Detection of some bacteria and mycotoxins in the baladi chicken eggs from backyard in Bahira Governorate

Fady Sayed Youssef[a], Ahlam E. Yonis[b], Gomaa Y. Elhaddad[b], Hossam Sh. Elsebaey[b], Nahed A.E.S. Naem[c], Fatma H. Amro[d], Maha S. Abd-Elhafeez[e]

[a]Department of Pharmacology, Faculty of Veterinary Medicine, Cairo University, 1221 Giza, Egypt, [b]Biotechnology Department, Reference Laboratory for Veterinary Quality Control on Poultry Production, Animal Health Research Institute Damanhur Branch, 22511, Agriculture Research Center, Egypt, [c]Bacteriology Department, Reference Laboratory for Veterinary Quality Control on Poultry Production, Animal Health Research Institute Damanhur Branch, 22511, Agriculture Research Center, Egypt, [d]Reference laboratory of veterinary Quality Control on Poultry Production (RLQP), Animal Health Research Institute (AHRI), Agriculture Research Center (ARC), Giza 12619, Egypt, [e]Chemistry, Toxicology and Feed Deficiency Department, Animal Health Research Institute (AHRI), Agriculture Research Center (ARC), Giza 12619, Egypt

Correspondence to Fady Sayed Youssef, PhD, Department of Pharmacology, Faculty of Veterinary Medicine, Cairo University, 1221 Giza, Egypt. Tel: +201094572140; fax: +025242492; e-mail: fadyalsalhany@cu.edu.eg, fadyalsalhany@gmail.com

Received: 29 June 2023
Revised: 22 July 2023
Accepted: 9 August 2023
Published: 2 February 2024

Egyptian Pharmaceutical Journal 2024, 23:110–121

Background
Public health risks result from the consumption of baladi eggs due to the Egyptian consumer's demand and preference for baladi chicken eggs that might be contaminated with bacteria and/or mycotoxins that might affect eggs quality and results in food-borne intoxication or infection to consumers.

Objective
The current study aimed to investigate the presence of certain bacteria (mainly Staphylococcus aureus, Escherichia coli, Klebsiella pneumonia and Salmonella) and shed light on the virulence genes and also detection of mycotoxins in baladi agg content, which act as potential public health problems for providing safe eggs suitable for human consumption.

Materials and methods
A total of 150 Baladi chicken eggs from backyard were arbitrarily collected from El-Behera Governorate, Egypt at 2022–2023 for recognition of S. aureus, E. coli, K. pneumonia, Salmonella and mycotoxins. Molecular detection of some bacterial virulence genes by Polymerase Chain Reaction (PCR), *including some* enterotoxins and hemolysin genes for some *S. aureus* isolates as (sea, seb, sec, sed and see, hla and hlb) and *E. coli isolates* virulence genes for (shiga toxin1, shiga toxin2, eaeA and astA). Quantification of ochratoxin A (OTA) and aflatoxins (AFS) in the eggs by a low-cost, high-recovery fluorescence detector (FLD) in conjunction with a straightforward, specific, and created High-Pressure Liquid Chromatography (HPLC) assay in accordance with green chemistry.

Results and conclusion
Bacteriological examination revealed isolation of *S. aureus, E. coli and K. pneumonia* at percent 26.6%, 58%, and34%, respectively. It was found that aflatoxin B1 (AFB1) is the predominant detected toxins (18%), and OTA amount was found to be lower than the of aflatoxins found. It was found that AFs and OTA were determined to be below the maximum allowable threshold in 98% of the egg samples examined.

Keywords:
aflatoxins, baladi chicken eggs, E. coli, FLD, HPLC, K. pneumonia, ochratoxin A, PCR, S. aureus, Salmonella, virulence

Egypt Pharmaceut J 23:110–121
© 2024 Egyptian Pharmaceutical Journal
1687-4315

Introduction

Eggs offer a distinctive, nutritive, easily absorbed source for humans at all ages, because they are the most abundant source of necessary minerals and vitamins as well as offering greater amounts of nutrients rather than calories in the food that humans consume [1,2]. also act as source of high-quality protein which contain all the amino acids needed for human diet [3]. making them a significant meal in the human diet and attractive to consumers.

Despite their small size and high retail price, baladi chicken eggs are preferred by Egyptian customers because they contain more nutrients than red and white farm eggs. Protein, vitamins, minerals, cholesterol, phosphorus, vitamin B12, vitamin B5, zinc, omega-3 fatty acids, in addition vitamin A are among the substances found in baladi eggs that act as supportive and enrichment mediums for various microbes to grow, to avoid any potential public health problems by providing safe eggs suitable for human consumption, control measures should not ignore home-produced eggs, as there is a risk of infection from their consumption [4].

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

Normally, there are no organisms in newly laid eggs. However, contamination may occur transovarian route as contamination with *Salmonellae* may occurs directly from an infected ovary of a laying hen [5]. After oviposition, contamination could occur at any point until consumption [6] through exposure to the different environment factors such as (dirt, dust, and bad storage condition), leading to contamination of egg shell then egg contents either by penetration or withdrawal through pores of the shells [7], and thus, enhance the infection and spoilage of egg [8].

Egg quality could be decreased by microbial contamination, which could result in deterioration and subsequent financial losses, or infections could be transmitted to consumers, creating a public health problem by causing cases of food-borne illness or intoxication. The severity of these illnesses might range from minor symptoms to potentially fatal circumstances [9,10]. In addition, the poultry sector is significantly impacted by the microbic pollution of eggs by a lot of microorganisms as *S.* T*yphimurium, E. coli, and Pseudo. aeruginosa,* which have flagella that allow them to penetrate through the pores [11].

One of the primary triggers of food-borne illness is *S. aureus* because it produces thermo-stable enterotoxins that could persist in the food circumstances and result in food-borne illness. stay protected in the food environment and cause food-borne illness. The third-leading reason for food-borne infections globally is *staphylococcal* food-borne illness [12]. Approximately fifty percent of *S. aureus isolates* yield enterotoxins, culminating in consumer food poisoning [13]. Numerous infections, including food poisoning, endocarditis, osteomyelitis, skin infections, and pneumonia, are brought on by *Staphylococcus* aureus [14]. Eggs and egg-derived products were responsible for around 11% of *staphylococcal* food-borne sickness cases [15].

S. aureus possesses many virulence factors and the most notable are the five major classical types of staphylococcal enterotoxins (SEs: SEA to SEE), the non-classical SE-like toxins (SEl: SEG to SEU), and other virulence genes such as toxic shock syndrome toxin 1 (TSST-1), exfoliative toxins and cytolytic toxins (leukocidin and hemolysins hla, hlb [16,17].

Also, eggs have been the source of many gastrointestinal infections, including *Escherichia coli* and *Salmonella* [18,19].

E. coli considered a pointer for both food quality and sanitation, when found in eggs, acts as a sign of inadequate or bad hygiene and results in egg spoilage [20].

Escherichia coli is a typical microbial flora of the chicken and human gastrointestinal tracts. Resistant E. coli strains from the gut frequently induce contamination of eggs during lay with multiresistant E. coli [21].

Klebsiella spp. can result in a range of illnesses acquired in the community and nosocomially, including infections of the soft tissue and wounds, the respiratory tract, and the urinary tract [22,23].

Poultry feed may contain fungi and their toxic byproducts (mycotoxins), especially in tropical regions. Since mycotoxins are substances that occur naturally, they cannot be avoided. It easily contaminates feed at harvesting, processing, transfer, or storing [24,25].

Consumer health could be affected by the aflatoxins in eggs, to which children are more vulnerable than adults. Aflatoxins were already linked to many human cancer cases and considered carcinogenic [25]. The furthermost conventional technique for assessment of AFS quantities in feed and food is the HPLC method, which, based on mycotoxins' physical and chemical characteristics, provides a precise, accurate, and specific way to quantity the mycotoxins levels in contaminated feed and food. Numerous investigations clarify genetic and analytical processes [26].

Table eggs are considered a rich source of protein fit for human consumption; therefore, it is essential to ensure that these eggs follow the food quality standards and are free from any food poisoning issues. Furthermore, due to the lack of studies conducted on baladi eggs compared to many studies on commercial eggs (white and brown), this current study has focused more on bacteriological and mycotoxin examination in baladi eggs using advanced techniques with the alignment of the necessary recommendations for providing safe eggs suitable for human consumption.

