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MOLECULAR CHARACTERIZATIONS OF SOME HAZARD BACTERIA ISOLATED FROM TABLE EGGS

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ABSTRACT

The objective of this study was to determine bacteriological status, incidence and molecular characterization of *E.coli, salmonellae* and *staph. aureus* in both baladi and farm table eggs. A total of 50 random chicken eggs contents samples (25 for each baladi and farm eggs) were collected from different supermarkets in Kafr-Elsheikh Governorate. Aerobic plate count showed mean values of $2.48x10^{4}\pm 0.39x10^{4}$ for baladi eggs and $6.15x10^{3}\pm 1.41x10^{3}$ for farm eggs, coliform count showed mean value of $9.53x10^{2}\pm 2.16x10^{2}$ for baladi eggs and $4.99x10^{2}\pm 0.72x10^{2}$ for farm eggs and staphylococcal count showed mean value of $2.05x10^{3}\pm 0.91x10^{2}$ for baladi eggs and $5.00x10^{2}\pm 0.30x10^{2}$ for farm eggs. Incidence of *E. coli* isolated from the examined samples of baladi and farm eggs contents (25 for each) were 4 isolates (16%) and 2 isolates (8 %), respectively. Serotypes of isolated *E. coli* were O1: H7 (1 isolate from farm eggs), O2: H6(1 isolate from baladi eggs), O44: H18 (1 isolate from baladi eggs), O78(2 isolate from baladi eggs and 1 isolate from farm eggs). Incidence of salmonellae isolated from the examined samples of baladi and farm eggs contents (25 for each) were 5 isolates (20%) from baladi eggs (2 isolates for *S. enteritidis* and 1 isolate for *S. typhimurium, S. kentucky* and *S. virchow*) and 3 isolates (12%) from farm eggs (2 isolates for *S. enteritidis* and 1 isolate for *S. kentucky*). Incidence of *staph.aureus* isolated from the examined samples of baladi and farm egg contents (25 for each) 2 isolates (8%) were isolated from each type. Multiplex PCR for identification of tetracycline resistance genes (tetB, tetC and tetD) of *E. coli* were (O78 and O1) Positive *E. coli* strains for tetB gene, (O78 and O44) Positive *e. coli* strains for both tetB and tetD genes, (O78) positive *E. coli* strain for tetC gene, (O2) positive *E. coli* strain for tetD gene. Multiplex PCR for identification of B-lactamase resistance genes of *Salmonella* species were (*S. enteritidis*) Positive strains for blaCMY gene, (*S. enteritidis and S. kentucky*) Positive strains for blaCTX gene, (*S. typhimurium*) Positive strain for blaCTX and blaCMY genes, (*S. enteritidis)* and *(S. virchow)* Negative strains for both genes. Multiplex PCR of enterotoxins genes (sea, seb, sec and sed) for characterization of *s. aureus*, 1 isolate positive *s. aureus* strain for sea and seb genes, 1 positive isolate *s. aureus* strain for sec gene, 1 isolate positive *s. aureus* strain for sea gene and 1 isolate negative *s. aureus* strain for enterotoxins genes. Farm eggs were bacteriologically better than baladi eggs. We recommend that strict hygienic measures to safe guard eggs from being deteriorated should be adopted in the farms and during handling and processing of eggs and not consumed raw.

Key word: *Table eggs, hazards bacteria, public health, food poisoning, multiplex PCR.*

INTRODUCTION

Chicken table egg is considered as the most valuable and perfect foodstuffs to human specially infants and elderly (Paskal *et al.,* 2014). At the same time, Eggs have been described as the most critical food vehicles of pathogenic microorganisms participating in the etiology of food borne diseases in humans (Stepien-Pysniak, 2010).

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Contamination of eggs and egg products with microorganisms can affect egg quality, which may lead to spoilage and pathogen transmission. This may induce cases of food-borne infection or intoxication to consumers, which constitute public health hazards. Several pathogenic microorganisms have been isolated from the surface of chicken egg shells and contents, among them, *Listeria monocytogenes, Escherichia coli* O157:H7, *Salmonella* and *Campylobacter* (Moore and Madden, 1993; Schoeni and Doyle, 1994; Hope *et al.,* 2002; Adesiyun *et al.,* 2005).

Eggs are liable to contamination either before laying (congenitally) or after laying when the

microorganisms reach the egg contents through penetration the shell and cause low egg quality, low shelf life and safety inducing public health hazards (Board and Fuller, 1994), in addition, fecal matter, improper washing, using of contaminated water and bad handling are the common sources of contamination. Egg can be contaminated by a variety of microbes such as *E. coli* and *Salmonella* (Ricke *et al.,* 2001). Contamination of the shell occurs from nest material, floor litter, avian fecal matter, collector's hands, packing materials and improper washing (Moats, 1980).

E.coli is a normal inhabitant of the intestinal tract of both man and animals and can penetrate the shell contaminating the egg contents (Mayes and Takeballi, 1983).

E. coli constitutes a major economic menace to poultry industry and consequently is of public health importance for human causing profuse watery diarrhea which is varying in its severity and persistence due to inflammation of the intestinal mucosa (Schiavoni and Vergora, 2000). The organism is taken as index of recent fecal contamination. Quiroga *et al.* (2000) stated that diarrhoegenic *E. coli* is the major agent involved in diarrhoeal disease in developing countries, could isolate *E. coli* from 35% of diarrhoeal and 26% of non diarrhoeal cases.

E. coli can multiply in egg content and cause infection when the number of the organism reaches 10^5 – 10^7 organisms/g (Eley, 1996). Bacterial contamination can happen at three main part of egg (egg yolk, albumen and shell memberan\egg shell) Bagrouz and Al-Jaff, (2005).

Escherichia coli can cause diarrheal disease in humans, referred to as diarrheagenic *E. coli.* These including enteropathogenic (EPEC), enterohaemorrahgic (EHEC), enteroinvasive (EIEC), enterotoxigenic (ETEC), Shiga toxin-secreating (STEC), diarrhea-associated haemolytic (DHEC), entero- aggregative (EAAggEC) and cytolethal distending toxin- recreating (CDTEC) *E. coli* strains. WHO (2009) reported also that each year, infections and persistent diarrhea in children in developing countries are not rare, as observed in infants living in the Nile delta area, who experienced between 4.6 and 8.8 diarrheal episodes, with ETEC accounting for 66% of these episodes. The production of cytotoxin in *E. coli* isolates have been extensively studied among isolates from humans and several animal species including poultry (Zahraei Salehi *et al.,* 2007).

