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Comparative Study Between Bacterial Status of Balady and Commercial Table Egg with Detection of Genes Producing toxin in Luxor Governorate

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Abstract

A total of two hundred chicken table eggs were collected from different location in Luxor governorate and divided into 4 groups (each group = 50 eggs) according to the source of eggs, balady (houses (A)& local market (B))and commercial (retail collection shops (C)& supermarkets (D)). Collected samples were examined bacteriologically for detection of shiga-like toxin producing *E. coli, Salmonella* spp. and coagulase positive *Staphylococcus aureus* (CPS) and its toxins and virulence genes. Results showed that the most predominant pathogen especially on egg shell was the CPS followed by *E. coli* and *Salmonella* spp. The incidence of CPS in the 4 groups was 66,48,12 & 28% from egg shell and 18,10, Zero& 6% from egg content, respectively. Also, *E. coli* was 42, 20, Zero&16 % from egg shell and 8,4,4& 4 % from egg content, respectively. While, *Salmonella* Spp. were 2, 4, Zero& 4% from egg shell and 6, 2, zero& 2% from egg content). The serological typing of *E. coli* revealed 13 serotypes (49 isolates) from table egg samples; O_1:H_7, O_2:H_6, O_{26}:H_{11}, O_{55}:H_7, O_{78}, O_{91}:H_{21}, O_{114}: H_4, O_{119}:H_6, O_{124}, O_{125}: H_{21},O_{126}:H_{21}, O_{158}& O_{163}:H_2. All isolated *E.coli* serotypes were isolated from balady table egg samples except strain O_{126}:H_{21}, 5 strains were isolated from commercial table egg samples as O_2:H_6,O_{55}:H_7,O_{78},O_{126}:H_{21} and O_{91}:H_{21}.Most *E.coli* strains were isolated from balady table egg samples were positive for shiga-like toxin genes (*stx*1, *stx*2, *eae*A). On the other hand, examined samples were positive for 10 *Salmonella* strains. The serological typing of *Salmonella* spp. revealed five serovars; *S.Enteritidis*(3 strains), *S.Typhimurium*(2 strains), *S.Kentucky* (3 strains), *S.Tesvie*(1 strain) and *S.Popuana* (1 strain). All 5 *Salmonella* serovars were isolated from balady table egg samples while only two *Salmonella* serovars were isolated from commercial table egg samples. PCR results of *Salmonella* virulence genes showed that *fimH* gene was detected in S.*Tsevie*. While *hilA* and *fimH* genes were detected in *S.Kentucky* and *stn*, *hilA* and *fimH* genes were detected in both *S.Typhimurium* and *S.Enteritidis*. Moreover, four types of enterotoxins produced by CPS isolated from balady and commercial table eggs were detected; SEA,SEB,SEC&SED. The incidence of CPS enterotoxins from balady table eggs was 84.6% and from commercial table eggs was 15.4%. The percentage of CPS enterotoxins from shell and content of balady table eggs was 17.5% and 7.1 %, respectively, while it was 10 % from only shell of commercial one. Finally, it could be concluded that as general, table eggs were contaminated by different pathogenic microorganisms and mostly occurred on egg shell. The balady table eggs were more contaminated with *E. coli,* Salmonella spp. and CPS than commercial one. The most isolated organism was CPS followed by shiga-like toxin producing *E. coli* and then Salmonella spp. On the other hand, commercial table eggs were better than balady table eggs especially those sold in retail shops and supermarkets. In the current study all isolates had the ability to produce toxins which considered more dangerous than the organism itself.

***Keywords**: Bacteria, balady table eggs, commercial table eggs, STE.C virulence genes, bacterial toxins .*

Introduction

Today, table eggs remain a staple food within the human diet, consumed by people throughout the world. They are consumed worldwide in the form of pastries, stews and beverages and are considered very nutritious and a cheap source of protein **(Blumenthal, 1990; Papadopoulou *et al.,* 1997).** Though eggs are considered as complete food for growth and sustenance, studies indicated that micro-organisms often contaminate eggs **(Abdullahi, 2010)**.

However, following exposure to environmental conditions for example, soil, feces and dirty nesting materials, eggs become contaminated with different types of micro-organisms **(Ellen *et al.,* 2000& Smith *et al.,* 2000).** Contamination of eggs and egg products with microorganisms can affect egg quality, which may lead to spoilage and pathogen transmission. This might induce food borne infection or intoxication to consumers **(Salihu *et al.,* 2015).**

Salmonella species have been considered one of the most important food-borne pathogens, around the world **(Martelli and Davies, 2012).** *Salmonella enterica* serovar Typhimurium and *Salmonella enteric serovar* Enteritidis are the most frequently isolated serovars from food-borne outbreaks throughout the world. *S.* Enteritidis and *S.* Typhimurium usually induce self limiting gastroenteritis or an asymptomatic carrier state in a wide variety of animal species **(Uzzau *et al.,* 2000).**

Escherichia coli are one of the common microbial flora of gut in farm animals, poultry and human being. Most of *E. coli* isolates are harmless, however, some strains are pathogenic and may cause serious food poisoning in human beings **(Begum *et al.,* 2014).**

Staphylococcus is considered a normal flora of chickens, isolated from the skin and feathers as well as in the respiratory and intestinal tracts **(Casey *et al.,* 2007). Kadariya *et al.,* (2014)** found that *Staphylococcal* food-borne disease (SFD) is one of the most common food-borne diseases worldwide resulting from the contamination of food by preformed *S. aureus* enterotoxins. Staphylococcal enterotoxins (SEs) excreted into food by enterotoxigenic strains of coagulase-positive Staphylococci (CPS), mainly *Staphylococcus aureus*, account for a large number of food-borne illnesses worldwide.