Materials and methods

Samples collection

From a backyard, 150 unbroken baladi chicken eggs (no cracks) were chosen at random from El-Behera Governorate, Egypt. Samples were kept at 4áµ C until analysis.

To remove the egg content aseptically the eggshell was disinfected at first with 70% alcohol; they were broken, and content were homogenized for 30 s.

Bacteriological examination

In a sterile beaker glass, each sample of albumen and yolk were blended to yield one sample. The egg content was enriched in BPW (Oxoid) at 37°C for 24 h, then culture them in cultural media prepared following the manufacturers instruction and sterilized as:

S. aureus was isolated on Baird parker agar and Mannitol Salt Agar (MSA) later recognized by biochemical identification rendering to [27]. *E. coli* isolation was achieved on MacConkey and Eosin Methylene Blue (EMB) then confirmed by biochemical tests (IMVIC) as stated by [27]. Serotyping of the identified isolates was done with polyvalent *E. coli* antisera (provided by Animal Health Research Institute, Dokki, Egypt).

K. pneumonia was isolated on MacConkey agar then determined through biochemical tests (lactose fermentation test, Indole production test, Voges-Proskauer test, Citrate utilization test) as stated by [28]. *Salmonella* was isolated and recognized according to [29,30].

Molecular identification of bacterial isolates virulence gene by PCR

DNA extraction

DNA was extracted from the identified bacterial colonies (from eight *S. aureus* isolates (randomly selected) and *E. coli* isolates (as one isolate from each detected serotype) by the QIAamp DNA Mini set (Qiagen, Germany) according to the instructions of manufacturer with few alterations. Briefly, 200 μl of colony suspensions were incubated for 10 min at 56°C with 200 μl of lysis buffer and 10 μl of proteinase K. Add to the lysate 200 μl of pure ethanol. Wash DNA with washing solution and centrifuged following the manufacturer's recommendations, then DNA was eluted with 100 μl of elution buffer supplied in the kit.

Oligonucleotide primers

Different primers used in PCR were supplied from Metabion (Germany) and are listed in (Table 1).

Nucleic acid amplification

DNA amplification of seven S. aureus virulence genes including enterotoxin gene (sea, seb, sec, sed, see) and hemolysin genes(hla and hlb) E. coli virulence genes including shiga toxin 1-2 (stx1, stx2,), E. coli attaching–envasive A (eaeA) and stable toxin A (astA) genes were identified. a 25-μl reaction containing 12.5 μl of Emerald Amp Max PCR Master Mix (Takara, Japan), 1 μl of each primer, 4.5 μl of water, and 6 μl of DNA template. A thermocycler model 2720 from Applied Biosystems was used to carry out the reaction.

Analysis of the PCR products

PCR products were separated via electrophoresis on 1.5% agarose gel (Applichem, Germany, GmbH) in 1× TBE buffer at room temperature using gradients of 5 V/cm. For gel analysis, 20 μl of the PCR products were loaded in each gel slot. Generuler 100 bp ladder (fermentas, Thermo) was used for determination of fragment sizes. The gel was then photographed by a gel documentation system (Alpha Innotech, Biometra) and the data was analyzed through computer software.

Investigation of mycotoxins

To estimate the existence of total aflatoxins (AFT) [sum of AF- B1, -G1, -B2, and -G2], and OTA, egg specimens' analysis was done using HPLC in conjunction with FLD.

Chemicals

Aflatoxins and Ochratoxin-certified references in acetonitrile solution (3 μg/ml), and Trifluoroethanoic acid (TFA) were obtained from The Sigma-Aldrich, Steinhaus, Germany. Immunoaffinity cartridges (IAC) for the clean-up step (AFLAPREP® and OCHRAPREP®) are from R-Biopharm Rhône Ltd., UK. Methyl cyanide (MeCN), Ethanoic acid (EA) and Methyl hydroxide (MeOH) of chromatography ultrapure grade, phosphate buffer saline (PBS), and the other used chemicals bought from Merck, Darmstadt, Germany, are analytical grade. and. A Milli-Q-system (Millipore, Mosheim, France) provided deionized water (DW); For the OTA analysis, the mobile phase (Mph) was DW, MeCN, and EA (51:47: 2), and for the AFs analysis, it was MeCN, MeOH, and DW (20:20:60) [36].

Sample extraction

The procedures of extraction were done according to Iqbal *et al.* [36] with slight alterations. The extraction was achieved in three steps before LC injection: sample preparation, purification, and derivatization.

(1) Preparation: A 3 g sample was homogenized for 10 min with 0.3 g sodium chloride and 10 ml of MeCN: DW (45:55) and then centrifugated at 3000 rpm at ambient 2 min Two mL of the filtrate mingled with 2 ml of DW for AFs extraction and for OTA extraction with 2 ml of PBS.

Table 1 Target genes, primers sequences, amplicon sizes and cycling conditions used for amplification of different virulence genes of *S.aureus* and *E. coli*

Target agent	Target gene	Primers sequences	Amplified segment (bp)	Primary denaturation	Amplification (35 cycles)			Final extension	Reference
					Secondary denaturation	Annealing	Extension		
S. aureus	***Sea***	GGTTATCAATGTGCGGGTGG	102	94°C 5 min	94°C 30 s	50°C 40 s	72°C 40 s	72°C 10 min	**Mehrotra *et al.*, 2000** [31]
		CGGCACTTTTTTCTCTTCGG							
	Seb	GTATGGTGGTGTAACTGAGC	164						
		CCAAATAGTGACGAGTTAGG							
	Sec	AGATGAAGTAGTTGATGTGTATGG	451						
		CACACTTTTAGAATCAACCG							
	Sed	CCAATAATAGGAGAAAATAAAAG	278						
		ATTGGTATTTTTTTTCGTTC							
	See	AGGTTTTTTCACAGGTCATCC	209						
		CTTTTTTTTCTTCGGTCAATC							
	Hla	GAAGTCTGGTGAAAACCCTGA	704	94°C 5 min	94°C 30 s	53°C 40 s	72°C 45 s	72°C 10 min	**Fei *et al.*, 2011** [32]
		TGAATCCTGTCGCTAATGCC							
	hlb	CAATAGTGCCAAAGCCGAAT	496	94°C 5 min	94°C 30 s	53°C 40 s	72°C 45 s	72°C 10 min	
		TCCAGCACCACAACGAGAAT							
E. coli	***Stx1***	ACACTGGATGATCTCAGTGG	614	94°C 5 min	94°C 30 s	58°C 40 s	72°C 45 s	72°C 10 min	Dipineto *et al.*, 2006[33]
		CTGAATCCCCCTCCATTATG							
	Stx2	CCATGACAACGGACAGCAGTT	779						
		CCTGTCAACTGAGCAGCACTTTG							
	eaeA	ATGCTTAGTGCTGGTTTAGG	248	94°C 5 min	94°C 30 s	51°C 30 s	72°C 30 s	72°C 7 min	Bisi-Johnso *et al.*, 2011 [34]
		GCCTTCATCATTTCGCTTTC							
	astA	CCATCAACACAGTATATCCGA	110	94°C 5 min	94°C 30 s	55°C 30 s	72°C 30 s	72°C 7 min	Piva *et al.*, 2003 [35]
		GGTCGCGAGTGACGGCTTTGT							

(2) Purification: The sample was passed slowly via a specific immunoaffinity cartridges at a flowrate of 1 drops/s then splashed at the same flow rate with water (1 ml). Elusion with 1 ml of MeOH for AFs and OTA residues. At last, the elute evaporated at 40°C under nitrogen stream. For OTA analysis, the residues were redissolved in 600 µl of the Mph before injection into HPLC, but for AFs analysis derivatization was done.
(3) Derivatization: After drying up, the derivatization of AFs was carried out with 100 µl of TFA in a dark environment for 15 min at ambient temperature with the vials' caps on. Then, add 500 µl of the mixture of MeCN: DW (1 : 9) mixture to the vials.