Salmonella enteritidis is able to invade the cells of the follicles before ovulation and multiply themselves after 2 h of infection Howard *et al*. (2005). Moreover, *Salmonella* spp. remains a potential threat to human health, as well as, broiler chickens. Avian salmonellosis has public health importance associated with gastroenteritis in man resulting from consumption of infected hen's and duck's eggs (Abouzeed *et al.,* 2000). Different *Salmonella* spp. could be isolated from human diarrhoeal swapsby several authers (Urio *et al.,* 2001 and Biendo *et al.,* 2003).

Salmonella human infection resulting from the consumption of contaminated eggs is still a major public health problem (Koen *et al.,* 2006).

Staphylococci are most common bacteria contaminating egg shells and egg contents from external or during formation and laying process (Abdullah, 2010).

Furthermore, among the pathogenic food poisoning organisms that affect the public health of humans due to consumption of eggs is *Staph. aureus* which is of serious concern to public health (Wyah, 1992). Its thermostable enterotoxins elaborated in large numbers of foods and animal products including eggs causing rapid onest of nausea, vomiting and diarrhea within 6 hours of ingestion of food. Several outbreaks of *Staph. aureus* food poisoning have been recorded, involving large number of individuals throughout the world (Ko and Chang, 1995). Also, there have been many research works that deal with *Staph. aureus* in hen's eggs (Sabreen, 2001 and Bastawrows *et al.,* 2002).

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.

MATERIALS AND METHODS

1. Collection of samples:

A total of 50 random samples of Baladi eggs and Farm eggs, 25 samples of baladi eggs (125 intact eggs) and 25 samples of farm eggs (125 intact eggs) were collected from different supermarkets in Kafrelsheikh governorate. The eggs were transported to the laboratory and each sample was placed in a sterile plastic bag and carried to the laboratory without undue to be examined microbiologically (Cultured within 6 hours of collection).

2. Preparation of samples:

The egg was prepared for evacuation of its content according to APHA (2004) recommended by El-Kholy *et al.* (2014). Each egg was washed with warm

water (32ºC) using a brush and soap, the egg was drained and immersed in 70% Alcohol for 10 min, then flamed after it has been removed from alcohol. A hole was made in the blunt end of the egg by using sterile scalpel. The content of each egg sample(content from 5 eggs were pooled to form one sample) was removed aseptically and received into a sterile mixer until the sample became homogenous and from the pooled egg content ten-fold serial dilutions up to 10^5 were aseptically prepared using 0.1% sterile peptone water. The prepared samples were subjected to the following examinations:

2.1. Aerobic Plate Count (APC) (APHA 2004): The technique was applied by using surface plating method on standard plate count agar. Plates showing colonies between 30 and 300 were selected and counted.

2.2. Total coliform count using three tubes most probable number (MPN) method: (FDA, 2002)

Lauryl sulphatetryptose broth (LST) and brilliant green lactose bile (2%) broth (BGLB broth) were used for presumptive and confirmed tests for total coliform, respectively.

2.3. *Staph aureus* count (APHA 2004): The surface plating technique of Baird–Parker ager plates was followed. Further, the suspected colonies were picked up and identified biochemically.

3. Isolation and identification of E. coli (Bailey and Scott, 1990) and (ISO, 2004):

For the isolation and identification of E. coli, 1 ml of homogenized egg contents was inoculated into MacConkey enrichment broth and incubated at 37°C for 24 hours, the positive enriched sample with gas production was cultured on Eosin Methylene Blue (EMB) Agar plates and incubated at 37 °C for 24 hours. Morphologically, typical colonies (at least 4 / plate) producing metallic sheen on EMB were stabbed into semisolid agar tubes for further identification. Biochemical tests according to Macfaddin (2000) were performed to confirm E. coli using Catalase, Indole, Methyl red, Voges- Proskauer, Nitrate reduction, Urease production, Simon citrate agar and various sugar fermentation tests. The purified colonies were confirmed by using API strips (BioMerieux, Mary- I'Etoile, France). The positive isolates were serologically identified according to Kok *et al.* (1996) by using rapid diagnostic E.coli Set1: O and Set 2: H antisera sets (DENKA SEIKEN Co., Japan).

4. Isolation and identification of Salmonellae (Mackie and McCartney, 1989 and Quinn *et al.* 2004):

For the isolation and identification of Salmonellae, 1 ml of homogenized egg contents was inoculated into 10 ml of Rappaport Vassilidis broth (enrichment broth) followed by S.S as selective plating media. The suspected colonies appeared as red colonies with or without black center were identified biochemically and serologically. The purified colonies were confirmed by using API strips (BioMerieux, Mary-I'Etoile, France).

In general, serological identification of Salmonellae was carried out according to Kauffman – White scheme (Kauffman, 1974) for the determination of somatic (O) and flagellar (H) antigens using Salmonella antiserum (DENKA SEIKEN Co., Japan).

5. Isolation and identification of *Staph. aureus* as recommended by Mekonnen *et al.* (2011): Isolation of *Staph. aureus* was done by streaking (0.1ml) of the enriched egg contents samples on mannitol salt agar and Baird Parker agar supplemented with egg yolk and potassium tellurite and the plate was incubated at 37 °C for 24–48 hours. Typical coagulase-positive *Staph.aureus* colonies are yellow colonies surrounded with halo zone on mannitol salt agar or jet black shining convex colonies surrounded by white halo zone, 1-1.5 mm in diameter on Baird Parker agar were considered to be presumptive *Staph. aureus.* Characteristic colonies were stabbed into semisolid agar tubes for further identification by conventional methods including Gram's stain and various biochemical tests including coagulase test with rabbit plasma, anaerobic utilization of glucose, catalase test, oxidase test, indole, nitrate reduction and hemolysis on sheep blood agar. The purified colonies were confirmed by using API strips (BioMerieux, Mary- I'Etoile, France).