Therefore, the present study was conducted to evaluate and compare the prevalence of shiga-like toxin producing *E. coli, Salmonella spp*. and coagulase *positive Staphylococcus aureus* (CPS) and its toxins and virulence genes in balady and commercial table eggs and their significance as public health hazards.

Materials and Methods

Samples Collection: A total of two hundred chicken table eggs were collected from different location in Luxor governorate and divided into 4 groups (each group = 50 eggs) according to the source of eggs. Group (A) was balady table eggs from village houses. Group (B) was balady table eggs from local markets. Group (C) was commercial table eggs from retail collection shops. Group (D) was commercial table eggs sold in supermarket. All collected samples were handled and immediately transferred to laboratory at Animal Health Research Institute, RLQP Luxor Branch., to be examined bacteriologically for detection of shiga-like toxin *E. coli*, *Salmonella* spp. and Coagulase positive *Staphylococcus* (CPS) and its toxins and virulence genes.

Preparation of samples (Roberts *et al.,* 1995):

For egg shell sample, it was prepared as a total of 180 mL sterile buffered peptone water were poured into the egg shell samples in plastic bags and mixed well .

For egg content sample, egg shell was sterilized by swabbing with 70% ethyl alcohol, flamed and broken with a sterile forcep from the broad ends. Each egg tested as one sample and the egg contents were poured on sterile jar and homogenized for 30 sec.

A ten fold serial dilution was prepared for egg shell and homogenized content samples and tested for bacterial isolation.

Detection of Shiga-like toxin producing *Escherichia coli* :

Isolation of *E. coli*: was carried out according to the method described by **(Lee and Arp 1998).**

Identification of *E. coli:* Suspected isolates of

E. coli were identified biochemically according to **(MacFaddin, 2000)**.

Serological identification of *E.coli* isolates: The isolates were serologically identified according to **(Kok *et al.,* 1996)** by using rapid diagnostic *E. coli* antisera sets **(DENKA SEIKEN Co., Japan)**.

Detection of Salmonellae :

Isolation of *Salmonella spp.*: was carried out according to **(ISO 6579-3:2014).**

Identification of *Salmonella spp.:* Suspected isolates of *Salmonella* were identified biochemically according to **(MacFaddin, 2000).**

Serological identification of *Salmonella* isolates: Serological identification of *Salmonellae* was carried out according to Kauffman – White scheme **International Organization for Standardization (ISO 6579 part 3, 2014)** for the determination of Somatic (O) and flagellar (H) antigens using *Salmonella* antiserum **(DENKA SEIKEN Co., Japan).**

Detection of Coagulase Positive *Staphylococcus aureus* (CPS) :

Isolation of coagulase positive *Staphylococcus aureus*: was carried out according to **(ISO 6888-1: 2003)**

Identification of coagulase positive *Staphylococcus aureus*: Suspected isolates of Coagulase Positive *Staphylococci* were identified according to **(Cruickshank *et al.,* 1975)** and biochemically identified according **(MacFaddin, 2000).**

Detection of enterotoxin of coagulase positive *Staphylococcus aureus* by **(Shingaki *et al.,* 1981):** The clear culture supernatant fluid was tested serologically by Reverse Passive Latex Agglutination technique "RPLA" using kits for the detection of *Staphylococcal* enterotoxins A, B, C and D (SET-RPLA, Denka Sekeu LTD, Japan).

Polymerase Chain Reaction (PCR):

Materials used for PCR:

Primer sequences of *E. coli* used for PCR identification system:

Application of PCR for identification of shiga toxins (stx1 & stx2) and intimin (eaeA) genes of *E. coli* was performed essentially by using primers (Pharmacia Biotech) as follow:

Primer	Oligonucleotide sequence (5′ → 3′)	Product size (bp)	References
stx1 (F)	5′ ACACTGGATGATCTCAGTGG ′3	614	**Dhanashree and Mallya (2008)**
Stx1 (R)	5′ CTGAATCCCCCTCCATTATG ′3		
Stx2 (F)	5′ CCATGACAACGGACAGCAGTT ′3	779	
Stx2 (R)	5′ CCTGTCAACTGAGCAGCACTTTG ′3		
***eae* A (F)**	5′ GTGGCGAATACTGGCGAGACT ′3	890	**Mazaheri *et al.* (2014)**
***eae A* (R)**	**5′** CCCCATTCTTTTTCACCGTCG **′3**		

1.6. Primer sequences of Salmonellae used for PCR system:

The primers for detection of virulence factors including Enterotoxin (*stn*), hyper-invasive locus (*hil*A) and fimbrial (*fim*H) genes of *Salmonella* species were synthesized as follow:

Target gene	Oligonucleotide sequence (5′ → 3′)	Product size (bp)	References
stn (F)	**5′** CTTTGGTCGTAAAATAAGGCG **′3**	260	**Makino *et al.*, (1999)**
Stn ®	**5′** TGCCCAAAGCAGAGAGATTC **′3**		
hilA (F)	**5′** CTGCCGCAGTGTTAAGGATA **′3**	497	**Guo *et al.*, (2000)**
hilA (R)	**5′** CTGTCGCCTTAATCGCATGT **′3**		
fimH (F)	**5′** GGA TCC ATG AAA ATA TAC TC **′3**	1008	**Menghistu(2010)**
fimH (R)	**5′** AAG CTT TTA ATC ATA ATC GAC TC **′3**		

DNA Extraction using QIA amp kit (Shah *et al.*, 2009):

After overnight culture on nutrient agar plates, one or two colonies were suspended in 20 ml of sterile distilled water, and the suspension was then heated at 100ºC for 20 minutes. Accurately, 50-200 µl of the culture were placed in Eppendorf tube and the manufacturer's instructions (Qiagen, Hilden, Germany) were applied.