Chromatography separation

Twenty µl was injected to HPLC system (Agilent, 1200, USA). The flow rate of the isocratic Mph was 1 ml/min. The mycotoxins were separated on reversed-phase column (250 mm, 4.6 mm i.d., 5 µm particle size, LiChrospher C18 ODS column, Agilent) at 40°C equipped with a FLD (Japan) The excitation wave lengths were 360 nm, the emission wave lengths were 440 nm [36].

Quality control for chromatographic analysis (Intra-lab verification)

According to [37,38] guidelines, validation criteria, including linearity, recovery, Limit of detections and quantifications (LOD and LOQ), and precision of AFs, and OTA were established. With three replicated analyses of fortified samples, method precision was decided in terms of inter-day precision at 6 days and repeatability (intra-day precision) on three levels (low, medium, and high). For all examined toxins, the calibration plot was concentration versus area under peak (AUP) with coefficient of determination. The accuracy and recovery were done by fortifying at three distinct levels ½ X, 1 X, and 2 X MRL of AFs and OTA in non-contaminated eggs.

Statistical assessment

Data were expressed as mean±S.D. using the commercially available software package (SPSS Inc., version 22.0, Chicago, IL, USA) to analyze the obtained data [39].

Results and discussions

Bacteriological investigation

Staphylococcus *spp.* was isolated at 70% (105/150) among them *S. aureus* represent as 38.1% (40 /105) while coagulase negative isolates represent as 61.9% (65/105) (Table 2). Eid *et al.* [40] reported a prevalence rate of 40% overall of coagulase-positive *Staphylococci* in both shell and contents of the eggs; our isolation rate was lower at 15% (7.5%), compared to theirs. Also, Stepień *et al.* [41] reported *S. aureus* at a percent of 45.7% from eggs, of which 38.7% and 2.5% were detected in the yolk and white, respectively, while Abdullah [13] documented *Staphylococcus* contamination of eggs on the shell, white, and yolk contents. Lower rate was reported by Ballah *et al.* [42] as prevalence rate was 25% for eggs gathered from various markets in Bangladesh. A significantly decreased rats found in baladi, white, brown farm hens' and duck egg by El-Kholy *et al.* [43] as the detection rate was 7(9.3%) and 4(5.3%) from white and brown poultry farms eggs shells, respectively while in egg contents rates was 2.7% and 1.3%, respectively, about 1(14.3%) of white poultry farms eggshells were contaminated with *S. aureus*.

S. aureus enterotoxins molecular analysis demonstrated that enterotoxin A (**sea**) and D(sed) were not detected at any of examined isolates, while enterotoxin b(seb) and e(see) were found only at one isolate 1/8 (12.5%), and enterotoxin c (sec) was found at 2 isolates 2/8 (25%). These enterotoxins are dangerous because they resist hydrolysis by stomach and jejunal enzymes and are heat-stable at 100°C for 30 min This might explain why staphylococcal food poisoning is the major cause of food-borne microbial intoxication globally [44]. Detection of hemolysin encoding gene (hla) and hemolysin encoding gene (hlb) were 4/8 (50%) and 7/8 (87.5%), respectively, (Table 3, Fig. 1).

Alpha-Hemolysin (encrypted by the Hla gene), water-soluble monomer secreted by most pathogenic strains of *S. aureus*, targets nearly all mammalian cells by forming stable, amphiphilic transmembrane pores [45,46]. Beta toxin coded by the *hlb* gene. One of the activities of this toxin in clinical conditions can be described as human lung, eye infection (cornea) and an ability to prevent the ciliary of nasal epithelium cells has been described [47,48].

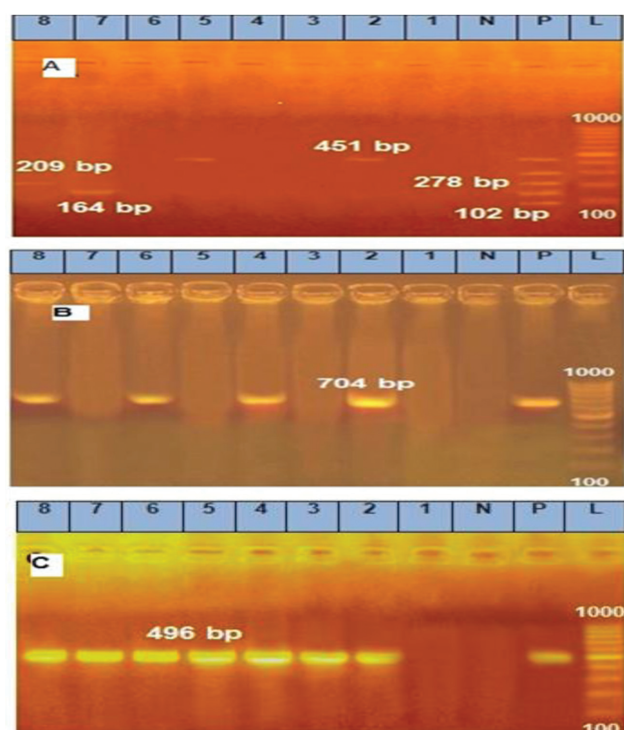
Table 2 Incidence of isolated bacteria from examined specimens

Total No. of examined samples	S. aureus	E. coli	K. pneumonia	Salmonella
150	NO. (%)	NO. (%)	NO. (%)	NO. (%)
	40 (26.6)	87 (58)	51 (34)	0 (0)

Table 3 Occurrence of some virulent genes and enterotoxins *of S. aureus*

Staph isolates No.	Sea	Seb	Sec	Sed	See	hla	Hlb
1	–	–	–	–	–	–	–
2	–	–	+	–	–	+	+
3	–	–	–	–	–	–	+
4	–	–	–	–	–	+	+
5	–	–	+	–	–	–	+
6	–	–	–	–	–	+	+
7	–	+	–	–	–	–	+
8	–	–	–	–	+	+	+

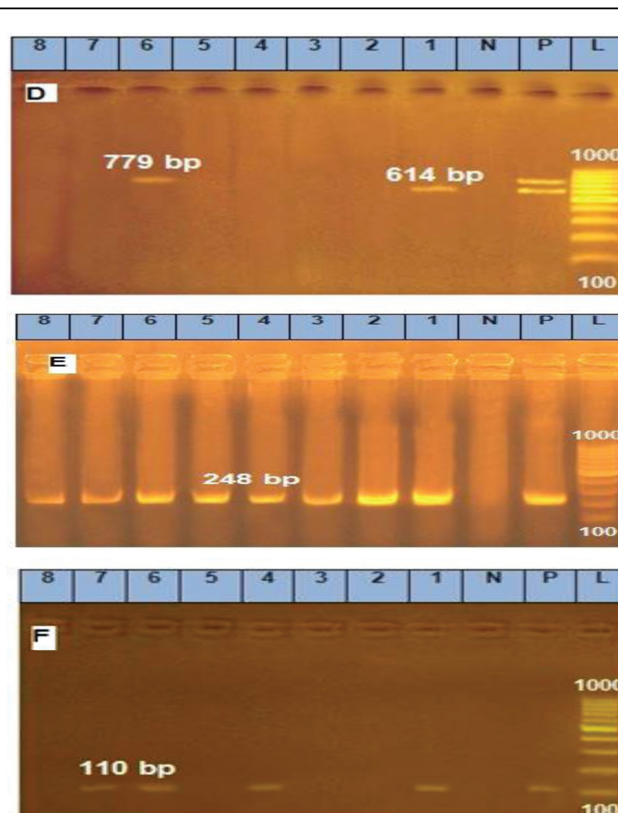
Figure 1



Electrophoresis of some investigated virulence genes and enterotoxins *of S. aureus* isolates, Lane (L):100 bp DNA ladder, Lane (P): Positive control, Lane (N): Negative control and tested isolates represent as 1 to 8 as: **A:** multiplex PCR of enterotoxin genes for sea (102 bp), seb (164 bp), sec (451 bp), sed (278 bp) and see (209), lanes (1, 3, 4, and 6): Negative for examined genes, lanes (2, 5): Positive sec gene, lane (7): Positive seb gene and lane (8) Positive see gene. **B**: Agarose gel electrophoresis of hla (704 bp) gene, lanes (1,3,5,7): Negative hla gene, lanes (2,4,6,8): Positive hla gene. **C**: Agarose gel electrophoresis of hlb (496 bp) gene, lane (1): Negative hlb gene, lanes (2, 3, 4, 5, 6, 7, 8): Positive hlb gene.

