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Prevalence of Non- O157 Shiga Toxin-Producing *Escherichia coli* and Enterotoxigenic *Staphylococci* in Ready-to-eat Meat Products, Handlers and Consumers in Cairo, Egypt

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Abstract: Shiga toxin-producing *Escherichia coli* and enterotoxin producing *Staphylococci* are potential food borne pathogens and their presence in ready to eat meat products and their handlers indicates that it is necessary to improve hygienic measures during manipulation of meat products. A total of 140 samples including 120 RTE meat products (beef luncheon, chicken luncheon and beef frankfurter, 40 samples, each) and 20 hand swabs of their handlers were collected from randomly selected supermarkets at Al-Salam city, Cairo, Egypt. These samples were examined for the presence of Shiga toxins producing *E. coli* (*Stx1* and *Stx2*) and enterotoxigenic *Staphylococcus aureus*. In addition, nasal swabs of RTE meat handlers were examined for enterotoxigenic *S. aureus*. Moreover, Stool of 50 diarrheic persons having the habit of eating RTE meat of the same categories examined were collected from private laboratories then examined for STEC. *E. coli* was detected in beef luncheon (20%), chicken luncheon (10%), stool samples (20%) and hand swabs (15%). Serological identification of *E. coli* isolates revealed the presence of *E. coli* O55:K59, O26:K60, O111:K58, O124:K72 and O128:K67 in beef luncheon, O55:K59 and O111:K58 in chicken luncheon, O55:K59, O124:K72 and O128:K67 in stool samples and O111:K58 and O124:K72 in hand swab. *Stx1* was detected in O26:K60 isolate, *Stx2* was detected in *E. coli* O128:K67 isolates, while *Stx1* and *Stx2* were detected in O111:K58 isolates. Moreover, *S. aureus* was detected in beef and chicken luncheon (10%, each), hand swabs (20%) and nasal swabs (30%). Further analysis of *S. aureus* isolates for toxigenic capabilities using ELISA technique revealed that the number of the enterotoxigenic strains were 3 (SEA, SED, SEA+SEC) in beef luncheon, 2 (SEA and SEC) in chicken luncheon, 3 (SEA, SEC and SED) in hand swabs and 3 (SEA, SEC and SEA+SED) in nasal swabs.

Key words: Ready-To-Eat Meat · Shiga Toxins- Producing *E. coli* · Enterotoxigenic *S. aureus* · Zoonoses

INTRODUCTION

Ready-to-eat (RTE) food products constitute a source of readily available and nutritious meals for wide scale consumers. Conversely, they are also considered as an ideal culture media for growth of many microorganisms and a major source of food borne pathogens [1].

Shiga toxin-producing *Escherichia coli* (STEC) represents one of the at least six different categories of diarrhoeagenic *E. coli* recognized [2]. Certain strains of STEC are frequently identified as causative agents of life-threatening diseases in humans, such as hemorrhagic colitis (HC) and hemolytic uraemic syndrome (HUS) [3]. A large number of STEC serogroups as non-O157:H7 (O26: H11, O111: H2, O103: H2, O128 and O145:H28) have been associated with food borne illnesses and their importance is increasing worldwide [4]. Transmission of infection to human occurs mainly through contaminated food of animal origin as well as cross contamination due to inappropriate food handling [5]. The majority of human diseases are associated with strains of STEC that produce either Shiga toxin 1 (*Stx1*) and/or Shiga toxin 2 (*Stx2*) which are encoded on lysogenic bacteriophages. A facet of STEC that makes them particularly worrisome food

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borne pathogens is their ability to survive the acidic milieu of various foods and human stomach [6].

Staphylococcal food poisoning is one of the most prevalent causes of gastroenteritis that is caused by the ingestion of food containing pre-formed toxins [7]. Despite the decrease in number of outbreaks reported annually in the last few decades, staphylococcal food poisoning is still reported as the third most prevalent cause of food borne illnesses worldwide [8]. Although staphylococci are commonly found on the skin of a wide variety of mammals and birds and on environmental surfaces, humans are thought to be the primary source of strains associated with food matrix staphylococcal intoxication [9]. Studies have shown that one of the most common types of food intoxication is caused by certain staphylococcal strains, mainly *Staphylococcus aureus* (*S. aureus*). Of the many extracellular toxins, staphylococcal enterotoxins (SE's) pose the greatest risk to consumer's health [10]. These enterotoxins are highly resistant to heat, therefore, measures to prevent the growth of *S. aureus* are critical because normal cooking's temperature will not destroy the toxins. Various typing methods have been developed to characterize *S. aureus* isolates. PCR and ELISA have been used as simple techniques for detecting enterotoxigenic strains [11].