DNA amplification:

Amplification reaction of *E. coli* (Fagan *et al.*, 1999):

The amplification was performed on a Thermal Cycler (Master cycler, Eppendorf, Hamburg, Germany). PCR assays were carried out in 1 ml of nucleic acid template prepared by using reference EHEC isolates (approximately 30 ng of DNA), 10 mM Tris-HCl (pH 8.4), 10 mM KCl, 3 mM MgCl2; 2 mM concentrations of each primer, 0.2 mM concentrations of each 29 -deoxynucleoside 59-triphosphate, and 4 U ofAmpliTaq DNA polymerase (Perkin-Elmer). Amplification conditions consisted of an initial 95°C denaturation step for 3 min followed by 35 cycles of 95°C for 20 sec, 58°C for 40 s, and 72°C for 90 sec. The final cycle was followed by 72°C incubation for 5 min. The reference strains were E. coli O157:H7 (positive for stx1, stx2 and *eaeA*) and E. coli (a non pathogenic negative control strain) that does not possess any virulence gene. The amplified DNA fragments were analyzed by 2% of agarose gel electrophoresis (Applichem, Germany, GmbH) in 1x TBE buffer stained with ethidium bromide and captured as well as visualized on UV transilluminator. A 100 bp plus DNA Ladder (Qiagen, Germany, GmbH) was used to determine the fragment size.

DNA amplification of virulence genes of Salmonella (Singh *et al.*, 2010):

The amplification was performed on a Thermal Cycler (Master cycler, Eppendorf, Hamburg, Germany) using 25 µl of PCR mixture. The reaction mix invariably consisted of 5 µl of the bacterial lysate, 5 µl of 10x assay buffer for Taq polymerase containing 1.5 mM MgCl2, 2 µl of 10mM dNTP mix 1 µl each of forward and reverse primer (10 pmol) and 1.25 U of Taq DNA polymerase made upto50 µl using sterile distilled water. The PCR cycling protocol was applied as following: An initial denaturation at 94°C for 60 sec, followed by 35 cycles of denaturation at 94°C for 60 sec, annealing at 64°C for 30 sec and extension at 72°C for 30 sec, followed by a final extension at 72° C for 7 min. Finally, 5 µl of each amplicon was electrophoresed in 1.5 % agrose gel and visualized on UV transilluminator.

All results discussed depending on the Egyptian Standards (3169/2007)for Table eggs.

Results and Discussion
All obtained results showen in tables 1-7

Table (1). Incidence of *E. coli, Salmonella spp. and coagulase positive Staphylococcus aureus* isolated from the examined Egg samples from Balady (Backyard)

Type of Egg	Egg source	Type of sample	No. of Samples	Positive Samples					
				E. coli		Salmonella spp.		coagulase positive Staph. aureus	
				No.	%	No.	%	No.	%
Balady (Backyard)	Group A (Houses)	Egg shell	50	21	42	1	2	33	66
		Egg content	50	4	8	3	6	9	18
	Group B (Local Market)	Egg shell	50	10	20	2	4	24	48
		Egg content	50	2	4	1	2	5	10
Total of samples			200	37	18.5	7	3.5	71	35.5

Table (2). Incidence of *E. coli, Salmonella spp. and coagulase positive Staphylococcus aureus* isolated from the examined Egg samples from Commercial (Farms):

Type of Egg	Egg source	Type of sample	No. of Samples	Positive Samples					
				E. coli		*Salmonella* spp.		coagulase positive *Staph. aureus*	
				No.	%	No.	%	No.	%
Commercial (Farms)	Group C (Retail Shops)	Egg shell	50	0	0	0	0	6	12
		Egg content	50	2	4	0	0	0	0
	Group D (Supermarket)	Egg shell	50	8	16	2	4	14	28
		Egg content	50	2	4	1	2	3	6
Total examined samples			200	12	6%	3	1.5%	23	11.5

E.coli was isolated from egg shell of group (A) in rate 42% and from egg content in rate 8% .