Multiplex Polymerase Chain Reaction (multiplex PCR)

1. Primer sequences used in the study:

1.1. Primer sequences of antibiotic resistance genes for *E. coli*:

The molecular characterization of tetracycline resistance genes (tetB, tetC and tet D) of *E.coli* was applied using the following primers (Pharmacia Biotech):

Primers	Oligonucleotide sequence (5′ → 3′) http://www.ncbi.nlm.nih.gov/pmc/articles/PMC14033 3/table/t2/ - t2fn1	Product size (bp)	References
tetB (F)	**5′** TTGGTTAGGGGCAAGTTTTG **′3**	659	**Ng *et al.* (2001)**
tetB (R)	**5′** GTAATGGGCCAATAACACCG **′3**		
tetC (F)	**5′** CTTGAGAGCCTTCAACCCAG **′3**	418	
tetC (R)	**5′** ATGGTCGTCATCTACCTGCC **′3**		
tetD (F)	**5′** AAACCATTACGGCATTCTGC **′3**	787	
tetD (R)	**5′** AAACCATTACGGCATTCTGC **′3**		

1.2. Primer sequences of *S. aureus* used for PCR identification system:
The demonstration of virulence factors including enterotoxin genes (A, B, C & D) of *S. aureus* was adopted by using the following primers:

Target gene	Oligonucleotide sequence (5′ → 3′) http://www.ncbi.nlm.nih.gov/pmc/articles/PMC14033 3/table/t2/ - t2fn1	Product size (bp)	References
sea (F)	**5′** TTGGAAACGGTTAAAACGAA**′3**	120	**Rall *et al.* (2008)**
sea (R)	**5′** GAACCTTCCCATCAAAAACA **′3**		
seb (F)	**5′** TCGCATCAAACTGACAAACG **′3**	478	
seb (R)	**5′** GCGGTACTCTATAAGTGCC **′3**		
sec (F)	**5′** GACATAAAAGCTAGGAATTT **′3**	257	
sec (R)	**5′** AAATCGGATTAACATTATCC **′3**		
sed (F)	**5′** CTAGTTTGGTAATATCTCCT **′3**	317	
sed (R)	**5′** TAATGCTATATCTTATAGGG **′3**		

1.3. Primers sequences of B-lactamase resistance genes of Salmonella species:
Accurately, the molecular characterization of B-lactamase resistance genes as virulence factors of Salmonellae were represented by cefotaxime (blaCTX) and cephalosporine (blaCMY) as follow:

Target gene	Oligonucleotide sequence (5′ → 3′) http://www.ncbi.nlm.nih.gov/pmc/articles/PMC14033 3/table/t2/ - t2fn1	Product size (bp)	References
blaCTX (F)	**5′** CGCTTTGCGATGTGCAG **′3**	550	**Ahmed et al. (2007)**
blaCTX (R)	**5′** ACCGCGATATGCTTGGT **′3**		
blaCMY (F)	**5′** GACGCCTCTTTCTCCACA **′3**	1007	
blaCMY (R)	**5′** TGGAACGAAGGCTACGTA **′3**		

2. 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 frozen at -20ºC till use.

3. Amplification of DNA:

3.1. Multiplex PCR detection of tet genes for *E. coli* (Karami *et al.*, 2006):

All isolates of tetracycline-resistant *E. coli* strains were assessed for carriage of the tetracycline resistance genes tetB, tetC and tetD. The method was optimized using the following reference *E. coli* strains with known tetgenes. The following procedure was used: a small amount of biomass from a bacterial colony was added to a sterile thin-walled reaction tube containing Taq Master Mix, primers, $MgCl_2$, and distilled water. The multiplex PCR included primers for tetB, tetC (0.25 µM each) and tet D (3 µM) and 1.5 mM $MgCl_2$. The tubes were sealed with a drop of mineral oil and heated to 95°C for 15 min in a thermocycler to activate the Taq DNA polymerase. After 5 min DNA template denaturation at 94°C, 25 PCR cycles followed, with DNA denaturation at 94°C for 1 min, primer annealing at 60°C for 1 min, and primer extension at 72°C for 1.5 min. Finally, PCR products were separated electrophoretically in a 2% agarose gel, visualized by staining with 0.5 µg/ml ethidium bromide, and examined in UV light. A 100 bp plus DNA Ladder (Qiagen, Germany, GmbH) was used to determine the fragment sizes.

3.2. Amplification of B-lactamase resistance genes of Salmonellae (Roschanski *et al.*, 2014):

PCR amplifications were performed in 50 µL reactions containing 25 µL DreamTaq Green PCR Master Mix (Thermo Scientific, St. Leon Roth, Germany), 2 µL of each forward and reverse primer (10 pmol), 20 µL of sterile water and 1 µL of DNA-mixture. The PCRs were performed with an initial denaturation step of 95°C followed by 35 cycles – denaturation at 95°C for 30 sec, annealing for 30 sec and elongation for 1 min. at 72°C – before finishing the run a final elongation step at 72°C for 5 min was attached. Similar multiplex PCR conditions were applied to the DNA templates of negative control isolates. The amplified products were electrophoresed on 2% agarose gel, stained with ethidium bromide (5 µg /100 ml) and captured as well as visualized on UV transilluminator.

3.3. Amplification of enterotoxin genes of *S. aureus* (Mehrotra *et al.*, 2000):

Ten µl of DNA sample was diluted in 990 µl of nuclease free water for PCR. The genomic DNA samples were amplified by PCR in a reaction mixture (25µl) containing 13.25 sterile dH_2O, 2.5ml 10 x buffer, 0.63ml 10mMNTPs, 1ml 25Mm $Mgcl_2$, 1.25 µl primer F(20pmol/ml), 1.25 µl primer R (20pmol/ml) and fill up to 25 µl PCR grade water. Concerning the primers used for demonstration of *S. aureus* enterotoxins (sea, seb, sec &sed), the amplification was performed on a Thermal Cycler (Master cycler, Eppendorf, Hamburg, Germany). DNA amplification was performed using the following conditions: initial denaturation for 5 min at 95°C followed by 30 cycles of denaturation (94°C for 2 min), annealing (55°C for 1 min), and extension (72°C for 2 min).A final extension step (72 °C for 5 min) was performed after the completion of the cycles. Amplified products were analyzed by 3% of agarose gel electrophoresis (Applichem, Germany, GmbH) in 1x TBEbuffer stained with ethidium bromide and captured as well as visualized on UV transilluminator at 254 nm.