The aim of this study was to investigate the prevalence of non-O157 STEC and enterotoxin producing *S. aureus* in some ready-to-eat meat products and human beings. A second aim was to characterize the virulence potential of any isolated STEC and enterotoxins producing *S. aureus*.

MATERIALS AND METHODS

Samples Collection: From June to August 2012, a total of 140 samples including 120 RTE meat products (beef luncheon, chicken luncheon and beef frankfurter; 40 samples, each) and 20 hand swabs of their handlers were collected from randomly selected supermarkets at Al-Salam city, Cairo, Egypt. RTE meat products and hand swabs were examined for the presence of STEC and enterotoxigenic *S. aureus*. In addition, nasal swabs of RTE meat handlers were collected and examined for enterotoxigenic *S. aureus*. Moreover, Stool samples of 50 diarrheic persons having the habit of eating RTE meat of the same categories examined were collected from private laboratories then examined for STEC.

Isolation of *E. Coli*: Briefly, 1 part of RTE meat products or stool samples and hand swabs were added to 9 parts of buffered peptone water (BPW), homogenized well, then incubated at 37°C for 24 h. A loopful of the pre-enriched homogenate was streaked onto MacConkey agar (Oxoid, CM7) and incubated for 24 h at 37 °C [12]. After incubation, suspected colonies were selected and identified biochemically by API 20 E (BioMerieux SA, France).

Serotyping of *E. coli* Isolates: *E. coli* isolates were serogrouped according to Kok *et al.* [13] using rapid diagnostic *E. coli* antisera sets (DIFCO Laboratories, Detroit Michigan 48232-7058, USA) at Food Analysis Center, Faculty of Veterinary Medicine, Benha University, Egypt. The serologically identified *E. coli* isolates were analyzed for the presence of *Stx1* and *Stx2* genes. Genomic DNA was extracted from each *E. coli* isolate using Bacterial DNA extraction kit (Spin-column) (BioTeke Corporation, Catalogue # DP2001) according to the manufacturer's instructions. The primers used for the detection of toxin genes have been published by Paton *et al.* [14] and Gannon *et al.* [15] as shown in Table 1.

Pcr Assay for Detection of *Stx1*: The reaction was performed in a volume of 20 µl containing 10 µl of readymade 2X power Taq PCR master mix (BioTeke Corporation, China), 20 µM *Stx1* primers and 2 µl of the purified DNA. The reaction conditions consisted of one cycle of 95 °C for 2 min, followed by 35 cycles of 94 °C for 1 min, 60 °C for 1 min, 72 °C for 2 min and a final extension at 72 °C for 5 min [15]. The reaction was carried out in Primus (MWG-Biotech) thermal cycler.

Pcr Assay for Detection of *Stx2*: A reaction volume of 20 µl containing 10 µl of readymade 2X power Taq PCR master mix (BioTeke Corporation, China) and 20 µM of primers (Alpha DNA, Canada) was used. The reaction conditions were: 25 cycles beginning with a 30-sec denaturation at 94 °C, primer annealing at 50 °C for 45 sec, followed by extension for 1 min 30 sec at 70 °C, a final extension for 10 min at 70 °C was also performed [16]. Amplification products were resolved in 1.2 (W/V) agarose gel, containing 5 µg ethidium bromides along with 100 bp molecular weight ladder (BioTeke Corporation, China). Agarose gels were run in 1X TBE, 5 µM ethidium bromide for at least 45 min at 100 volts, then visualized under Ultraviolet Transilluminator (Spectroline) and photographed.

Isolation of *S. aureus*: RTE meat samples, hand and nasal swabs were pre-enriched in BPW. A loopful of

pre-enriched samples in BPW were inoculated onto Baired parker agar medium (Oxoid, CM0275) [17] and incubated

Table 1: Oligonucleotide primers of *Stx1* and *Stx2* genes of *E. coli*:

Virulence factor	Oligonucleotide sequence (5'-3')	Predicted size of amplified products (bp)	Reference
Stx1	TTC GCT CTG CAA TAG GTA TTC CCC AGT TCA ATG TAA GAT	555	[14]
Stx2	CCA TGA CAC CGG ACA GCA GTT CCT GTC AAC TGA GCA CTT TG	779	[15]

at 37 °C for 24 h. The suspected colonies were subjected to biochemical identification using catalase test, coagulase test (Test tube method) and oxidase test [18].