Lower findings have been reported by **Samah *et al.*, (2015)**; 13.5% (egg shell) and 7.5% (egg content). On the other hand , higher findings have been reported by **Maha (2013)** who isolated *E. coli* isolated by 82% (egg shell) and 32% (egg content). Results from Group B (local market), revealed an incidence of 20% *E. coli* from egg shell and 4% from egg content, which were lower than that found by **Sabarinath *et al.*, (2009)**; who reported 33% from egg shell and 25% from egg content; and relatively similar to results obtained by **Elafify *et al.*, (2016)**; 12% (egg shell) and 4% egg content. On the other hand, these results were higher than results obtained by **Hamzah (2014);** who recorded 6% (egg shell), and 2% (egg content) and **Bahobail *et al.*, (2012)**; 12% (egg shell) and was not detected from egg content. From group C (retail collection shops), the incidence of *E. coli* reached 4% from egg content and did not detected in egg shells. These results were lower than that found by **Fardows & Shamsuzzaman, (2015);** 40% (egg shell), **Salihu *et al.*, (2015);** 56.3% (egg shell)& 43% (egg content), and **Amal *et al.*, (2015);** 29% (egg shell) & 37.5% (egg content). While, these results were really similar to results obtained from **Hamzah (2014)**; 7% (egg shell) and 1% (egg content). On the other hand, **Cader *et al.*, (2014)** could not detect *E. coli* from egg shell nor egg content. Results from Group D (supermarket), the incidence of *E. coli* reached the 16% (egg shell) and 4% (egg content). These results were similar to that found by **Elafify *et al.*, (2016)**; who recorded 16% (egg shell) and 4% (egg content) and **Sabarinath *et al.*, (2009);** 16.6% (egg

shell) but did not detected from egg content. The results were higher than that obtained from **Hamzah (2014)**; 2% (egg shell) but did not detected from egg content), **Maha (2013)**; reported an isolation of 4% (egg shell) but did not detect from egg content, and **Bahobail *et al.,* (2012)**; 6.1% (egg shell) & 1.7% (egg content)

In the present study incidence of *Salmonella* Spp. Was higher than those reported by **Hamzah (2014)** who found the incidence by 1% (egg shell) and not detect from egg content. Both **Bahobail *et al.,* (2012) & Sabarinath *et al.,* (2009)** could not detect *Salmonella* Spp. From neither egg shell nor content. Results from Group (C). Samples were free from Salmonella spp. from both egg shell and content which were similar to results found by **Hamzah (2014)** and **Cader *et al.,* (2014)**.

While, the results were lower than that obtained by **Fardows & Shamsuzzaman (2015)** who found 6.6% (egg shell), and **Salihu *et al.*, (2015)** who found 13.7% (egg shell) and 16.8% (egg content). For Group D, results were 4% (egg shell) and 2% (egg content). These results were higher than that reported by **Hamzah (2014); Bahobail *et al.,* (2012); Mahdavi *et al.*,(2012)** & **Sabarinath *et al.,* (2009**), where all of them could not detect *Salmonella* Spp. From both egg shell and content.

The recent study revealed that coagulase positive *Staphylococcus. aureus* (CPS) were higher than that found by **Samah *et al.,* (2015)** who reported isolation rates of CPS from a total of 80 (40%) isolates were detected; 29 (14.5%) isolates were detected from egg shell,15 (7.5 %) isolates from egg content, and 36(18%) isolates from both shell and content, out of the total examined 200 eggs 15 (7.5%) were harbor both Coagulase Positive *Staphylococci* and *E. coli.*

On the other hand, results from **Table (2)** shown the incidence of CPS isolates from samples of Group (C) and Group (D) which were 12%&28 from egg shell and 0 & 6 % from egg content, respectively. The current results were lower than that results obtained by **Chaemsanit *et al., (*2015)** who found 40/49 isolates from market layer eggs were carried CPS and concluded that CPS were the predominant flora of eggshells.

Stepien-pysniak *et al.,* (2009) reported a relatively high degree of contamination of table eggs with *Staphylococcus* organisms , which in 1125 bacteriological tests conducted on whites, yolks and shells of eggs from three sources, *Staphylococci* were found in 514 cases. Thirteen strains were isolated from the whites, but *Staphylococci* were found more often in yolks (199 strains). The highest percentage of *Staphylococci* bacteria was found on the surface of the egg shell (302 strains). In France. *Staphylococcal* food poisonings reported in a two-year period (1999-2000), the food involved identified eggs and egg products (11%) and poultry (9.5%) **(Haeghebaert *et al.*, 2002). Abdullahi (2010)** reported the highest degree of eggshell contamination with Gram-positive bacteria particularly *Staphylococcus* spp. (75%), *E. coli* (9%) and *Salmonella* spp. (11%). **Muna and Hayder (2014)** reported the presence of *Staphylococcus aureus* in 10.52% of table eggs samples collected from supermarkets and also maintained that it could not be detected in samples from farms.

In this study It was found that the eggshells were predominantly contaminated with CPS especially from balady table eggs of backyard and were more contaminated compare to commercial one. The contamination was mostly from environment and bad handling and storage conditions. On the other hand, the lower contamination rate of commercial table eggs might be because of the cleaning process of eggs before marketing and its hygienic handling.