RESULTS

Table 1: Statistical analytical results of different bacterial count (/ml) in the examined samples of egg contents (n=25).

EggType	Baladi eggs			Farm eggs		
Bacterial count	**Min**	**Max**	**Mean ± S.E***	**Min**	**Max**	**Mean ± S.E***
APC	$1.4x10^3$	$7.5x10^4$	$2.48x10^4 \pm 0.39x10^4$ a	$4.9x10^2$	$1.1x10^4$	$6.15x10^3 \pm 1.41x10^3$ a
Coliform count	$1.0x10^2$	$2.4x10^3$	$9.53x10^2 \pm 2.16x10^2$ b	$1.0x10^2$	$2.0x10^3$	$4.99x10^2 \pm 0.72x10^2$ b
Staph. count	$1.0x10^2$	$4.0x10^3$	$2.05\ x10^3 \pm 0.91x10^2$ c	$1.0x10^2$	$9.0x10^2$	$5.00x10^2 \pm 0.30x10^2$c

The different superscripts in the same row indicated significant differences
S.E*= Standard error of mean

Table 2: Incidence of *E. coli* isolated from the examined samples of egg contents (n=25).

Egg Type	Baladi eggs		Farm eggs		Strain characteristics
***E.coli* Strains**	**No.**	**%**	**No.**	**%**	
O1 :H7	-	-	1	4	**EPEC**
O2 :H6	1	4	-	-	**EIEC**
O44:H18	1	4	-	-	**EPEC**
O78	2	8	1	4	**ETEC**
Total	4	16	2	8	

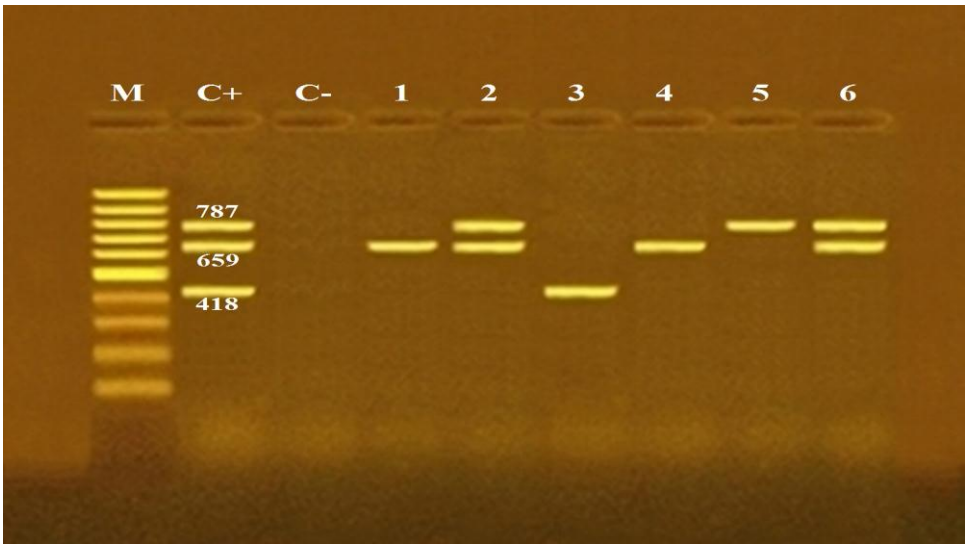
EPEC = Enteropathogenic ***E.coli*** **ETEC** = Enterotoxigenic ***E.coli***
EIEC = Enteroinvasive ***E.coli***

Table 3: Incidence of Salmonellae isolated from the examined samples of egg contents (n=25).

Egg Type	Baladi eggs		Farm eggs	
Salmonella Strains	**No.**	**%**	**No.**	**%**
S. Enteritidis	2	8	2	10
S. Typhimurium	1	4	0	0
S. Kentucky	1	4	1	4
S. Virchow	1	4	0	0
Total	5	20	3	12

Table 4: Incidence of *Staph. aureus* in the examined samples of egg contents (n=25).

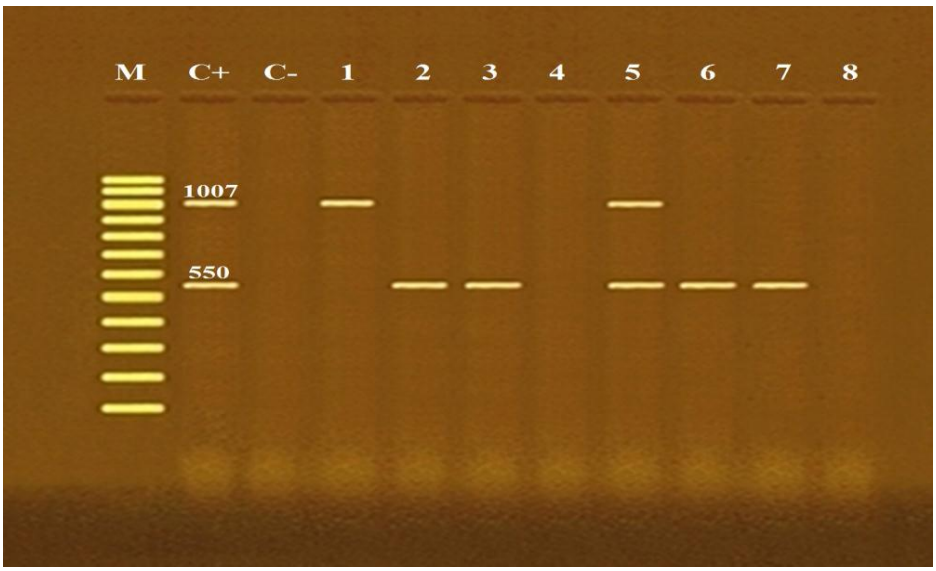
	Baladi eggs		Farm eggs	
Egg Type	**N0.**	**%**	**N0.**	**%**
S. aureus	**2**	**8**	**2**	**8**



Photograph (1): Agarose gel electrophoresis of multiplex PCR for identification of tetB (659 bp), tetC (418 bp) and tetD (787 bp)