The results of *S. aureus* hemolysin genes were so interesting, as 87.5% of the isolates were positive for *hlb*, while 27.27% were positive for the *hla* gene. Diverse virulence gene profiles were also reported from different food categories worldwide, such as milk products in South Africa [49]. and retail chicken in Egypt [50]. According to the findings of this study and previous published papers, different

Figure 2



Electrophoresis of some *E. coli* virulence genes, Lane (L): 100 bp ladder (DNA marker), Lane (P): Control positive, lane (N): Control negative as: **D:** duplex PCR of *stx1* (614 bp) and *stx2* (779 bp) genes, lane (1): Positive stx1 gene, lane (6): Positive *stx2* gene, lanes (2,3,4,5,7,8): Negative stx1 and stx2 genes. **E:** uniplex PCR of *eae* gene (248 pb), lanes 1 to 8: Positive *eae* gene. **F**: uniplex PCR of *astA* gene (110 pb), lanes (1,4, 6,7): Positive *astA* gene, lanes (2,3, 5,8): Negative astA gene.

toxins contribute to S. aureus' pathogenic potential and pose a health concern to consumers.

E. coli incidence was 58% (87 /150) that 8 different serotypes namely O93(11 isolates), O85 (10 isolates), O2 (9 isolates), O77(8 isolates), O142 (7 isolates), O154 (5 isolates), O11(3 isolates) and O21 (2 isolates) were identified while (32 isolates) were untypal. The occurrence of *E. coli* in our research was 58%, which is near the results of Gole *et al.* [51], with an incidence of 60.78%. Also it approach to the results of Hanaa [52] which revealed that out of 150 examined egg samples, 63 (63%) was positive for *E. coli* from hen of which the incidences in egg shell, egg albumin and egg yolk were 40%, 8% and 15%, respectively. A different result was noted by Sabrinath *et al.* [53], who determined that 13.3%, 45.8%, and 45.8%, respectively, *E. coli* was present in egg contents gathered from both small and large farms. A lower incidence (19%) was found by Ghasemian Safaei *et al.* [54], while siriphap *et al.* [55] determined low prevalence of *E. coli as 12%* in

hen's egg collected from supermarkets in Thailand to found only 91 isolates out from 750 examined egg. EPEC strains' refers to eae-haboring diarrheagenic *E. coli* that lacks the Shiga toxin genes yet can cause severe damage to intestinal cells [56].

Our investigation revealed that molecular identification of E. coli (Figure 2) virulence genes *eaeA* were detected at a rate of (100%). As 8/8 of tested E. coli isolates were positive (Table 4). These completely adverse results were reported by others [57,58], as none of the isolates analyzed included the eae A gene. The *eae*A gene (attaching and effacing of enterocyte gene gene) was evaluated to speculate the virulence of the isolated E. *coli* strains. There were 100% positive results for this gene in all tested isolates. This result assured the virulence of these isolates because the *eaeA* gene encodes for intimin protein which act as a bacterial adhesion molecules that result in the emersion of the A/E lesions [59]. The high incidence rate of *eae*A gene detection was reported by many authors as [60] who detected *eae*A gene in 95.9% of the tested E. *coli* O157:H7 isolates.

Also our investigation revealed that stx1 gene was detected at a rate of 12.5%, as only 1/8 of the examined isolates have been positive for stx1, and the same result was reported for stx2. *This* agrees with Galal *et al.* [61] who detected either stx1 or stx2 with *eae*A gene in 3/19 (15.78%) of the samples. but Zahraei Salehi *et al.* [62] reported a reduced stx1 detection rate, with just 1 (8.33%) of the 12 tested isolates being positive, but higher rates for *stx2* were found in 9 (75%) isolates. A higher rate was found by Elafify *et al.* [63] as out of 20 isolates (20%) positive for stx1, all tested isolates (5 out of 5) were positive for stx2, and both stx1 and stx2 genes were found by Samanta *et al.* [64].

Table 4 Presence of some virulence genes of *E. coli* isolates

		virulence genes			
E. coli sample	Serotype	Stx1	Stx2	eaeA	astA
1	O93	+	−	+	+
2	O85	−	−	+	−
3	O2	−	−	+	−
4	O77	−	−	+	+
5	O142	−	−	+	−
6	O154	−	+	+	+
7	O11	−	−	+	+
8	O21	−	−	+	−

While *astA* gene was detected at a rate of 50%, as 4/8 evaluated isolates were positive the gene. astA gene encodes a heat-stable toxin of enterotoxigenic e. coli (ETEC) that result in the inhibition of ion exchange and sodium reabsorption, contributing to the release of water and salt into the gut and hence net fluid loss, leading to watery and secretory diarrhea [65,66].

K. pneumonia was found in 51/150 with percent 34%, while *Salmonella* was not detected in our samples. *S. aureus* was detected in our investigation at a rate of 26.6%, which is lower than the results of [67], who isolated *S. aureus* from the content of baladi eggs in percentages of 74.4%.

Salmonella spp. was not isolated during this study; this agrees with Awny *et al.* [68], and Yenilmez [69], but disagrees with Marek *et al.* [70] who claimed that 11% of table eggs had *Salmonella* in them.

The incidence of *K. pneumonia* was 34%, resembling the rate of 31% declared by Fatima Zahra *et al.* [71] during a study of table eggs from the informal sector, while a lower rate of 20% was found in the formal sector from egg content, and a lower rate of 15% of Klebsiella *spp.* was reported by Al Momani *et al.* [72].

Detection of mycotoxins

Intra-lab verification

The mycotoxins method qualification results were shown in (Table 5), the intra-day precision coefficient of variance (CV%) not exceed 0.47% (2% that set by USP, 2019) [73] and the inter-day precision does not surpass 1.2% (6% that set by ICH, 2005) [74]

Table 5 Intra-Intralab verification sheet of analyzed mycotoxins

	AFB1	AFB2	AFG1	AFG2	OTA
RT (min.)	1.979	1.071	0.591	0.451	11.214
Range (ppb)			0.15-6		
Correlation coefficient (R^2)	0.99997	0.99998	0.99985	0.99971	0.99970
LOD (ppb)	0.011484	0.009015	0.024965	0.012764	0.005287
LOQ (ppb)	0.034452	0.027044	0.074895	0.038291	0.01586
Accuracy	97.9±5	98.5±3.5	96.7±18.2	93.9±12.4	94.5±12
Intra-day precision (CV%)	0.12	0.33	0.18	0.32	0.47
Inter-day precision (CV%)	0.62	0.87	1.1	1.2	0.94

of the used assay, which is highly precise and exceptionally accurate.

Analysis using the linear least square method showed that concentrations and AUP are linearly related. In the range 0.50–20.0 μg/ml it has been found to be linear for all analyzed mycotoxins with a correlation coefficient more than 0.99 as shown in (Fig. 3).

The retention times (RT) of the Afla -G2, -G1, -B2, -B1, and OTA standards at 0.451, 0.591, 1.071, 1.979, and 11.214 min as shown in (Figs 4 and 5) demonstrate the great resolution of the chromatograms.

Specificity and selectivity: As shown in (Figs 4 and 5), HPLC Chromatograms of pure standards and fortified blank eggs at the same levels, demonstrating no invasive peaks were separated at the same retention times (RT) of the toxins peaks and no matrix interventions were visible on the chromatograms.