Detection of Staphylococcal Enterotoxins: For detection of enterotoxigenic capabilities, each isolate of *S. aureus* was incubated in Brain Heart infusion broth (DIFCO, USA) for 12 h at 37°C. The broth culture was then centrifuged at 3500 r.p.m. for 10 minutes. Sterile filtration of the supernatant was applied. The enzyme immunoassay (ELISA) was carried out in an ELISA plate reader (ELX800, BioTeke Instruments, Bad Friedrichshall, Germany) where the absorbance was measured at 450 n using the RIDASCREEN set A, B, C, D, E (Art No. R4101, R-Biopharm AG, Darmstadt, Germany) [19].

RESULTS

E. coli was detected in 12 RTE meat products out of 120 examined (10%). The isolated *E. coli* were 8/40 beef luncheon (20%) and 4/40 chicken luncheon (10%). Beef frankfurter samples were free from *E. coli*. *E. coli* isolated from beef luncheon were serologically identified as O55:K59, O26:K60, O111:K58, O128:K67 (1 isolate, each) and O124:K72 (4 isolates). However, those isolated from chicken luncheon were identified as O55:K59 (3 isolates) and O111:K58 (1 isolate). PCR analysis of *E. coli* serotypes revealed the presence of *Stx1* gene in O26:K60 isolate, *Stx2* gene in O128:K67 isolates and both toxins in O111:K58 isolates (Table 2 and Figure 1).

Also *E. coli* was detected in 10/50 stool samples (20%) and 3/20 hand swabs of RTE meat handlers examined (15%). *E. coli* isolates from stool were serologically identified as O55:K59 (4 strains, 8%), O124:K72 (5 strains, 10%) and O128:K67 (1 strains, 2%). However, those from hand swab were O111:K58 (1 strain) and O124:K72 (2 strains). *Stx2* was detected in O128:K67 isolate from diarrheic stool, while *Stx1* and *Stx2* were detected in O111:K58 from hand swabs (Table 3 and Figure 1).

S. aureus was detected in beef and chicken luncheon (10%, each) Table (4). Further analysis of *S. aureus* isolates from RTE meat products samples for toxigenic capabilities using ELISA technique revealed the presence of 3/4 enterotoxigenic strains (SEA, SED, SEA+SEC) in beef luncheon, 2 (SEA and SEC) in chicken luncheon (Table 4).

Table 2: Prevalence of *Stx1* and *Stx2* producing *E. coli* in RTE meat products

					Shiga toxins production	
Examined samples	No. of positive samples (%)	The identified serotypes	No. (%)*	*E. coli* biotype	*Stx1*	*Stx2*
Beef luncheon (n=40)	8 (20)	O55:K59	1(2.5)	EPEC	-	-
		O26:K60	1(2.5)	EHEC	+	-
		O111:K58	1(2.5)	EHEC	+	+
		O124:K72	4(10)	EIEC	-	-
		O128:K67	1(2.5)	ETEC	-	+
Chicken luncheon (n=40)	4 (10)	O55:K59	3(7.5)	EPEC	-	-
		O111:K58	1(2.5)	EHEC	+	+
Beef frankfurter (n=40)	-	-	-	-	-	-

*The percentage of the identified serotypes was calculated from the total examined samples

Table 3: Prevalence of *Stx1* and *Stx2* producing *E. coli* in hand swabs of RTE meat handlers and stool samples of consumers.

						Shiga toxin production	
Examined Samples	No. of positive samples (%)	The identified serotypes	No.	Percent to total examined samples	*E. coli* biotype	*Stx1*	*Stx2*
Stool samples (n=50)	10 (20)	O55:K59	4	8	EPEC	-	-
		O124:K72	5	10	EIEC	-	-

		O128:K67	1	2	ETEC	-	+
Hand swabs (n=20)	3 (15)	O111:K58	1	5	EHEC	+	+
		O124:K72	2	10	EIEC	-	-

Table 4: Occurrence of enterotoxigenic *S. aureus* in RTE meat products and hand and nasal swabs of their handlers.