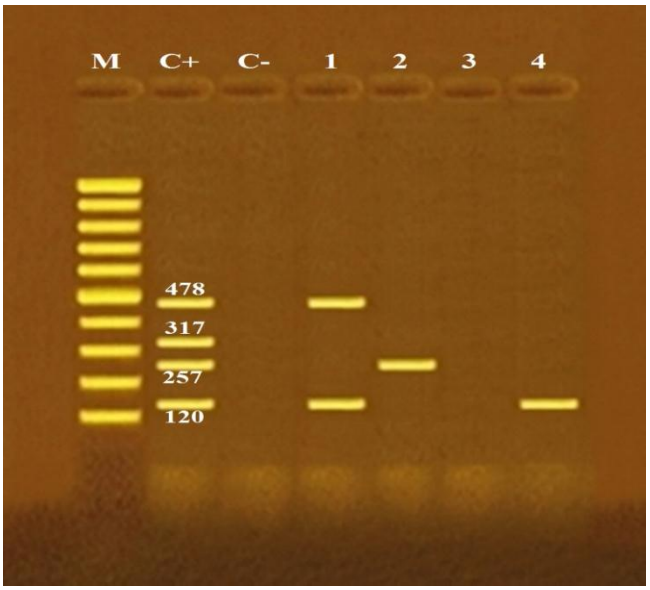
Resistance genes of *E. coli.*

Lane M: 100 bp ladder as molecular size DNA marker.
Lane C+: Control positive *E. coli* for tetB, tetC and tetD genes.
Lane C-: Control negative.
Lanes 1 & 4 (O78 and O1): Positive *E. coli* strains for tetB gene.
Lanes 2 & 6 (O78 and O44): Positive *E. coli* strains for both tetB and tetD genes.
Lane 3 (*E. coli* O78): Positive *E. coli* strain for tetC gene.
Lane 5 (*E. coli* O2): Positive *E. coli* strain for tetD gene..



Photograph (2): Agarose gel electrophoresis of multiplex PCR for identification of B-lactamase resistance genes of Salmonella species represented by blaCTX (550 bp) and blaCMY (1007 bp).

Lane M: 100 bp ladder as molecular size DNA marker.
Lane C+: Control positive for blaCTX and blaCMY genes.
Lane C-: Control negative.
Lane 1 (*S. Enteritidis*): Positive strain for blaCMY gene.
Lanes 2, 3 (*S. Enteritidis*), 6 and**7 (*S. Kentucky*):** Positive strains for blaCTX gene.
Lane 5 (*S.Typhimurium*): Positive strain for blaCTX and blaCMY genes.
Lanes 4 (*S. Enteritidis*) and **7 (*S. Virchow*):** Negative strains for both genes.



Photograph (3): Agarose gel electrophoresis of multiplex PCR of sea (120 bp), seb (478 bp), sec (257 bp) and sed (317 bp) enterotoxin genes for characterization of *S. aureus*.

Lane M: 100 bp ladder as molecular size DNA marker.
Lane C+: Control positive for sea, seb, sec and sed genes.
Lane C-: Control negative.
Lane 1: Positive *S. aureus* strain for sea and seb genes.
Lane 2: Positive *S. aureus* strain for sec gene.
Lane 4: Positive *S. aureus* strain for sea gene.
Lane 3: Negative *S. aureus* strain for enterotoxins.

DISCUSSION

In Egypt eggs most commonly used unwashed. Special attention has been paid for raw or undercooked eggs because the hens act as natural reservoirs of a variety of pathogens. The contamination occurs through the shell; But humidity, temperature and storage time are critical for migration of bacteria from the surface of the shell to the inner structures of the egg (Evencio *et al.*, 2012).

Food-borne diseases caused by microorganisms are a large and growing public health problem. Most common foodborne pathogens associated with food of animal origin are *Salmonella, Campylobacter, Listeramonocytogenes, Staphylococcus aureus* and *E.coli* (Akbar and Anal 2011, Ghasemian, (2011) and Akbar and Anal, (2013a). Freshly laid eggs are generally devoid of organisms. Exposure to environmental conditions as temperature and humidity influence the bacterial penetration and thus enhance the infection and spoilage (Theron *et al.*, 2003).

Foodborne illness is a major public health problem and the main cause of diarrheal diseases affecting all developed and developing countries (Akbar and Anal, 2014).

In table (1) results ofAerobic plate count showed mean values of baladi eggs $2.48x10^{4}\pm 0.39x10^{4}$ and for farm eggs $6.15x10^{3}\pm 1.41x10^{3}$, coliform count showed a mean value of baladi eggs $9.53x10^{2}\pm 2.16x10^{2}$ and for farm eggs $4.99x10^{2}\pm 0.72x10^{2}$ and staphylococcal count showed mean values of baladi eggs $2.05x10^{3}\pm 0.91x10^{2}$ and for farm eggs $5.00x10^{2}\pm 0.30x10^{2}$and it was lower than the accepted $10x10^{5}$ gm/cfu as recommended by the International Commission on the Microbiological Specification for Food (ICMSF 1996). Statistical analytical results between baladi eggs and farm eggs was highly significant ($p < 0.01$) and these results were lower than Eman *et al*. (2015) they recorded that the mean value of total colony count was $5.36x10^{6} \pm 1.07x10^{5}$cfu/gm., while the mean values of coliforms and Coagulase Positive Staphylococci were $2.61x10^{7} \pm 5.22x10^{5}$ MPN/gm. and $1.94x10^{7} \pm 3.88x10^{5}$cfu/gm., respectively, from chicken table eggs.

El-kholy *et al*. (2014) Evaluate the microbiological quality of poultry farms eggs in Beni-Suef city and results were The aerobic plate count (present in 50% of egg contents) and coliform count(present in 47.06 % of egg content) were $1.1x10^{3} \pm 3x10^{2}$ and $1.5x10^{3}\pm 8.4x10^{2}$cfu/ml for egg content respectively, the high counts of coliforms may be due to bad sanitary conditions and/or delay in eggs collection from nests which were contaminated with fecal matters, Jull (1984).

Results in Table 2 revealed that incidence of *E. coli* isolated from the examined samples of baladi and farm egg contents (25 for each) were 4 isolates (16%) and 2 isolates (8 %) respectively. Serotypes of isolated *E. coli* were O1: H7 (1 isolate from farm eggs), O2 : H6(1 isolate from baladi eggs), O44: H18 (1 isolate from baladi eggs), O78(2 isolate from baladi eggs and 1 isolate from farm eggs).