Table (3). Incidence of different serotypes of shiga-like toxin producing *E.coli* isolated from both balady and commercial table egg samples:

Sero-types	No. of strains	No. of Escherichia coli contaminated samples							
		Balady (No. = 100) table egg				Commercial (No. = 100) table egg			
		Group A (Houses) (No. = 50)		Group B (Local Market) (No. = 50)		Group C (Retail Shops) (No. = 50)		Group D (Supermarket) (No. = 50)	
		Shell	Content	Shell	Content	Shell	Content	Shell	Content
O1:H7	4	1	1	1	1	0	0	0	0
O2:H6	7	3	1	1	0	0	0	2	0
O26:H11	1	0	0	1	0	0	0	0	0
O55:H7	3	0	1	0	0	0	2	0	0
O78	11	5	1	3	0	0	0	2	0
O91:H21	6	3	0	1	0	0	0	0	2
O114:H4	1	1	0	0	0	0	0	0	0
O119:H6	2	2	0	0	0	0	0	0	0
O124	1	0	0	1	0	0	0	0	0
O125:H21	5	3	0	1	1	0	0	0	0
O126:H21	4	0	0	0	0	0	0	4	0
O158	2	2	0	0	0	0	0	0	0
O163:H2	2	1	0	1	0	0	0	0	0
Total No. of Positive Isolates	49	21	4	10	2	0	2	8	2

Different serogroups of *E. coli* have been detected by **Maha (2013)** who could isolateO_{114} (2 strains), O_{125} (3 strains) from balady table eggs; and O_{114} (1 strain), O_{125} (3 strains) and O_{126} (2 strains) from commercial table eggs. Also, **Elafify*et al.,* (2016)**who found 9 different *E. coli* serogroups namely O_1, O_2, O_{26}, O_{44}, O_{78}, O_{114}, O_{124}, O_{125} and O_{128} from commercial table eggs collected at Mansoura governate. Moreover, **Elbayoumi *et al.,* (2018a)** who detected O_{114}:H_4 (one strain), O_{127}:H_4 (2 strains), O_{128}:H_2 (2 strains), and O_{158}(one strain).

Table (4). The prevalence of virulance genes (*stx*1, *stx* 2 and *eae* A) in enteropathogenic *E. coli* stains isolated from Balady and Commercial Table egg samples:

Serotypes (No.=13)	Types of Shiga toxin genes		
	***Stx*1**	***Stx*2**	***eae*A**
O26:H11	+	+	+
O1:H7	+	+	-
O78	+	+	-
O91:H21	+	+	-
O119:H6	+	+	-
O158	+	+	-
O55:H7	+	-	-
O114:H4	+	-	-
O163:H2	+	-	-
O2:H6	-	+	-
O125:H21	-	+	-
O126:H21	-	+	-
O124	-	-	-

The present study was designated to recognize some virulence genes that may play a role in virulence of enteropathogenic *E. coli* by using one of the recent development molecular biological techniques (PCR). the genes were shiga toxin 1 gene (*stx1*), shiga toxin 2 gene(*stx2*) and intimin gene (*eae*A). Shiga toxins were central to the pathogenesis of bloody diarrhea and hemolytic uremic syndrome through cytopathic effect on vascular endothelial cells of kidney, intestine, central nervous system and other organs **Brogden *et al.*, (2000) and Ethelberg *et al.*, (2004).** Although *Stx1* and *Stx2* have similar structures and modes of action their toxicities appear to be distinct. *Stx2* was 1000 times more cytotoxic than *Stx1* towards human renal microvascular endothelial cells, the putative target of Shiga toxins in the development of HUS (**Louise and Obrig,1995**). In addition, a subset of STEC strains considered to be highly virulent for humans because they have capacity to produce attaching and effacing lesions on intestinal mucosa, a property encoded on a pathogenicity island termed the locus for enterocyte effacement (LEE).The *eaeA* gene has been used as a convenient diagnostic marker for LEE-positive STECs (**Boerlin *et al.*, 1999** and **Scheutz*et al.*, 2012)**. STEC represent a hazardous public health problem worldwide causing various human gastrointestinal tract diseases, including watery or bloody diarrhea and might develop a life-threatening disease, such as hemorrhagic colitis (HC), Thrombotic Thrombocytopenic Purpura (TTP) and Hemolytic Uremic Syndrome (HUS). The latter is characterized by thrombocytopenia, microangiopathic hemolytic anemia and acute renal failure **(Pennington, 2010).**

The current study results from **Table (4)** and **Fig (1)**13 serological strains of *E. coli* were isolated. Twelve strain were positive for shiga-like toxin genes (*stx1*, *stx2*, *eaeA*).

In previous similar study, carried by **Elafify *et al.*, (2016)** found that most serological *E. coli*s trains isolated from commercial table eggs were positive for *eaeA* gene as O_2, O_{26}, O_{44}, O_{78}, O_{124} and O_{125}, while some strains as O_1, $O_{44}$and O_{128} were positive for *stx2*. Also, other serological strains were positive for *stx1* and *eaeA* genes as O_1 and O_{44},while, strains O_{114} and O_{125} were positive for *stx2* and *eaeA* genes. Two strains only;O_{78} and O_{128}; were positive for *stx1*, *stx2* and *eaeA* genes. Also, **Galal *et al.*, (2013)** detected *stx1*, *stx2* and *eaeA* genes in 2/9 (10.5%) of egg samples. **Maha (2013)** detected stx2 gene only in serological strains as O_{44}, O_{114}, O_{125}, $O_{126}$and O_{127}, were isolated from table eggs of chicken and all strains was negative for *stx1* and *eaeA* genes.