Source	No. of samples Examined	*S. aureus* positive samples (%)	Enterotoxins type (No. of isolates producing the respective toxin)					
			SEA	SEC	SED	SEA+SEC	SEA+SED	Total (%)*
Beef luncheon	40	4 (10)	1	-	1	1	-	3 (75)
Chicken Luncheon	40	4 (10)	1	1	-	-	-	2 (50)
Beef frankfurter	40	-	-	-	-	-	-	-
Total	120	8 (6.7)	2	1	1	1	-	5 (62.5)
Hand swabs	20	4 (20)	1	1	1	-	-	3 (75)
Nasal swabs	20	6 (30)	1	1	-	-	1	3 (50)

* The percentage of the enterotoxigenic *S. aureus* isolates were calculated to the total positive samples.

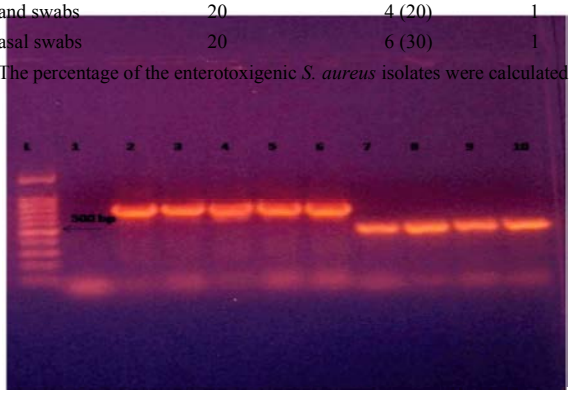

Fig. 1: PCR results of *E. coli* serotype positive for *Stx1* and *Stx2*. L: 100 bp Ladder; Lane1: Negative control; Lanes 2-6, *E. coli* samples positive for Shiga toxin 2 gene *(Stx2)* at 779 bp; Lane 2:O111:K58 from hand swab; Lane 3:O111:K58 from beef luncheon; Lane 4:O111:K58 from chicken luncheon; Lane 5:O128:K67 from beef luncheon; Lane 6:O128:K67 from stool samples; Lanes 7-10, *E. coli* samples positive for Shiga toxin 1 gene *(Stx1)* at 555 bp; Lane 7: O111:K58 from hand swab; Lane 8: O111:K58 from beef luncheon; Lane 9: O111:K58 from chicken luncheon; Lane 10: O26:K60 from beef luncheon.

DISCUSSION

Several reports have documented the isolation and serotyping of *E. coli* in beef meat products [20- 22] and chicken meat products [23, 24]. However, limited information is available on the genotypic characterizations of *Stx1* and *Stx2* producing *E. coli*. Such studies are important because STEC cause life threatening infections when transmitted to man through contaminated food of animal origin. The results of the present study demonstrated that only 8/40 beef luncheon (20%) were positive for *E. coli* (Table 2). Similar prevalence of *E. coli* in beef products was recorded in Giza, Egypt by AL-Mutairi [22] who detected *E. coli* in 15/75 beef meat products (20%). His samples were processed kofta, sausage and shawerma that were sold by street vendors who were not observing the basic rules of personal hygiene. Higher prevalence rate (30.8%) of *E. coli* in beef meat products were recorded in Sharkia, Egypt by Mohamed *et al.* [25]. The samples they examined were frozen and baladi sausage which were not subjected to heat treatment during manufacturing. On the contrary, lower prevalence (2%) was recorded by Hassanien [26] in Qalyobia, Egypt.

In Egypt, there are few reports describing isolation of STEC other than *E. coli* O157:H7 from beef meat products, even though the transmission of non-O157 STEC to human has been associated with consumption of beef meat products [27]. In this study, non-O157 STEC (O26:K60, O111:K58 and O128:K67) were isolated from beef luncheon (Table 2). Non-O157:H7 STEC were previously recorded in beef meat products [21, 22, 28]. Hussein and Bollinger [20] found non-O157 STEC to be more prevalent in beef products than *E. coli* O157. They also stated that the prevalence rates of non-O157 STEC ranged from 1.7 to 58% in packing plants, 3 to 62.5% in supermarkets and an average of 3% in fast food restaurants.