E.coli is one of the major problems in chicken production influencing heavier losses and sever drop in egg production, about 5.5 % mortality and 10-20% drop in eggs was observed with *E.coli* infections Qu *et al.* (1997). Samah *et al.* (2015) declared a total of 36 *E. coli* isolates with prevalence rates of 18%. Among the total isolates, 21 (10.5%) isolates, 9 (4.5 %) isolates, and 6(3.0%) isolates were detected from over the shell, egg contents, and both shell and contents, respectively. Arathy *et al*. (2011) could detect an overall isolation rate of 12.2%, while 8% of the isolates were detected from shell and 5% from yolk samples, respectively. Isolation rate was reported by (Adesiyun *et al*., 2005) who recorded (37.0%) as an overall isolation rate, (28.3%) as an isolation rate from egg shell, while they recorded isolation rate from egg content samples (3.8%). Lower isolation rates were recorded by (Saitanu *et al.*, 1994) who isolated *E. coli* from egg contents with a rate of 1.2%.Ansah *et al*. (2009) Isolated *Escherichia coli* from egg content. Resistant *E. coli* strains from the gut often cause contamination of eggs during lay with multi resistant *E. coli* (Turtura *et al.*, 1990) El-kholy *et al*. (2014) recorded that *E.coli* could be detected in 11.76% of contents of poultry farm eggs.

Jehan *et al.* (2014) Isolated *E.coli*, in both baladi and farm eggs were in the following percentage (22) and (19) respectively. Egg shell contamination is the main reason for *E. coli* infection. Poor hatcher sanitation can leave a residue of *E. coli* from the previous hatch leads to yolk infections which occur during hatching process (Eric, 2011) and (Maha 2013) reported that *Esherichia coli* population can be used as measures of quality and sanitary processing condition (Kornacki and Johnson, 2001). Also, it is an ideal indicator organisms of fecal contamination, in human and animal feces, 90-100% of coliform organisms isolated are *E.coli* (Hurst *et al.*, 2002). The conventionally identified *E.coli* strains isolated from different types of table eggs were serotyped into 7 different serotypes included O44, O111, O114, O125, O126, O127 and O128. *E. coli* serotyping is an important technique for making the proper diagnosis and epidemiological investigations during food borne outbreaks. Thus serotyping alone cannot be relied on for categorizing a strain of *E. coli* and the identification of specific virulance characteristics/genes must also be performed (Barlow *et al.*, 1999). Ansaruzzaman *et al.* (2007) reported that classification of ETEC strains, largely based on

O-antigen type, Colonization Factor (CF) expression pattern and toxin profile. *Escherichia coli* is the major micro-organism isolated both from the surface and in the content of the egg, this may be attributed to the fact that *Escherichia coli* are normal inhabitants of intestinal tracts of birds (Singleton and Sainsburg, 1981). They have also been known to contaminate the surface of egg while the mechanical process can spread the bacteria through the eggs. Contaminations with the pathogen while in the field occur through improperly decomposed manure and poor hygienic practice of farm workers. *Escherichia coli* can bring about urinary tracts infections, pneumonia meningitis and peritonitis in humans (Schoeni and Doyle, 1994). Eman *et al*. (2015) isolated*E. coli* from chicken table eggs 12 isolates(28.58%).

Photo1 showed that Multiplex PCR for identification of tetracycline resistance genes (tetB, tetC and tetD) of *E. coli* were (O78 and O1) Positive *E. coli* strains for tetB gene ,(O78 and O44) Positive *E. coli* strains for both tetB and tetD genes, (O78) positive *E. coli* strain for tetC gene, (O2) positive *E. coli* strain for tetD gene. Diarrassouba *et al*. (2007) Recorded that Fifty-two E. coli isolates (70.3%) and nine Salmonella isolates (14.52%) were multi-resistant to at least nine antibiotics. The multi-resistant isolates were evaluated for the presence of tetracycline resistance, integron class 1, and bla CMY-2 genes by PCR. Of the 74 *E. coli* isolates, 55 isolates were resistant to amoxicillin and ceftiofur. Among these 55 resistant *E. coli* isolates, 45 (81.8%) and 22 (40.0%) were positive for bla CMY-2 and qacEΔ1-SulI genes, respectively. Tetracycline resistance was found in 56 isolates (75.8%) among which 12 (21.4%) and 24 (42.9%) gave positive results for tetA and tetB, respectively.

Musgrove *et al.* (2008) isolated *E. coli* from eggs and analyzed for their antibiogramagainst 16 antibiotics including ampicillin, tetracycline, gentamicin, and kanamycin. They reported that *E. coli* (73.20%) were susceptible to all antimicrobial used. They reported that some *E. coli* (29.90%) isolates exhibited resistance against tetracycline. Adesiyun *et al.* (2005) Isolated *E. coli* in 4.3% of egg contents of farm eggs also, they added that the frequency of *E. coli* founded in eggs depending on their rearing sources. Study presence of 14 tetracycline resistance determinants from *E. coli* isolated from animal origin and analyses indicated that 97% of these strains contained at least 1 of 14 tetracycline resistance genes [tetA, tetB, tetC, tetD, tetE, tetG, tetK, tetL, tetM, tetO, tetS, tetA(P), tetQ, and tetX] examined, while the most common genes found in these isolates were tetB (63%) and tetA (35%), tetC, tetD, and tetM were also found, *E. coli* isolates from chickens were the only strains to have tetM, 31% of e.coli were highly resistant to tetracycline. Samah *et al*. (2015) reported the following results from baladichicken a total of 36 *E. coli* strains were isolated with an overall isolation rate of 18%, were judged by PCR, for *E. coli* strains, the *blaTEM, sul1* and *tetA(A)* genes were tested for the B-lactams, trimethoprim sulphamethazone and tetracyclines, respectively and *E. coli* virulence was tested by PCR through the testing of*iss, eaeA, stx1, stx2, tsh* and *papC* genes.