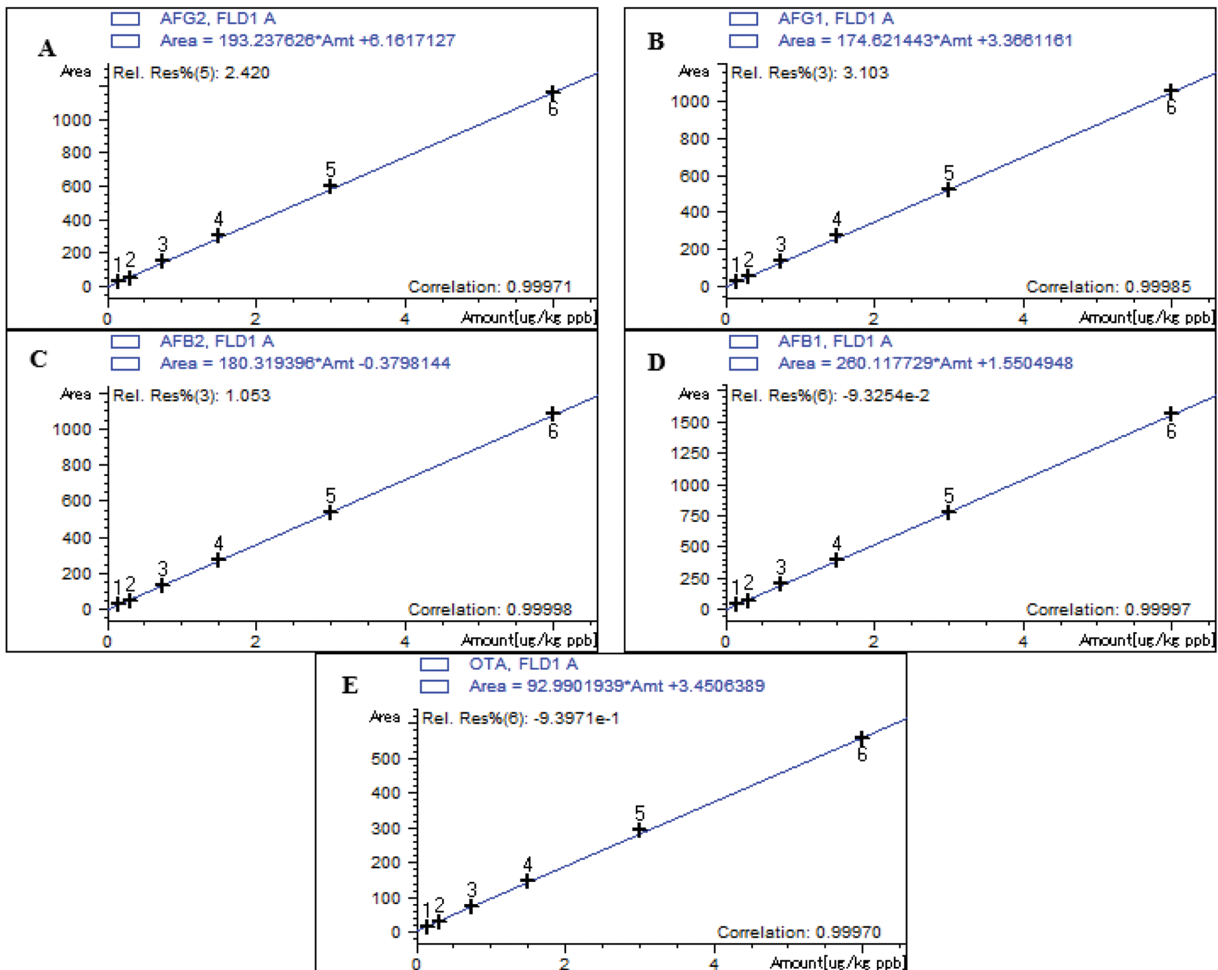
Concentrations of mycotoxins

The incidence and concentrations of mycotoxins residues in examined baladi chicken eggs were summarized in (Table 6 and Figs 6–8), showing that AFB1 is the predominant detected toxin (18%).

In the current investigation, 150 chicken baladi eggs were gathered, and the prevalence of AFs and OTA was examined. According to the findings, AFs and OTA were present in egg samples in 18% and 8%, respectively. AFT (total of AFB1, AFG1, AFB2, and AFG2) and OTA reached maximum values of 6 and 0.39 ppb, respectively. The mean AFB1 concentration in the eggs was 1.02 ppb, with values ranging from 0.02 to 4.5 ppb.

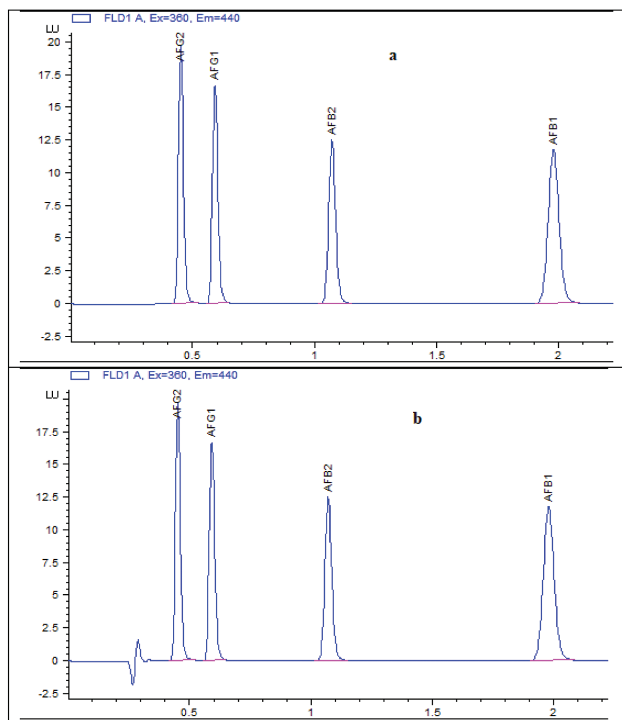
Restricted information about AFs or OTA presence in eggs was reported in numerous studies. According to Iqbal *et al.* [36], AFs and OTA were present in 28 and 35%, respectively, of the 80 eggs (n=80) that were obtained from Pakistan. In a paper by Amirkhizi *et al.* [75], AFB1 was present in 58% of Iranian egg samples

Figure 3



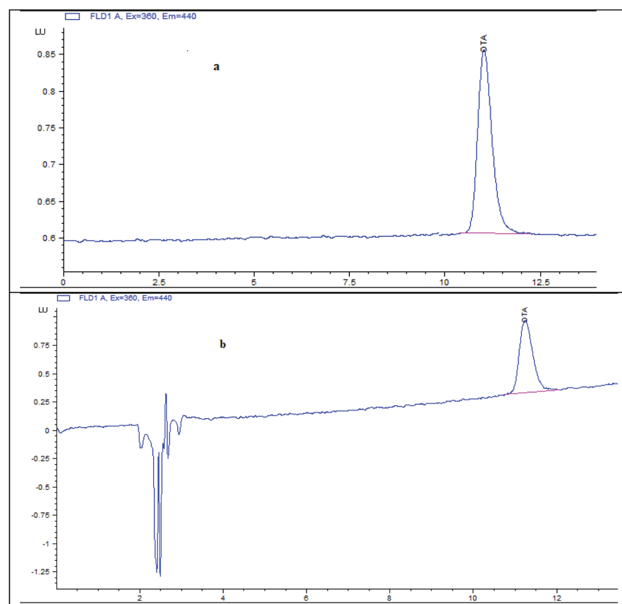
Caliberation plots for AFS (**A**–**D**) and OTA (**E**).

Figure 4



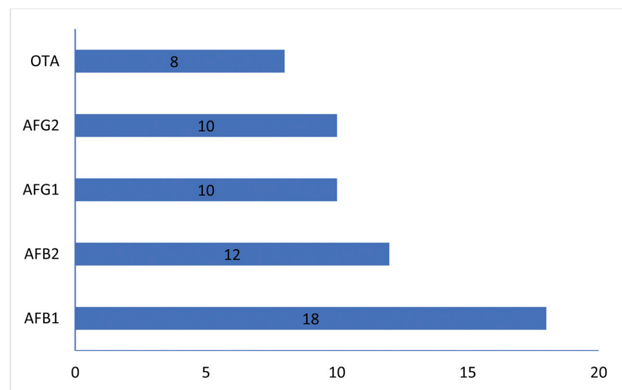
Chromatograms of aflatoxins at a conc. of 0.15 ppb in acetonitrile solvent (**a**) and in blank egg (**b**).

Figure 5



Chromatograms of OTA at a conc. of 0.15 ppb in acetonitrile solvent (**a**) and in blank egg (**b**).