Regarding the prevalence of *E. coli* in chicken luncheon, Table 2 reveals the isolation of *E. coli* from 10% of the examined samples (4/40). These isolates were identified as O55:K59 (3 isolates) and O111:K58 (1 isolate). Lower prevalence (2%) was recorded by Zahran [29] in Qalyobia, Egypt. On the contrary, higher prevalence (25%) was recorded by Sharaf and Sabra [30] in Al-Taif, Saudia Arabia. El-Nawawi *et al.* [24] isolated O44 from

chicken meat products in Egypt.

Absence of *E. coli* isolates in beef frankfurter (Table 2) may be attributed to purchasing of beef frankfurter samples in their original intact package; consequently they were not subjected to cross contamination by slicing machines or contaminated hands. These results agreed with Javadi *et al.* [31].

E. coli O55:K59 and O111:K58 isolated from beef and chicken luncheon were categorized as enteropathogenic *Escherichia coli* (EPEC) and enterohaemorrhagic *Escherichia coli*- Shiga toxigenic *Escherichia coli* (EHEC-STEC), respectively. Alonso *et al.* [23] in Argentina reported a predominance of EPEC contamination in chicken meat and chicken meat products. The occurrence of EPEC and STEC in chicken meat may be related to the evisceration process, mainly due to the rupture of the intestine, or possible cross contamination during food processing and handling. The presence of STEC O55:K59 and O111:K58 in beef and chicken luncheon in this study may be attributed to that the grinding machine used to prepare chicken luncheon is shared with bovine meat or due to addition of bovine fat to chicken meat for thorough mixing of the latter.

Table 3 shows that *E. coli* was detected in 10/50 stool samples (20%). *E. coli* isolates from stool were serologically identified as O55:K59 (4 strains, 8%), O124:K72 (5 strains, 10%) and O128:K67 (1 strains, 2%). In comparison to this study, higher prevalence rate (51.5%) of *E. coli* was recorded in Cairo by Behiry *et al.* [32]. However, lower prevalence (6%) was previously recorded by Bodhidatta *et al.* [33]. Generally, the variation in the prevalence rate of *E. coli* from one study to another may be accounted for differences in number and health status of human cases examined, localities and hygienic measures.

Also *E. coli* was detected in 15% of hand swabs of RTE meat handlers and were identified as O111:K58 (1 strain) and O124:K72 (2 strains) (Table 3). Samaha *et al.* [34] and Mohamed *et al.* [25] isolated *E. coli* from hand swabs of food handlers in Egypt. Their respective results were 7.5 and 32%. The difference in the prevalence rates may be related to the kind of food they handled.

E. coli O128:K67 isolated from diarrheic stools and beef luncheon was categorized as ETEC and was found to contain *Stx2* that is more related to HUS than *Stx1* producing strains [35]. Meanwhile, *E. coli* O124:K72 isolated from stool, hand swabs and beef luncheon was categorized as enteroinvasive *E. coli* (EIEC). Humans are a major reservoir of EIEC and the serotypes most frequently associated with illness include O143, O144, O152, O164 and O167. Among these serogroups, O124 is commonly encountered [36]. ETEC were recorded in stool samples in previous studies [37].

PCR analysis of *E. coli* isolates for the presence of *Stx1* and *Stx2* genes clarified the presence of *Stx1* gene in O26:K60 isolate, *Stx2* gene in O128:K67 isolates and both toxins in O111:K58 isolates (Figure 1). Isolation of the *Stx1* and *Stx2* producing *E. coli* O111:K58 from beef and chicken luncheon and hand swabs of food handlers in the current study suggests cross contamination from handlers to food and vice versa. Moreover, isolation of O124:K72 from beef luncheon, hand swabs of RTE meat handlers and stool of diarrheic persons trigger the transmission cycle from infected handlers to food products and finally to consumers.

Enterotoxigenic *S. aureus* is one of the causative agents of food borne intoxication. For this reason determination of its prevalence in foods is important with respect to assessing public health risk. Bryan [38] observed that of 175 staphylococcal outbreaks, 29% were traced to ham and 8% to chicken dishes. In this study, *S. aureus* was detected in beef and chicken luncheon (10%, each) Table (4). In previous study, coagulase positive *S. aureus* was detected in 7/110 (6.4%) hamburger patties in Turkey [39].