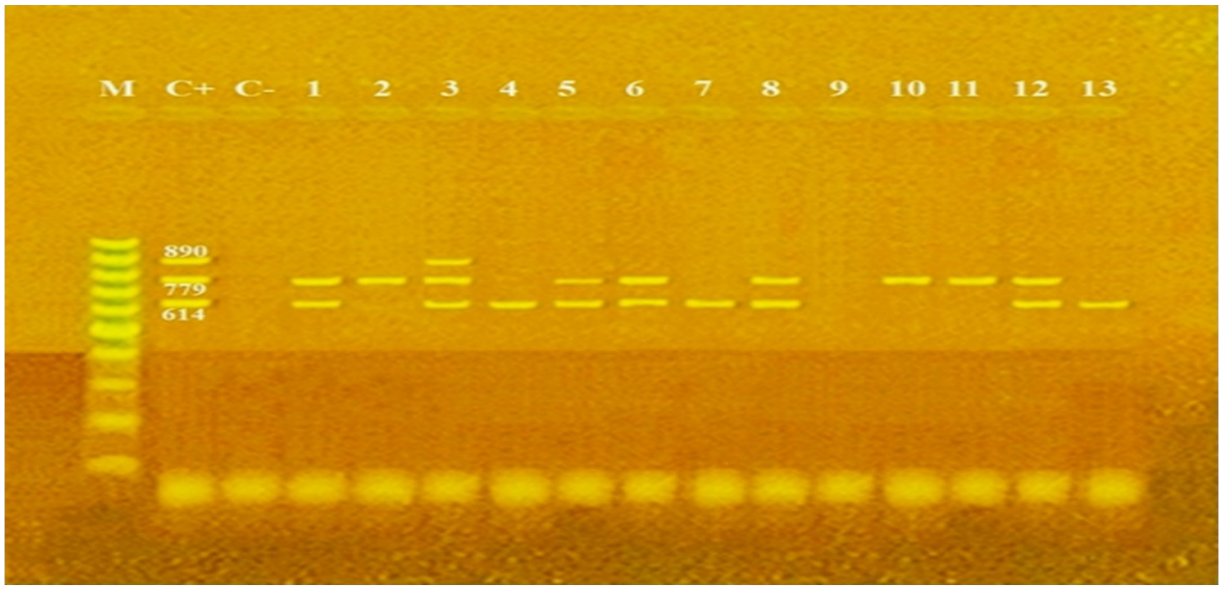

Fig. (1): Shown the gene demonstration (stx1, stx2 &eaeA) of different shiga-like toxin producing E. coli strains. **Lanes 1, (O1), 5 (O78), 6 (O91), 8 (O119) & 12 (O158):** Positive *E. coli* strains for *stx1* and *stx2* genes. **Lanes 2 (O2), 10 (O125) & 11 (O126):**Positive *E. coli* strains for *stx2* gene. **Lane 3 (O26):** Positive *E. coli* strain for *stx1*, *stx2* and *eaeA* genes. **Lanes 4 (O55), 7 (O114) & 13 (O163):**Positive *E. coli* strains for *stx1* gene. **Lane 9 (O124):** Negative *E. coli* strain for *stx1*, *stx2* and *eaeA* genes. **Lane M:** 100 bp ladder as molecular size DNA marker. **Lane C+:** Control positive *E. coli* for *stx1*, *stx2* and *eaeA* genes. **Lane C-:** Control negative.

Table (5). Incidence of different serotypes of *Salmonella* spp. in both balady and commercial table egg samples:

Serotypes	No.	No. of *Salmonella* spp. contaminated samples							
		Balady (No. = 100)				Commercial (No. = 100)			
		Group A (Houses) (No. = 50)		Group B (Local Market) (No. = 50)		Group C (Retail Shops) (No. = 50)		Group D (Supermarket) (No. = 50)	
		Shell	Content	Shell	Content	Shell	Content	Shell	Content
***S.* Enteritidis**	3	0	0	0	1	0	0	2	0
***S.* Typhimurium**	2	0	1	1	0	0	0	0	0
***S.* Kentucky**	3	0	2	0	0	0	0	0	1
***S.* Tsevie**	1	0	0	1	0	0	0	0	0
***S.* Papuana**	1	1	0	0	0	0	0	0	0
Total No. of Positive Isolates	10	1	3	2	1	0	0	2	1

Salmonella is one of the most common causes of food-borne infection worldwide. In Australia, the egg industry is periodically implicated in cases of *Salmonella* food poisoning (**Chousalkar *et al.*, 2010).** Uncooked or partially cooked foods containing raw egg as an ingredient accounted for 14% of food-borne outbreaks in 2006, 13% in 2007, and 28% in the first quarter of 2008 **(OzFoodNet Working Group, 2010).** It has been shown that some *Salmonella* serovars, such as *S.* Enteritidis, have the capacity to infect developing eggs within the oviduct, and therefore contaminated eggs act as an ecological amplifier **(Gast *et al.*, 2011).**

From **Table (5)** is showing that 10 strains of *Salmonella* spp. were isolated from the examined samples that revealed 5 serovars; *S.* Enteritidis (3 strains), *S.* Typhimurium (2 strains), *S.* Kentucky (3 strains), *S.* Tesvie (1 strain) and *S.* Popuana (1 strain). From balady table egg samples (no. 100), isolated *Salmonella* serovars were *S.* Enteritidis, *S.* Typhimurium,

S. Kentucky, *S.* Tesvie and *S.* Popuana from both egg shell (3%) and egg content (4%). On the other hand, samples from commercial table eggs (no. 100) showed low no. of *Salmonella* serovars where only *S.* Enteritidis and *S.* Kentucky were isolated by 2% from egg shell and 1% from egg content.

