findings were obtained by **(Morshdy *et al*., 2014 and Hassanien *et al*., 2015)**, in chicken-shawarma and beef-shawarma samples respectively, and also by **(El-Mossalami *et al*., 2008 and Abu-Zaid *et al*., 2013)**, for beef-shawarma only. On the other hand, non-detectable findings were revealed by **(Mohamed, 2001 and Sharaf and Sabra,2012)**,toisolate*Staphylococcus aureus* from beef-shawarma and chicken-shawarma samples respectively. *Staphylococcus aureus* is one of the most important etiological agent predominantly associated with sporadic staphylococcal food poisoning around the world **(Balaban and Rasooly, 2000)**, and in many countries, it is the main bacterial agent causing foodborne diseases. It is a versatile human pathogen capable of causing staphylococcal food poisoning, toxic shock syndrome, pneumonia, postoperative wound infection and nosocomial bacteremia **(Lund, 2014)**. *S. aureus* can be carried on hands, nasal passage or throats. Most foodborne outbreaks are resulted from contamination from food handlers and production of heat stable toxins in food **(Nester*etal*., 2001; Soriano *et al*., 2002; FSIS, 2003 and Kadariya *et al*., 2014)**. Ingestion of heat stable enterotoxins-containing food causes nausea, vomiting, abdominalcramps,gastroenteritisand diarrhea within 1-6hrs post-consumption of contaminated

food **(Stewart, 2003)**. Recorded data in Table, Fig. (3), indicated that out of each 40 chicken-shawarma and beef-shawarma samples, 5 samples (12.5%) and 3 samples (7.5%) were positive for *Salmonella spp.* respectively, using conventional culture method, while by real-time PCR technique, 4 samples (10%) and 3 samples (7.5%) were positive for *Salmonella spp.* respectively. The results in (Table 6), declared that *Salmonella* isolates from chicken-shawarma were serologically identified as *Salmonella* Enteritidis (60%), *Salmonella* Typhimurium (20%) and untypable serovars (20%). While, the serovars isolated of *Salmonella* from beef-shawarma were *Salmonella* Typhimurium (66.67%) and *Salmonella* Dublin (33.33%). Elevated data were declared by **(Nimri *et al.*, 2014 and Ahmed *et al.*, 2015)**, for both shawarma types served to consumer at fast food restaurants in many localities. While, for chicken-shawarma a considerably higher finding was obtained by **(Banna and Nawas, 2016)**. Elsewhere, **(Ayaz *et al.*, 1985)**, declared that beef-shawarma showed higher positive result. Also, in beef-shawarma lower finding was reviewed by **(Al-Mutairi, 2011)**. On contrary, *Salmonella* spp. hasn't been detected in any of the investigated shawarma samples by **(Mohamed, 2001; Alhaddad *et al.*, 2013; El-Dosoky *et al.*, 2013 and Nimri *et al.*, 2014)**, through using conventional microbiological method. Moreover, in obtainable literatures there is no similar data with the current study of *Salmonella* spp. detection in shawarma, but, significantly higher result 80% was achieved by **(Nimri *et al.*, 2014)**, using PCR assay. Ready-to-eat foods should be free of *Salmonella* as consumption of food containing this pathogen may result in foodborne illness **(Buyukyoruk *et al.*, 2014)**. The incidence of foodborne salmonellosis has increased over the last 20 years and resulted in major public health problem globally. *Salmonella* serotypes are considered potentially pathogenic for human and most foodborne salmonellosis are caused by non-host-adapted serotypes **(FDA, 2012 and WHO, 2013)**. *Salmonella* Enteritidis became the major cause of the foodborne nontyphoidal salmonellosis, causing a worldwide pandemic in humans **(Ward *et al.*, 2000 and Patrick *et al.*, 2004)**. *Salmonella* serotypes, Enteritidis and Typhimurium are the most important causes of foodborne salmonellosis in humans worldwide **(Majowicz *et al.*, 2010 and Hendriksen *et al.*, 2011)**. The results tabulated in Table, Fig.(4), indicated that out of each 40 chicken-shawarma samples, 7 samples (17.5%) were positive for *Campylobacter jejuni*, using conventional culture method, while by real-time PCR technique, 6 samples (15%) were positive for *Campylobacter jejuni* respectively. Although, in contrast result which was obvious by **(Nimri *et al.*,2014)**, who failed to recover *Campylobacter* spp. from either type