Further analysis of *S. aureus* isolates from RTE meat products samples for toxigenic capabilities using ELISA technique revealed the presence of 3/4 enterotoxigenic strains (SEA, SED, SEA+SEC) in beef luncheon, 2 (SEA and SEC) in chicken luncheon (Table 4). In spite of the low prevalence of *S. aureus* in RTE meat products the proportion of enterotoxigenic strains is considered high. The obtained results agreed with Bania *et al.* [40] who reported that 15-80% of *S. aureus* strains isolated from various sources are enterotoxigenic. It is very likely that contamination of RTE meat products occurred during handling and mixing of ingredients. This stresses the need to instruct handlers on how to implement good manufacturing practices.

Hands and mucous membranes of the nasopharynx are considered the predominant colonization sites of staphylococci. The source of *S. aureus* intoxication is any kind of food which has come into contact with food handlers' hands contaminated with *S. aureus* and subsequently not properly stored [41]. *S. aureus* was detected in 4 hand swabs (20%) and in 6 nasal swabs (30%) of meat handlers. The enterotoxin typing of *S. aureus* isolates revealed the presence of 3/4 enterotoxigenic strains (75%) in hand swabs (SEA, SEC

and SED) and 3/6 enterotoxigenic strains (50%) in nasal swabs examined (SEA, SEC and SEA+SED). Enterotoxigenic *S. aureus* was previously detected in hand swabs of food handlers; SEA [42], SEB [43], SEC [44], SED [45]. A recent study in Botswana reported that 57.5% of the food handlers harbored *S. aureus* and 21% of them possessed toxigenic strains [46]. Isolation of enterotoxigenic *S. aureus* from nasal swabs agreed with Udo *et al.* [43] and Soriano *et al.* [45]. Isolation of enterotoxigenic *S. aureus* from nasal and hand swabs of RTE meat handlers, chicken and beef luncheon substantiate the role played by food handlers in dissemination of such bacteria through foods they handle.

CONCLUSION

It could be concluded that *E. coli* and *S. aureus* harboring toxin genes are prevalent in Beef and chicken luncheon and humans in Egypt. The obtained data illustrate the need to keep a careful watch over which pathogens are causing human disease, understanding STEC and enterotoxigenic *S. aureus* epidemiology and a control strategy plan. Furthermore, it is convenient for the authors to suggest beef frankfurter as a safer meat product depending on the obtained results of this study and similar ones that illustrate absence of both non-O157 STEC and enterotoxigenic *S. aureus*.

REFERENCES

1. Gracey, J.F., 1986. Meat Hygiene. 8th Eds. the English Long Book Sic and Baillier, Tindall, London, pp: 100-1.
2. Kaper, J.B., J.P. Nataro and H.L.T. Mobley, 2004. Pathogenic *Escherichia coli*. Nat. Rev. Microbiol., 2: 123-140.
3. Nataro, J.P. and J.B. Kaper, 1998. Diarrheagenic *Escherichia coli*. Clin. Microbiol. Rev., 11: 142-201.
4. Bettelheim, K.A., 2007. The non-O157 shiga toxigenic (verocytotoxigenic) *Escherichia coli*; under-rated pathogens. Crit. Rev. Microbiol., 33: 67-87.
5. Gyles, C.L., 2007. Shiga toxin-producing *Escherichia coli*: an overview. J. Anim. Sci., 85: E45-62.
6. Brudzinski, L. and M.A. Harrison, 1998. Influence of incubation conditions on survival and acid tolerance response of *Escherichia coli* O157:H7 and non-O157:H7 isolates exposed to acetic acid. J. Food Prot., 61: 542-546.
7. Jablonski, L.M. and G. Bohach, 1997. *Staphylococcus aureus*. In: Doyle MP, Beuchat LR, Montville TJ, (Eds), Food microbiology fundamentals and frontiers. American Society for Microbiology Press, Washington, DC, pp: 353-357.
8. Zhang, S., J.J. Iandolo and G.C. Stewart, 1998. The enterotoxin D plasmid of *Staphylococcus aureus* encodes a second enterotoxin determinant (sej). FEMS Microbiology Letters, 168: 227-233.
9. Rosec, J.P., J.P. Guiraud, C. Dalet and N. Richard, 1997. Enterotoxin production by *Staphylococci* isolated from foods in France. Int. J. Food Microbiol., 35: 213-221.
10. Balaban, N. and A. Rasooly, 2000. Staphylococcal enterotoxins. Int. J. Food Microbiol., 61: 1-10.
11. Aydin, A., M. Sudagidan and K. Muratoglu, 2011. Prevalence of staphylococcal enterotoxins, toxin genes and genetic-relatedness of foodborne *Staphylococcus aureus* strains isolated in the Marmara Region of Turkey. Int. J. Food Microbiol., 148: 99-106.
12. Cruickshank, R., J.P. Duguid, B.P. Marmion and H.A. Swain, 1975. Medical microbiology. The practice of medical microbiology. 12 Editions, Vol. II. Churchill, Edinburgh.
13. Kok, T., D. Worswich and E. Gowans, 1996. Some serological techniques for microbial and viral infections. In Practical Medical Microbiology (Collee, J.; Fraser, A.; Marmion, B. and Simmons, A. eds.), 14th ed. Edinburgh, Churchill Livingstone, UK.
14. Paton, A.W., L. Beutin and J.C. Paton, 1995. Heterogeneity of amino acid sequences of *Escherichia coli* Shiga - like toxin type-I operons. Gene, 153: 71-74.
15. Gannon, V.P., R.K. King, Y.J. Kim and E.J. Thomas, 1992. Rapid and sensitive method for detection of Shiga-like toxin-producing *Escherichia coli* in ground beef using the polymerase chain reaction. Appl. Environ. Microbiol., 58: 3809-3815.
16. Franck, S.M., B.T. Bosworth and H.W. Moon, 1998. Multiplex PCR for enterotoxigenic, attaching and effacing and Shiga toxin-producing *Escherichia coli* strains from calves. J. Clin. Microbiol., 36: 1795-1797.
17. ISO-6888-1, 1999. Microbiology of food and animal feeding stuffs- Horizontal method for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species). Part 1.Technique using Baird-Parker agar medium. Ed ². Geneva: International Organization for Standardization.