Figure 6



Incidence of mycotoxins in eggs.

at an average concentration of 0.30–16.36 ppb. Herzallah [76] demonstrated AFs pollution with an average of 1.23 ppb in 10 eggs. According to Shehata *et al.* [77], there were 30%, 16.6%, and 20%, respectively, of baladi, brown farm eggs, and white farm eggs that assessed positive for AFT residues. The average AFs residue levels in baladi were 6.7 ppb, with a range of 0.9–14.3 ppb. An average level of AFs residues in farm brown eggs was 3.2 ppb, with a range of 0.34–7.3 ppb, while the average levels in farm white eggs were 4.34 ppb, with a range of 0.75–9.1 ppb, respectively. Our findings, however, showed a lower occurrence, which the ration's nature is to blame for.

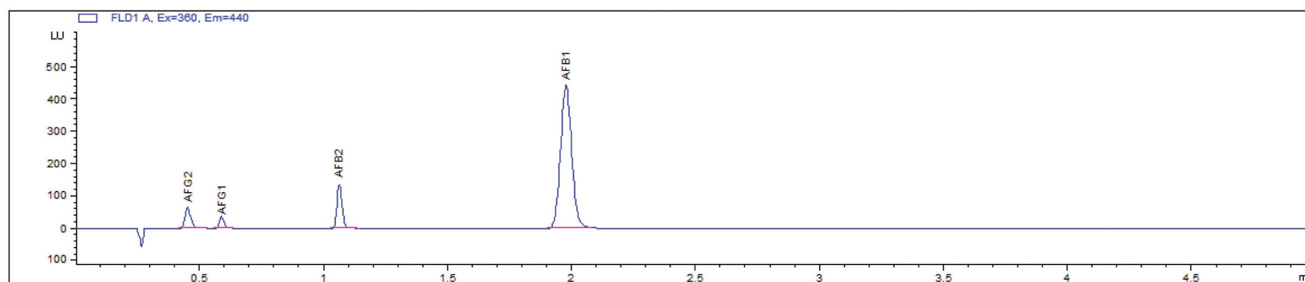
Different plant species, fungal species, and ecological variables like temperature, moisture, and the presence of insects all play a role in the fluctuations in mycotoxins' occurrence and levels in eggs [78]. The type and concentration of mycotoxin in the eggs are influenced by the sort of feed that the birds consume.

Table 6 Concentrations of mycotoxins (ppb) in 150 baladi chicken eggs

Conc.	+ve samples	mean±SD	LOD-0.499	0.5–0.99	1–2.99	3–6	≥EU MRL**	≥US FDA MRL***
AFB1	27 (18%)	1±1.27	14	4	6	3	5 (3.3%)	0
AFB2	18 (12%)	0.3±0.37	15	0	3	0	0	0
AFG1	15 (10%)	0.14±0.16	14	1	0	0	0	0
AFG2	15 (10%)	0.058±0.056	15	0	0	0	0	0
AFT	27 (18%)	1.3±1.7	14	2	8	3	3 (2%)	0
OTA	12 (8%)	0.049±0.04	12	0	0	0	0	0

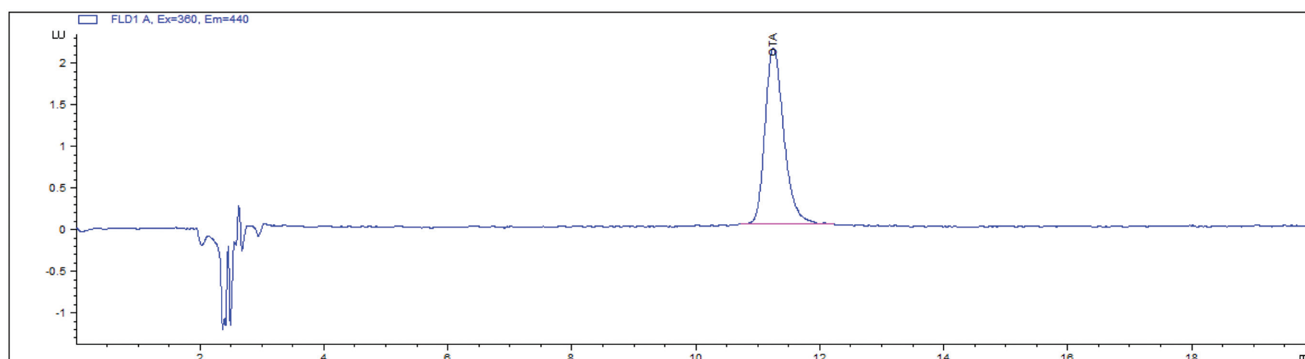
*LOD for AFB1 0.0115, AFB2 0.009, AFG1 0.025, AFG2 0.013, OTA 0.016 ppb. **EU MRL 2 ppb for AFB1, 4 ppb for AFT, and 2 ppb for OTA (**EC 1881/2006**). ***US FDA MRL 20 ppb for AFT.

Figure 7



Chromatogram of egg sample with 6 ppb of AFT.

Figure 8



Chromatogram of egg sample with 0.39 ppb of OTA.

Since grains and legumes make up the majority of the ingredients in poultry diets, the concentration of mycotoxins varying according to the degree of contamination [79]. The amount of OTA in the eggs was found to be lower than the amount of aflatoxins found; these findings concur with those of [80] who informed that the amount of OTA was substantially lesser than that of AFB1 and zearalenone.

Conclusion

From our study, we found the presence of some bacteria in egg contents, which may be the result of unsanitary production and handling practices, poor storage conditions, or careless cleaning before marketing, this may allow germs to spread to the egg's contents by penetrating the eggshells' pores and reaching the inside surface. A public health problem occurs if eggs are handled, eaten raw, unpasteurized, or exploited in egg-related products. Therefore, egg consumption should be approached with caution to prevent or at the very tiniest minimize food-borne infection. Preventive measures must be followed to avoid bacteria development on or within eggs, the storage and marketing procedures must also adhere to high hygiene standards. Despite the fact that AFs and OTA were determined to be below the maximum allowable threshold in 98% of the egg samples examined, according to the health risk assessment, AFB1 and OTA exposure put both adults and children at risk for health problems. It is suggested to implement control and monitoring strategies to reduce the content of mycotoxins in chicken eggs in Egypt given the rise in poultry egg consumption in that country and the possible dangers of mycotoxin exposure, particularly for youngsters.

Acknowledgements

Financial support and sponsorship

Nil.

Conflicts of interest

The authors declare there are no conflicts of interest.

References

1 Ebubekir A, Ahmet S, Ekerog L. Effect of egg shape index on mechanical properties of chicken eggs. J Food Eng 2008; 85:606–612.

2 Ansah T, Dzoagbe GSK, Teye GA, Adday S, Danquah JK. Microbial quality of table eggs sold on selected markets in the Tamale municipality in the Northern Region of Ghana. Livest Res R Dev 2009; 21:8.

3 Puglisi MJ, Fernandez ML. The health benefits of egg protein. Nutrients 2022; 14:2904.

4 Hardy Zielicka A, Zarowna D, Szych J, Madajczak G, Sadkowska-Todys M. Ensuring safety of home-produced eggs to control salmonellosis in Poland: lessons from an outbreak in September 2011. Euro Surveill 2012; 17. pii:20319.

5 Gast RK, Jones DR, Guraya R, Anderson KE, Karcher DM. Research Note: Contamination of eggs by *Salmonella* E*nteritidis* and Salmonella Typhimurium in experimentally infected laying hens in indoor cage-free housing. Poult Sci 2021; 100:101438.

6 Indhu B, Muthusami S, Thirunavukkarasu N. Studies on microflora and their role on eggshell contamination and infection. Int J Pharm Chem Bio Sci 2014; 4:518–521.

7 Schoeni JL, Glass KA, Mcdermott JL, Wong ACL. Growth and penetration of Salmonella enteritidis, Salmonella Heidelberg and Salmonella typhimurium in eggs. Int J Food Microbiol 1995; 24:385–396.

8 Theron H, Enter PV, Lues JF. Bacterial growth on chicken eggs in various storage environments. Food Res Int 2003; 36:969–975.

9 Kaneko KI, Hayashidani H, Ohtomo Y, *et al.* 'Bacterial contamination of ready-to-eat foods and fresh products in retail shops and food factories'. J Food Prot 1999; 62:644–649.

10 Safaei HG, Jalali M, Hosseini A, Narimani T, Sharifzadeh A, Raheim E. The prevalence of bacterial contamination of table eggs from retails markets by *Salmonella spp*., *Listeria monocytogenes*, *Campylobacter jejuni* and *Escherichia coli* in Shahrekord, Iran. Jundishapur J Microbiol 2011; 4:249–253.