Results in Table 3 and photo 2 revealed Incidence of salmonellae isolated from the examined samples of baladi and farm eggs contents (25 for each) were 5 isolates (20%) from baladi eggs (2 isolates for *S. enteritidis* and 1 isolate for *S. typhimurium*, *S. kentucky* and *S. virchow*) and 3 isolates (12%) from table eggs (2 isolates for *S. enteritidis* and 1 isolate for *S. kentucky*). Multiplex PCR for identification of B-lactamase resistance genes of *Salmonella* species were (*S. enteritidis*) Positive strains for blaCMY gene, (*S. enteritidis and S. kentucky*) Positive strains for blaCTX gene, (*S. typhimurium*) Positive strain for blaCTX and blaCMY genes, (*S. enteritidis)* and *(S. virchow)* Negative strains for both genes. Salmonella can be regarded as two types of infections. The first is primarily of importance for public health by causing food borne illness. The other type causes severe disease leads to great economic losses in poultry industry (Anbessa and Shiferaw, 2013). Chousalkar *et al*. (2010) and Samah *et al*. (2015) detected no Salmonella neither from on the shell nor from the egg contents. Salmonella spp. was isolated by other researcher as (Mona *et al*., 2014) who detected Salmonella in eggs at a rate of (1.5%). However, (Camilleri, 1992) stated that failure to detect salmonella spp. from eggs does not imply that local flocks are not infected by salmonella. Other researcher detected lowest incidence of Salmonella in table eggs with 0.07% in egg content by De Reu *et al.* (2006) who found 0.18% and Begum *et al.* (2010) who reported variable and very low incidence of Salmonella. On contrary, Abdul Aziz *et al.* (2012) failed to isolate Salmonella spp. these results variation could be attributed to different control measures applied against these bacteria.

Egg-associated Salmonellosis is a public health problem, the use of antibiotics in animals disrupts normal flora of intestine, resulting in emergence of antibiotic-resistant Salmonellae and their prolongedfecal shedding into the environment (Ahmed *et al.*, 2011). Resistance genes may be transferred from microorganisms to others, or may be due to mutations and selection pressure in microorganisms. *Salmonella* are potentially dangerous because of their ability to producing potent enterotoxin (Singh *et al.*, 2010). Soo Jin *et al*., (2001) Detected β – lactamase gene distributed among *salmonella* isolates from animal origin using multiplex PCR. Eman *et al.* (2008) isolated salmonellae from baladi in percentage (3.3) *salmonella* from farm eggs in percentage (3) respectively. Salihu *et al.* (2015) Isolated *salmonella* (13.5%) from contents of farm eggs at retail outlets in Sokoto metropolis Nigeria, and they recorde that the high percentage of isolation

of *salmonella* from the egg contents may be due to the organism's presence in the ovary or oviducts before the shell forms around it. Our results agrees with (Kinde *et al.,* 2000). Van *et al.* (2005) reported that the bacterium infects the eggs by either vertical transmission during development of these eggs within the ovary or horizontal transmission through trans-shell contamination.

In Table 4 and photo 3 Incidence of *s.aureus* isolated from the examined samples of baladi and farm egg contents (25 for each) were 2 isolates (8%) isolated from each type. Multiplex PCR of enterotoxins genes (sea, seb, sec and sed) for characterization of *s. aureus*, 1 isolate positive *s. aureus* strain for sea and seb genes, 1 positive isolate *s. aureus* strain for sec gene, 1 isolate positive *s. aureus* strain for sea gene and 1 isolate negative *s. aureus* strain for enterotoxins genes. Table eggs were bacteriologically better than baladi eggs. Abdullah (2010) reported the highest degree of egg contamination with Staphylococcus spp and eggs laid in dirty environment contained more bacteria than eggs lay in clean environment. The surrounding environment and storage condition including temperature and storage duration can influence the level of bacterial contamination (Stepien 2010). However, some of the common forms of Staphylococci are associated with poultry infections. Increasing attention has been given to the role of poultry and poultry products, including eggs, as a potential source of infections in humans induced by antibiotic-resistant Staphylococcus strains (Abulreesh and Organji 2011). *Staphylococcus aureus* and other spp. are important Pathogens in human and veterinary medicine, beside their importance in regard to food hygiene because of their ability to form staphylococcal enterotoxins (SEs). Jannatul Fardows *et al*. (2016) Recorded that out of 150 egg shells, 120 (80%) yielded growth of different bacteria, of them, Staphylococcus spp. were 80 (66.67%), out of 80 Staphylococcus spp., 30 (25%) were *Staphylococcus aureus* and sensitive to ciprofloxacin. Staphylococcus is considered to be a normal flora of chickens, isolated from the skin and feathers as well as in the respiratory and intestinal tracts Andrew Bryan *et al.* (2004) and Casey *et al.* (2007). Goto *et al*. (2007) recorded that the enterotoxin genes of 30 strains of *Staph. aureus* were detected with LAMP assay to each enterotoxin, SEA, SEB, SEC and SED, completely accorded with the results of polymerase chain reaction (PCR) assay. Enterotoxin production, determined by a reverse passive latex agglutination assay, strongly correlated with the presence of the corresponding genes and the sensitivity of the LAMP assay was generally higher than that of conventional PCR assay and it rapidly detected enterotoxigenic *Staph. aureus* strains within 60 min. Lee *et al*. (2007) reported that verifying the actions of toxin genes in humans is very important. Therefore, the expression of enterotoxin genes in *S. aureus* was confirmed by a reverse transcription real-time PCR that facilitates detection on an RNA level. Consequently, comparing the expression of various staphylococcal enterotoxin genes can provide a more effective evaluation of the toxic hazards of *S. aureus*. Siriporn *et al*. (2015) Isolated *Staphylococcus aureus* (18.4%), from table eggs soled in markets. Arathy *et al.* (2011) reported bacteria of 15 different genus, included Staphylococcus spp., Staphylococcus spp. (18.40% in market and 28.40% farm layer) was predominantly found associated to egg.