18. Cheesbrough, M., 2000. Microbiological tests in District Laboratory Practice in tropical countries. Part 2. Cambridge university press. United Kingdom. pp: 1-266.
19. Ewald, S., 1988. Evaluation of enzyme-linked immunosorbent assay (ELISA) for detection of staphylococcal enterotoxin in foods. Int. J. Food Microbiol., 6: 141-153.
20. Hussein, H.S. and L.M. Bollinger, 2005. Prevalence of Shiga toxin-producing *Escherichia coli* in beef. Meat Sci., 71: 676-689.
21. Balagué, C., A.A. Khan, L. Fernandez, A.L. Redolfi, V. Aquili, P. Voltattorni, C. Hofer, G. Ebner, S. Dueñas and C.E. Cerniglia, 2006. Occurrence of non-O_{157} Shiga toxin-producing *Escherichia coli* in ready-to-eat food from supermarkets in Argentina. Food Microbiol., 23: 307-313.
22. AL-Mutairi, M.F., 2011. The incidence of *Enterobacteriaceae* causing food poisoning in some meat products. Adv. J. Food Sci. Technol., 3: 116-121.
23. Alonso, M.Z., P.M.A. Lucchesi, E.M. Rodríguez, A.E. Parma and N.L. Padola, 2012. Enteropathogenic (EPEC) and Shiga toxigenic *Escherichia coli* (STEC) in broiler chicken and derived products at different retail stores. Food Control, 23: 351-355.
24. EL-Nawawi, F.A., O.A. Attala and S. Saleh, 2012. Enteropathogens of public health importance in imported frozen meat and chicken. Int. J. Microbiol. Res., 3: 59-63.
25. Mohamed, E.M.M., A.I.A. Maysa E.M.M. Labib, A.A. Magda and A.G. Rasha, 2009. Studies on Some Zoonotic bacterial Food Borne Infection. Vet. Med. Assoc., 69: 1.
26. Hassanien, F.S., 2004. Bacterial hazards associated with consumption of some meat products. Benha Vet. Med. J., 15: 1441-54.
27. Martin, A. and L. Beutin, 2011. Characteristics of Shiga toxin-producing *Escherichia coli* from meat and milk products of different origins and the association with food producing animals as main contamination sources. Int. J. Food Microbiol., 146: 99-104.
28. Mahmoud, A.M.M., 2012. Molecular characterization of diarrheagenic *Escherichia coli* isolated from meat products sold at Mansoura city, Egypt. Food Control 25: 159-164.
29. Zahran, A.D., 2004. Using Gamma irradiation as an option for controlling bacteria contaminating some food of animal origin. PhD Thesis, Faculty of Veterinary Medicine, Benha University, Egypt.
30. Sharaf, E.M. and M.S. Sabra, 2012. Microbiological loads for some types of cooked chicken meat products at Al-Taif Governorate, KSA. World Appl. Sci. J., 17: 593-597.
31. Afschin, J., S. Saeid and D. Abdullah, 2011. Study on microbial properties of Frankfurters during shelf life. Annals of Biological Research,, 2: 407-412.
32. Behiry, I.K., E.A. Abada, E.A. Ahmed and R.S. Labeeb, 2011 Enteropathogenic *Escherichia coli* associated with diarrhea in children in Cairo, Egypt. Scientific World Journal, 11: 2613-2619.
33. Bodhidatta, L., N. Vithayasai, B. Eimpokalarp, C. Pitarangsi, O. Serichantalergs and D.W. Isenbarger, 2002. Bacterial enteric pathogens in children with acute dysentery in Thailand: increasing importance of quinolone- resistant *Campylobacter*. Southeast Asian J. Trop. Med. Public Health, 33: 752-757.
34. Samaha, H.A., Y.N. Haggag, A.A. Draz and L.N. Mohamad, 2004. The role of poultry in transmitting some zoonotic bacterial and fungal diseases. Alex. J. Vet. Sci., 21: 1-13.
35. Paton, A.W. and J.C. Paton, 2002. Direct detection and characterization of Shiga toxigenic *Escherichia coli* by multiplex PCR for *Stx1*, *Stx2*, eae, ehxA and saa. J. Clin. Microbiol., 40: 271-274.
36. Doyle, M.P., T. Zhao, J. Meng and S. Zhao, 1997. Escherichia coli O_{157}:H_7. In: Food Microbiology, Fundamentals and Frontiers, ed. M.P. Doyle, Beuchat LR, Montville TJ, Pp. 171-191. American Society for Microbiology Press, Washington, D.C.
37. Arif, S.K. and L.I.F. Salih, 2010. Identification of different categories of diarrheagenic *Escherichia coli* in stool samples by using multiplex PCR technique. Asian J. Med. Sci., 2: 237-243.
38. Bryan, F.L., 1988. Risks associated with vehicles of foodborne pathogens and toxins. J. Food Prot., 51: 498-508.
39. Aycicek, H., S. Cakiroglu and T.H. Stevenson, 2005. Incidence of *Staphylococcus aureus* in ready-to-eat meals from military cafeterias in Ankara, Turkey. Food Control, 16: 531-534.
40. Bania, J., A. Dabrowska, K. Korzekwa, A. Zarczynska, J. Bystron, J. Chrzanowska and J. Molenda, 2006. The profiles of enterotoxin genes in *Staphylococcus aureus* from nasal carriers. Lett. Appl. Microbiol., 42: 315-320.
41. Gilbert, R.J. and T.J. Humphrey, 1998. Food borne bacterial gastroenteritis. In: Hausler WJ, Sussman M,