The results shown that isolated *Salmonella* serovars of balady table eggs were more than commercial eggs. Also, all 5 types of *Salmonella* serovars were isolated in balady table egg samples while only two *Salmonella* serovars were found in commercial table egg samples. *Salmonella* serovars were detected from both egg shell and egg content of samples from balady and commercial table eggs.

From similar studies, **Fardows and Shamsuzzaman, (2015),** reported that 4 *Salmonella* serovars were isolated from commercial table eggs (no. of examined eggs 150); *S.* Enteritidis (6 strains), *S.* Typhimurium (4 strains), *S.* Typhi (1 strain) and *S.* Paratyphi A (1 strain) from egg shell and *S.* Enteritidis (5 strain) from egg content. In addition, they isolated 4 unidentified *Salmonella* strains from egg shell and content. **Jebelli *et al.*, (2012)** reported that in chickens it had been shown that both *S.* Typhimurium and *S.* Enteritidis infect the reproductive tract and contaminate eggs but *S.* Enteritidis persists after egg are laid.

Table (6). The prevalence of *stn* (260 bp), *hilA* (497 bp) and *fimH* (1008 bp) virulence genes for *Salmonella spp*. isolated from examined samples:

Serotypes (No.=5)	Types of Shiga toxin genes		
	stn	***hilA***	***fimH***
***S.* Typhimurium**	+	+	+
***S.* Enteritidis**	+	+	+
***S.* Kentucky**	-	+	+
***S.* Papuana**	+	-	-
***S.* Tsevie**	-	-	+

The present study was directed to recognize some virulence genes of *Salmonella* by using one of the recent development molecular biological techniques (PCR). The genes were enterotoxin gene (***stn***), hyper-invasive locus gene (***hilA***) and fimbrial gene (***fimH***), they were applied on random isolated *Salmonella* species:*S.*papuana,*S.*Enteritidis,*S.*Typhimurium,*S.* Kentuckyand*S.*Tsevieas shown from **Table (6)**. From **Table (6)**, PCR results showed that *fimH* gene was detected in *S.* Tsevie. While, *hilA* and *fimH* genes were detected in *S.* Kentucky. The examined genes *stn, hilA* and *fimH* genes were detected in both *S.* Typhimurium and *S.* Enteritidis as shown also in **Fig (2).**

Similar to current study, **Elbayoumi *et al.*, (2018b)** reported that *stn, hilA* and *fimH* genes were detected in *S.* Typhimurium, *S.* Enteritidis and *S.* Kentucky from some poultry products collected from different supermarkets at El Menofiya Governorates.

On other previous studies**, Singh (2011)** found *stn* gene in 16 isolates of *S. typhimurium* from ground beef (8), intestine (6) and muscles (2) samples. **Minami *et al.*, (2010)** found *stn* gene in all 34 *Salmonella* serovars isolates.