of shawarma sandwiches furthermore, **Easa, (2010)** could isolate *Campylobacter jejuni* from chicken-shawarma when evaluate the microbial quality of fast and traditional fast food at Cairo, Egypt, by means of traditional culture method. *Campylobacter jejuni* is now recognized as one of the main causes of bacterial foodborne disease in many developed countries **(Ryan and Ray, 2004)**. Over 90% of human campylobacteriosis are caused by *C. jejuni* with low mortality rate of 0.1% **(FDA/CFSAN, 2003; Janssen *et al*., 2008 and CDC, 2014)**. *C. jejuni* infection in human, results in an acute self-limiting gastrointestinal illness characterized by fever, abdominal cramps, nausea and/or vomiting headache and diarrhea which frequently become bloody **(Humphrey *et al*., 2007 and Blaser and Engberg, 2008**). Occasionally, *C. jejuni* spreads to the blood stream causing serious life-threatening complications particularly in patients who are immunocompromised or among the very young and elderly including meningitis, endocarditis, hepatitis, septic arthritis and miscarriage or neonatal sepsis **(Pacanowski *et al*., 2008 and Nielsen *et al*., 2010)**. Other potential long-term autoimmune sequels associated with *campylobacter* enteritis may develop typically within few weeks after the onset of diarrheal illness. The most important post-infectious consequences are Guillain-Barre syndrome and reactive arthritis **(Pope *et al*., 2007 and Nyati and Nyati, 2013)**. Unfortunately, campylobacteriosis is often a pediatric disease for children less than 5 years of age in developing countries including Egypt **(Rao *et al*., 2001 and Coker *et al*., 2002)**. Ultimately, in this study *Listeria monocytogenes* could not be detected in all examined chicken-shawarma and beef-shawarma sandwiches samples collected from different public restaurants in various districts at Damietta, Egypt. This finding contrasts with that of **(Zaghloul*et al*.,2014)**, who reported that *L. monocytogenes* was detected in meat-shawarma sandwiches at Cairo, Egypt, by 7% and 11% using different microbiological methods including classic selective conventional and chromogenic media respectively. Elsewhere, **El-Shenawy *et al*., (2011)** was recorded for isolation of *L.monocytogenes* in chicken-shawarma and beef-shawarma sandwiches samples by 12% and 21% respectively. But, similar result was reviewed by **(Nimri *et al*., 2014)**, who failed to detect *Listeria* spp. from either type of shawarma. Moreover, **Easa, (2010)**, could isolate *L. monocytogenes* from chicken-shawarma when evaluate the microbial quality of fast and traditional fast food at Cairo, Egypt.

CONCLUSION

The results of this investigation showed that, some of the ready-to-eat shawarma sold in public restaurants in Damietta city, which randomly collected have pose a considerable risk to

human health due to the presence of pathogenic *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* spp., in chicken-shawarma and beef-shawarma and *Campylobacter jejuni* in chicken-shawarma samples. While, *Listeria monocytogenes* was not detected in all investigated samples. In conclusion, availability of epidemiologic data, by similar studies about the pathogens and their transmission in food, will provide basic evidence about the threats of not applying standard measures and will serve as a basis for adopting new regulations that will ensure safety of the consumer which may avoid and reducing outbreaks. Real-time polymerase chain reaction was more rapid and sensitive method, useful for the rapid detection of virulent genes of some foodborne bacterial pathogens transmitted by ready-to-eat shawarma in a reasonable period of time.

RECOMMENDATION

Hazards are current and challenging or difficult to control by many available preventive dealings. It is now generally documented that it is impossible to fully guarantee the safety of food but, national and international monitoring programs exist to safeguard that, the levels present are satisfactory **(Hastein *et al*., 2006)**. It is possible to reduce the chance of accidents happening by applying a well-designed HACCP program **(ICMSF, 1988)**, together with good industrial practices (GMP) and good hygiene practices (GHP), which include sound cleaning and disinfection measures **(NACMCF**, **1992 and FAO, 1994 and 1999)**. To improve the quality of shawarma and to safeguard the consumer health the following recommendations must be considered: a selection of high quality raw materials, effective heat treatment, the leftover slices must not have allowed to be used in the next day, the leftover shawarma block must be stored at -18ºC and reheated at 75ºC, adequate cleaning and sanitization of utensils day-by-day observance of appropriate personal, food handling of cooked food. Meanwhile, the non-governmental organizations together with the governmental health agencies encourage taking simple measures to educate those workers and vendors at restaurants on topics of food safety and hygienic practices. On the other hand, careful handling/preparing, washing, cleaning and above all personal hygiene awareness would help to minimize contamination and subsequently prevent consumers from foodborne illness.The governmental authority must be supplied a standard for a microbiological guideline for the Egyptian shawarma. Due to the presence of potential hazard in ready-to-eat foods products, the detection of pathogens is critical to safeguard public health. Thus, microbiological food testing is important to ensure the safety of food products **(Dwivedi and Jaykus, 2011)**.

Consideration may also be given to investigate the health status of food handlers who may have been suffering from illnesses or asymptomatic carriers of the foodborne pathogens **(Buyukyoruk *et al*., 2014)**.

ACKNOWLEDGEMENT

The authors would like to express their deepest heartfelt thanks and gratitude to **(Dr. Amr Elgohary)**, the research member of Damietta Sea' Port Food Inspection Lab., AHRI, Egypt and Food Safety Dept. Toxicology Lab. MOA, Saudi Arabia for his valuable services and assistance in accomplishing the practical part of this research.

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