(Vol. ed.), Topley and Wilson's Microbiology and Microbial Infections. Hodder Arnold, London, pp: 539-65.

42. Mehrotra, M., G. Wang and W.M. Johnson, 2000. Multiplex PCR for detection of genes for *Staphylococcus aureus* enterotoxins, exfoliative toxins, toxic shock syndrome toxin 1 and methicillin resistance. J. Clin. Microbiol., 38: 1032-1035.
43. Udo, E.E., M.A. AL-Bustan, L.E. Jacob and T.D. Chugh, 1999. Enterotoxin production by coagulase positive staphylococci in restaurant workers from Kuwait city may be a potential cause of food poisoning. J. Med. Microbiol., 48: 819-823.
44. Fueyo, J.M., M.C. Martín, M.A. González-Hevia and M.C. Mendoza, 2001. Enterotoxin production and DNA fingerprinting in *Staphylococcus aureus* isolated from human and food samples. Int. J. Food Microbiol., 67: 139-145.
45. Soriano, J.M., G. Font, H. Rico, J.C. Moltó and J. Mañes, 2002. Incidence of enterotoxigenic staphylococci and their toxins in foods. J. Food Prot., 65: 857-860.
46. Loeto, D., M.I. Matsheka and B.A. Gashe, 2007. Enterotoxigenic and antibiotic resistance determination of *Staphylococcus aureus* strains isolated from food handlers in Gaborone, Botswana. J. Food Prot., 70: 2764-2768.