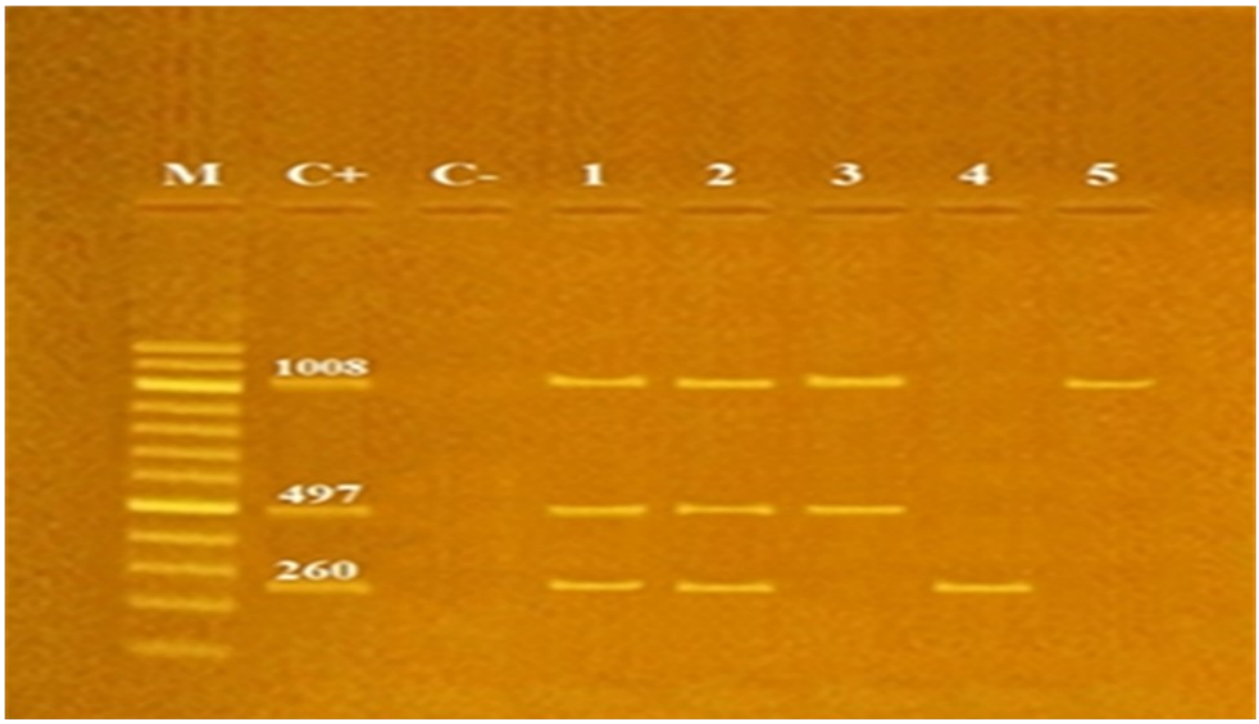

Figure (2): Shown the virulence genes (*stn, hilA* and *fimH*) for characterization of *Salmonella* species isolated from examined samples. **Lanes 1:** (*S.* Typhimurium) **& 2** (*S.* Enteritidis)**:** Positive strains for *stn, hilA* and *fimH* genes. **Lane 3:** (*S.* Kentucky): Positive strain for *hilA* and *fimH* genes. **Lane 4:** (*S.* papuana)**:** Positive strain for *stn* gene. **Lane 5:** (*S.* Tsevie)**:** Positive strain for *fimH* gene. **Lane M:** 100 bp ladder as molecular size DNA marker. **Lane C+:** Control positive strain for *stn, hilA* and *fimH* genes. **Lane C-:** Control negative.

Table (7). The incidence of Enterotoxin produced from coagulase positive *Staphylococcus aureus* isolated from balady table egg samples:

Type of samples	No. of samples (a)	No. isolates (b)	Types of enterotoxins and No. of detection (no. =2)				Total No. of enterotoxins ©	% of enterotoxins (c/b)
			SEA	SEB	SEC	SED		
Balady	Shell (no=100)	57	5	0	3	2	10	17.5 %
	Content (no.=100)	14	0	1	0	0	1	7.1 %
No. and % of isolates and enterotoxins		71/200 (35.5%)	5/11 (45.5%)	1/11 (9.0%)	3/11 (27.3%)	2/11 (18.2%)	11/11 (100%)	

Table (8). The incidence of Enterotoxin produced from coagulase positive *Staphylococcus aureus* isolated from commercial table egg samples:

Type of samples	No. of samples (a)	No. isolates (b)	Types of enterotoxins and No. of detection (no. =2)				Total No. of enterotoxins (c)	% of enterotoxins (c/b)
			SEA	SEB	SEC	SED		
Commercial	Shell (no=100)	20	1	0	0	1	2	10 %
	Content (no.=100)	3	0	0	0	0	0	0%
No. and % of isolates and enterotoxins		23/200 11.5%	1/2 (50%)	0/2 (0%)	0/2 (0%)	1/2 (50%)	2/2 (100%)	

All of CPS enterotoxins were very important as maintained by **Le Loir *et al.*, (2003)** who concluded that food-borne diseases were of major concern worldwide. To date, around 250 different food-borne diseases have been described, and bacteria are the causative agents of two thirds of food-borne disease outbreaks. Among the predominant bacteria involved in these diseases, *Staphylococcus aureus* was a leading cause of gastroenteritis resulting from the consumption of contaminated food. *Staphylococcal* food poisoning was due to the absorption of Staphylococcal enterotoxins preformed in the food.

In the current study table(7,8) the detection of enterotoxins from isolated CPS from balady table eggs and commercial table eggs was 11/11 (100%) and 2/2 (100%).

In France, Among the Staphylococcal food poisonings reported in a two-year period (1999 - 2000), in which the food involved had been identified were eggs and egg products for 11% and poultry for 9.5% **(Haeghebaert *et al.*, 2002).**

In this study, from **Table (7& 8)** the percentage of the 4 enterotoxins produced from CPS from shell and content of balady table eggs was 17.5% (10/57) and 7.1% (1/14), respectively and the SEA was the most detected type by 45.5% (5/11).While, the percentage of only two types of enterotoxins produced (SEA and SED) was 10% (2/20) from only shell of commercial one by equally percentage for two type of Staphlococcal enterotoxins.

Johler *et al.*, (2016) maintained that there are five major staphylococcal enterotoxins: SEA, SEB, SEC, SED, and SEE in food. From previous studies, the enterotoxin most frequently encountered in different types of foods was SEA **(Ayulo *et al.*, 1994, Marin *et al.*, 1992 &Sokari, 1991),** followed bySEC **(Ombui *et al.*, 1992 and Rosec *et al.*, 1997)**, SEB **(Ng and Tay, 1993)**, and SED **(Harvey *et al.*, 1982)**.

Conclusion

From the present study it could be concluded that all previous results, table eggs carried virulent toxins producing bacteria which are biological hazard to consumer. The table eggs were contaminated by different pathogenic microorganisms mostly occur on egg shells. The balady table egg were more contaminated with *E. coli*, *Salmonella* spp. and coagulase positive *Staphylococcus* than commercial one. The most isolated organism was coagulase positive *Staphylococcus aureus* then shiga-like toxin producing *E. coli* and followed by *Salmonella* spp. The contamination may be due to contact with soil, dust and dirty nesting material. The commercial table eggs were better than balady table eggs especially those sold in retail shops.

Recommendation

Some suggestive measures should be considered to keep the examined table eggs free from pathogens as possible:

Increase the awareness especially in local market in villages.

Good refrigeration and cooking of table eggs to avoid public health hazards.

Strict monitoring of local market and supermarkets where balady table eggs were sold by veterinary authority.

Routine microbiologically examination should be done periodically

The pathogenic isolates from table eggs must have further investigation especiallyto determine the possible shiga toxin production in routine examination.

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