11 Damena A, Mikru A, Adane M, Dobo B. Microbial profile and safety of chicken eggs from a poultry farm and small-scale vendors in Hawassa, Southern Ethiopia. J Food Qual 2022; 1:1–16.

12 Boerema A, Clemens R, Brightwell G. Evaluation of molecular methods to determine enterotoxigenic status and molecular genotype of bovine, ovine, human and food isolates of Staphylococcus aureus. Int J Food Microbiol 2006; 107:192–201.

13 Abdullah IN. Isolation and identification of some bacterial isolates from table egg. Al-Anbar J Vet Sci 2010; 3:59–67.

14 Kehrenberg C, Cuny C, Strommenger B, Schwarz S, Witte W. Methicillin-resistant and –susceptible Staphylococcus aureus strains of clonal lineages ST398 and ST9 from swine carry the multidrug resistance gene cfr. Antimicrob Agents Chemother 2009; 53:779–781.

15 Haeghebaert S, Le Querrec F, Gallay A, Bouvet P, Gomez M, Vaillant V. Les toxi-infections alimentaires collectives en France, en 1999 et 2000. Bull Epidemiol Hebd 2002; 23:105–109.

16 Hennekinne JA, de Buyser ML, Dragacci S. Staphylococcus aureus and its food poisoning toxins: Characterization and outbreak investigation. FEMS Microbiol Rev 2012; 36:815–836. [CrossRef] [PubMed]

17 Argudin MA, Mendoza MC, Gonzalez-Hevia MA, Bances M, Guerra B, Rodicio MR. Genotypes, exotoxin gene content, and antimicrobial resistance of Staphylococcus aureus strains recovered from foods and food handlers. Appl Environ Microbiol 2012; 78:2930–2935. [CrossRef] [PubMed]

18 Hope BK, Baker R, Edel ED, Houe AT, Schlosser WD, Ehiting RM, *et al.* An overview of the *Salmonella enteritidis* risk assessment for shell eggs and egg products. Risk Anal 2002; 22:203–218.

19 Adesiyum A, Offiah N, Seepersadsingh N, Rodrigo S, Lashley V, Musai L, Georges K. Microbial health risk posed by table eggs in Trinidad. Epidemiol Infect 2005; 133:1049–1056.

20 Berrang M, Frank J, Jeffrey R, Stan J, Cox N. Eggshell membrane structure and penetration by Salmonella typhimurium. J Food Prot 1999; 62:73–76.

21 Turtura GC, Massa S, Chazvinizadeh H. Antibiotic resistance among coliform bacteria isolated from carcasses of commercially slaughtered chickens. Int J Food Microbiol 1990; 11:351–354.

22 Podschun R, Ullmann U. *Klebsiella spp*. as nosocomial pathogens: epidemiology, taxonomy, typing methods, and pathogenicity factors. Clin Microbiol Rev 1998; 11:589–603.

23 Navon-Venezia S, Kondratyeva K, Carattoli A. *Klebsiella* pneumoniae: a major worldwide source and shuttle for antibiotic resistance. FEMS Micro Rev 2017; 41:252–275.

24 Liau B, Jong T, Lee M, Chang C. Supercritical fluid extraction and quantification of aflatoxins in feedstuffs by liquid chromatography. J Chromatography 2007; 21:667–673.

25 Yaling W, Tongjie C, Guozhong L, Chunsan Q, Huiyong D, Meiling Y, Gerd S. Similtaneous detection of airborne aflatoxin, ochratoxin and zearalenone in poultry houses by immunoaffinity column and high-performance liquid chromatography. Environ Res 2008; 107:139–144.

26 Hassan AM, Abdel-Aziem SH, El-Nekeety AA, Abdel-Wahhab MA. Panax ginseng extract modulates oxidative stress, DNA fragmentation and up-regulate gene expression in rats sub chronically treated with aflatoxin B 1 and fumonisin B 1. Cytotechnology 2015; 67:861–871.

27 Quinn PJ, Markey BK, Carter ME, Donnelly WJ, Leonard F. Veterinary Microbiology and Microbial Disease. Ames, USA: Iowa State University Press 2002. 124–126

28 Arya LK, Kumar M, Priya P, Saurabh K, Kumari N. Isolation and identification of Klebsiella pneumoniae from a milk sample. Indian Vet J 2020; 97:15–17.

29 ISO 6579–1:2017/Amd.1. Microbiology of the food chain. Horizontal method for the detection enumeration and serotyping of Salmonella. International Standards Organization 2020; 1–11.

30 ISO 6579. 2002. Microbiology of food and animal feeding stuff – Horizontal method for the detection of Salmonella. International Standards Organization, Geneva

31 Mehrotra M, Wang G, Johnson WM. Multiplex PCR for Detection of Genes for Staphylococcus aureus Enterotoxins, Exfoliative Toxins, Toxic Shock Syndrome Toxin 1, and Methicillin Resistance. J Clin Microbiol 2000; 38:3.

32 Fei W, Hongjun Y, Hong-bin H, Changfa W, Yundong G, Qifeng Z, *et al.* Study on the hemolysin phenotype and the genetype distribution of staphylococcus aureus caused bovine mastitis in Shandong dairy farms. Intern J Appl Res Vet Med 2011; 9:2011.

33 Dipineto L, Santaniello A, Fontanella M, Lagos K, Fioretti A, Menna LF. Presence of Shiga toxin-producing Escherichia coli O157:H7 in living layer hens. Lett Appl Microbiol 2006; 43:293–295.

34 Bisi-Johnson MA, Obi CL, Vasaikar SD, Baba KA, Hattori T. Molecular basis of virulence in clinical isolates of Escherichia coli and Salmonella species from a tertiary hospital in the Eastern Cape, South Africa. Gut Pathog 2011; 3:9.

35 Piva IC, Pereira AL, Ferraz LR, Silva RSN, Vieira AC, Blanco JE, *et al.* Virulence Markers of Enteroaggregative*Escherichia coli* Isolated from Children and Adults with Diarrhea in Brasília, Brazil. J Clin Microbiol 2003; 41:1827–1832.

36 Iqbal SZ, Nisar S, Asi MR, Jinap S. Natural incidence of aflatoxins, ochratoxin A and zearalenone in chicken meat and eggs. Food Control 2014; 43:98–103.

37 ICH (International Conference on Harmonization of Technical Requirements for Registration of Pharmaceutical for Human Use) Validation of Analytical Procedures: Text and Methodology Q2 (R1) 2005;1(20), 05

38 USP C. <1225> Validation of compendial procedures & <621> Chromatography. Rockville, Rockville, MD: United States Pharmacopeia; 2019.

39 Morgan GA, Barrett KC, Leech NL, Gloeckner GW. IBM SPSS for introductory statistics: Use and interpretation. New York: Routledge; 2019.

40 Eid S, Nasef A, Soad Erfan M. Ahmed. Multidrug resistant bacterial pathogens in eggs collected from backyard chickens. Assiut Vet Med J 2015; 61:87–103.

41 Stępień-Pyśniak D, Marek A, Rzedzicki J. Occurrence of bacteria of the genus Staphylococcus in table eggs descended from different sources. Pol J Vet Sci 2009; 12:481–484.

42 Ballah FM, Islam MS, Rana ML, Ferdous FB, Ahmed R, Pramanik PK, *et al.* Phenotypic &Genotypic Detection of Biofilm-Forming *Staphylococcus aureus* from Different Food Sources in Bangladesh. Biology 2022; 11:949.1–13.

43 El Kholy A, Elshater M, Abd El Gawad M, Zeinhom M. Prevalence of enterotoxigenic *S. aureus* in table eggs in El-Fayoum City, Egypt. J Vet Med Res 2022; 29:1–5.

44 Holmberg SD, Blake PA. Staphylococcal food poisoning in the United States. new facts and old misconceptions. JAMA 1984; 251:487–489.

45 Seilie ES, Wardenburg JB. '*Staphylococcus aureus* pore-forming toxins: the interface of pathogen and host complexity'. Semin Cell Dev Biol 2017; 72:101–116.