The degree of contamination with bacteria depending on the source of eggs (Stepien-Pysniak *et al.,* 2009; Shareef *et al.,* 2009) the safety of eggs depends on the number of bacterial cells on shell and content of eggs for presence of factors that initiate pathogen multiplication (Ricke *et al.,* 2001). The risk of illness resulting from consumption of contaminated eggs depends not only on the number of bacterial cells in eggs, but also on the type of bacteria (Bradshaw *et al.,* 1990). *Staphylococcus aureus* has been reported by Stepien-Pysniak *et al.* (2009) demonstrated that Staphylococcus spp. dominated in the yolks 38.8%, on the shells 58.9 and 2.5% in white of table eggs. Variance in prevalence of bacterial contamination from shell and content might attributed to penetration rate. These results supported by Al-Ali *et al.* (2012) who found that Salmonella spp. were the highest in penetration rate followed by *Stapylococcus aureus* and *Escherichia coli* through egg pours. Samah *et al*. (2015) reported the following results from baladichicken egg, eighty (40%) Coagulase Positive Staphylococci isolates were detected and the antibiotic susceptibility results were judged by PCR, for mecA, *blaZ* and tetK genes were tested for the evaluation of the susceptibility of Coagulase Positive Staphylococci against oxacillin, B-lactams, and tetracyclines, respectively and the virulence of Coagulase Positive Staphylococci was estimated through testing of the enterotoxins genes in addition to coagulase, *spa* and *hlg* genes

CONCLUSION AND RECOMMENDATIONS

From results obtained in this study we can concluded that farm table eggs were more hygienic than baladi table eggs (Aerobic plate count, coliform count, staphylococcal count and Incidence of *E. coli* and *Salmonellae* were higher in baladi eggs than farm eggs). Isolation of *E.coli*, *Salmonellae* and *Staph.aureus* from both baladi and farm eggs may be attributed to lack of sanitary conditions, so eggs should be considered as a vehicle for transmission of potentially pathogenic bacteria. It is important to handle eggs in such a way that microorganisms present do not have chance to multiply and to prevent eggs from becoming contaminated with other microorganisms (WHO, 2009). Improving hygienic practices during routine handling of eggs should be followed to limit the spread of such bacteria to humans. While less than 100 cell/ml of bacterial

counts can be achieved if some better hygienic practices implemented, such as introduction of cooling system for eggs during production, transportation and during distribution process. Increase awareness of public health of producers, sellers and consumers. Also, antibiotic sensitivity tests should be done to the isolated bacteria to detect effective antibiotic in treatment and for saving our time, costs of treatment and decreasing our losses. It is concluded that table eggs should not be consumed raw. Also we can recommend strict hygienic measures to safe guard eggs from being deteriorated should be adopted in the farms and during handling and processing of eggs.

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التوصيف الجزيئى لبعض البكتريا الخطيره المعزوله من بيض المائده.

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تم اجراء هذه الدراسة لمعرفه مدى التواجد والتوصيف الجزيئى لميكروبات الاى كولاى والسالمونيلا والمكور العنقودى الذهبى. تم تجميع ٥٠ عينه (٢٥ من البيض البلدى و٢٥ من بيض المزارع) من السوبر ماركت من اماكن مختلفه من محافظه كفر الشيخ وكان متوسط عدد الميكروبات الهوائيه فى البيض البلدى ٢.٤٨×١٠^٤ ± ٠.٣٩×١٠^٤ وفي بيض المزارع ٦.١٥×١٠^٣ ± ١.٤١×١٠^٣ وكان متوسط عدد الميكروبات القولونيه فى البيض البلدى ٩.٥٣×١٠^٢ ± ٢.١٦× ١٠^٢ وفى بيض المزارع ٤.٩٩×١٠^٢ ± ٠.٧٢×١٠^٢ وكان متوسط عدد المكور العنقودى الذهبى فى البيض البلدى ٢,٠٥×١٠^٣ ± ٠.٩١×١٠^٢ وفى بيض المزارع ٥×١٠^٢ ± ٠.٣٠×١٠^٢ تم عزل ميكروب الاى كولاى من البيض البلدى ٤ معزولات (١٦%) ومن بيض المزارع ٢ معزوله (٨%) وكان التصنيف السيرولوجى لهذه المعزولات هو معزوله واحده من البيض البلدى O2:H6 ومعزوله واحده من بيض المزارع وO1:H7 ومعزوله واحده من البيض البلدى , O44:H18 معزولتان واحده من البيض البلدى والاخرى من بيض المزارع .O78 كما تم عزل ميكروب السالمونيلا وكانت نسبه العزل من البيض البلدى ٥ معزولات (٢٠%) معزولتان من السلمونيلا انترتيدس ومعزوله من السلمونيلا تيفيميوريمو معزوله واحده من السلمونيلا كينتاكى ومعزوله واحده من السلمونيلا فيرشا وبينما تم عزل ميكروب السالمونيلا من بيض المزارع وكان عدد المعزولات ٣ (١٢%) معزولتان من السلمونيلا انترتيدس ومعزوله من السلمونيلا كينتاكى. تم عزل ميكروب المكور العنقودى الذهبى بمعدل ٢ معزوله (٨%) من كل من البيض البلدى وبيض المزارع. تم عمل اختبار مالتبلكس لتفاعل البلمره المتسلسل على ميكروب الايكولى للبحث عن الجينات المسئوله عن مقاومه التتراسيكلين (tetB, tetC,tetD) وكانت معزولات (O78,O1) تحتوى على الجين(tetB) ومعزولات (O78,O44) تحتوى على جينات (tetB,tetD) ومعزوله O78 على جين (tetD)تم عمل اختبار مالتبلكس لتفاعل البلمره المتسلسل على ميكروب السلمونيلا للبحث عن الجينات المسئوله عن المقاومه للبيتالكتامز (blaCTX,blaCMY) وكانت معزوله السلمونيلا انترتيدز تحتوى على جين (blaCMY) وكانت معزوله السلمونيلا انترتيدس ومعزوله سلمونيلا كينتاكى يحتويان على جين (blaCTX) وجين (blaCMY) وكانت هناك معزولتان من السلمونيلا انترتيدس وفرشا ولا يحتويان على اى من الجنين محل الدراسه. كما تم عمل اختبار مالتبلكس لتفاعل البلمره المتسلسل على ميكروب المكورالعنقودى الذهبى للكشف عن الجينات المسئوله عن انتاج السموم المعويه وهى جينات (sea, seb , sec, sed) وكانت هناك معزوله واحده تحتوى على جين sea و seb ومعزوله واحده تحتوى على جين sea ومعزوله واحده تحتوى على جين sec ومعزوله واحده لا تحتوى على اى من الجينات محل الدراسه. كان بيض المزارع أفضل من الناحيه البكتيريه من البيض البلدى كما اننا ننصح باستخدام اجراءات صحيه صارمه لحمايه البيض من الفساد فى المزرعه واثناء تناول وتجهيز البيض والذى لايجب ان يستهلك نيئا .