46 Du Y, Liu L, Zhang C, Zhang Y. 'Two residues in *Staphylococcus aureus* & alpha;-hemolysin related to hemolysis and self-assembly'. Infect Drug Resist 2018; 11:1271–1274.

47 O'Callaghan RJ, Callegan MC, Moreau JM, Green LC, Foster TJ, Hartford OM, *et al.* Specific roles of alphaâ toxin and betaâ toxin during *Staphylococcus aureus* corneal infection. Infect Immun 1997; 65: 1571–1578.

48 Hayashida A, Bartlett AH, Foster TJ, Park PW. *Staphylococcus aureus* betaâ toxin induces lung injury through syndecanâ 1. Am J Pathol 2009; 174:509–518.

49 Akindolire MA, Babalola OO, Ateba CN. Detection of antibiotic resistant Staphylococcus aureus from milk: A public health implication. Int J Environ Res Public Health 2015; 12:10254–10275. [CrossRef] [PubMed]

50 Sallam KI, Abd-Elghany SM, Elhadidy M, Tamura T. Molecular characterization and antimicrobial resistance profile of methicillin-resistant Staphylococcus aureus in retail chicken. J Food Prot 2015; 78:1879–1884. [CrossRef] [PubMed]

51 Gole VC, Chousalkar KK, Roberts JR. Survey of *Enterobac-teriaceae* contamination of table eggs collected from layer flocks in Australia. Int J Food Microbiol 2013; 164:161–165.

52 Hanaa A el H. Elsheikh. Multidrug resistant bacteria in balady eggs. Faculty of Veterinary Medicine. Egypt: Damanhour University; 2023.

53 Sabarinath A, Guillaume V, Guillaume B, Mathew V, De Allie C, Nath Sharma R. Bacterial contamination of commercial chicken eggs in Grenada, West Indies WIVJ 2009; 9:4–7.

54 Ghasemian Safaei H, Jalali M, Hosseini A, Narimani T, Sharifzadeh A. The prevalence of bacterial contamination of table eggs from retails markets by Salmonella spp., Listeria monocytogenes, Campylobacter jejuni and Escherichia coli in Shahrekord, Iran. Jundishapur J Microbiol 2011; 4:4.

55 Siriphap A, Suwancharoen C, Laenoi W, Kraivuttinun P, Suthienkul O, Prapasawat W. First study on virulence genes, antimicrobial resistance, & integrons in *Escherichia coli* isolated from cage, free-range, & organic commercial eggs in Phayao Province, Thailand. Vet World 2022; 15: 2293–2301.

56 Kaper JB. Defining EPEC. Revista de Microbiologia 1996; 27:130–133.

57 Shimaa HA. Some Advanced Studies on avian pathogenic E. coli in broiler chickens at Sharkia Governorate. M. V. Sc. Thesis. Fac. Vet. Med. Zagazig Univ. 2013

58 Emara HAM, Al-Ebshahy EM, Torky HA. Virulence Genes and Antibiotic Resistance of *Escherichia coli* Isolates from Dead-In Shell Chicken Embryos. AJVS 2019; 62:144–150.

59 Kilici A, Ertas HB, Muz A, özbey G, Kalender H. Detection of the eaeA Gene in Escherichia coli from Chickens by Polymerase Chain Reaction. Turk J Vet Anim Sci 2007; 31:215–218.

60 El-Jakee JK, Mahmoud RM, Samy AA, El-Shabrawy MA, Effat MM, Gad El-Said WA. Molecular characterization of e. coli isolated from chicken, cattle and buffaloes. Int J Microbiol Res 2012; 3:64–74.

61 Galal HM, Hakim AS, Dorgham SM. Phenotypic and virulence genes screening of *Escherichia coli* strains isolated from different sources in delta Egypt. J Life Sci 2013; 10:352–361.

62 Zahraei Salehi TS, Afarchi A, Peighambari SM, Mahzounie M, Rabbani Khorasgani M. Detection of *stx1*, *stx2*, *eae*, espB and hyl genes in avian pathogenic *Escherichia coli* by multiplex polymerase chain reaction. J Vet Res 2007; 62:37–42.

63 Elafify M, Elsherbini M, Abdelkhalek A, Al-Ashmawy M. Prevalence and molecular characterization of enteropathogenic *Escherichia coli* isolated from table eggs in Mansoura. J Adv Vet Anim Res 2016; 3: 1–7.

64 Samanta I, Joardar SN, Das PK, Sar TK. Comparative possession of shiga toxin, intimin, enterohaemolysin and major extended spectrum beta lactamase (ESBL) genes in *Escherichia coli* isolated from backyard and farmed poultry. Iran J Vet Res 2014; 16:90–93.

65 Wang H, Zhong Z, Luo Y, Cox E, Devriendt B. Heat-stable enterotoxins of enterotoxigenic Escherichia coli and their impact on host immunity. Toxins 2019; 11:24. [CrossRef]

66 Vidal RM, Muhsen K, Tennant SM, Svennerholm A-M., Sow SO, Sur D, *et al.* Colonization factors among enterotoxigenic Escherichia coli isolates from children with moderate-to-severe diarrhea and from matched controls in the Global Enteric Multicenter Study (GEMS). PLoS Negl Trop Dis 2019; 13:e0007037. [CrossRef]

67 El-gendi MARWA, Amin M. 'isolation and characterization of methicillin resistant staphylococcus aureus (mrsa) from table eggs. Assiut Vet Med J 2019; 65 I:1–11.

68 Awny C, Amer AA, Abo El-Makarem HS. Microbial hazards associated with consumption of table eggs. AJVS 2018; 58:139–146.

69 Yenilmez F, Bulancak A. Microbiological quality of table eggs sold at different sales location. Çukurova Tarım ve Gıda Bilimleri Dergisi 2020; 35:115–124.

70 Marek A, Stępień-Pyśniak D, Rzedzicki J. Occurrence of Bacteria of the genus staphylococcus in table eggs descended from different sources. Polish J Vet Sci 2009; 12:481–484.

71 Fatima Zahra EF, Nassik S, Sabrine N, Ahlam K, Nadia C, Kawtar A, *et al.* Bacteriological Quality Of Table Eggs In Moroccan Formal And Informal Sector. Int J Food Sci 2022; 2022:1–9.

72 Al Momani W, Janakat S, Khatatbeh M. Bacterial contamination of table eggs sold in Jordanian markets. Pak J Nutr 2018; 17:15–20.

73 USP C. <1225> Validation of compendial procedures & <621> Chromatography. Rockville, Rockville, MD: United States Pharmacopeia; 2019.

74 ICH (International Conference on Harmonization of Technical Requirements for Registration of Pharmaceutical for Human Use) Validation of Analytical Procedures: Text and Methodology Q2 (R1) 2005;1(20),05

75 Amirkhizi B, Arefhosseini SR, Ansarin M, Nemati M. Aflatoxin B1 in eggs and chicken livers by dispersive liquid-liquid microextraction and HPLC. Food Addit Contam Part B 2015; 8:245–249.

76 Herzallah SM. Determination of aflatoxins in eggs, milk, meat and meat products using HPLC fluorescent and UV detectors. Food Chem 2009; 114:1141–1146.

77 Shehata AA, Ali H, Ghazali NH. Detection of Aflatoxin and antibacterial residues in different types of table eggs with studying of the effect of heat treatment. Benha Vet Med J 2014; 27:177–187.

78 Frenich AG, Romero-González R, Gómez-Pérez ML, Vidal JLM. Multi-mycotoxin analysis in eggs using a QuEChERS-based extraction procedure and ultra-high-pressure liquid chromatography coupled to triple quadrupole mass spectrometry. J Chromatogr A 2011; 1218: 4349–4356.

79 Adegbeye MJ, Reddy PRK, Chilaka CA, Balogun OB, Elghandour MM, Rivas-Caceres RR, Salem AZ. Mycotoxin toxicity and residue in animal products: Prevalence, consumer exposure and reduction strategies-A review. Toxicon 2020; 177:96–108.

80 Omar SS, A. Haddad M, Fakhri Y. Prevalence and concentration of mycotoxins (Aflatoxin B1, Ochratoxin A, Deoxynivalenol and Zearalenone) in the boiled poultry eggs and probabilistic health risk assessment: a case study in Jordan. Int J Environ Anal Chem 2021; 103:1–13.