Prevalence of Enterotoxigenic *S. aureus* in Table Eggs in El-Fayoum City, Egypt

Adel M. El-Kholy¹ · Mohamed A.H. El-Shater² · Marwa M. Abdel-Gawad³* · Mohamed M.A. Zeinhom¹

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Abstract

This study was designed to determine the prevalence of enterotoxigenic *S. aureus* in table eggs in El-Fayoum city, Egypt. A total of 250 table egg samples (75 Baladi hens’, 75 white farm hens’, 75 brown farm hens’ and 25 duck egg samples) were collected randomly from poultry farms, groceries, supermarkets, and street vendors in El-Fayoum city, Egypt. Each Baladi hen’s egg sample was represented by five eggs, while each farm hen’s and duck egg sample was represented by three eggs. The shells and contents of eggs were examined for the presence of *Staphylococcus* spp., coagulase (coa), and staphylococcal enterotoxins (Ses) genes. The obtained results revealed that the examined samples of shells and contents of Baladi hens’, poultry farms’ (white and brown), and ducks’ eggs were contaminated with *Staphylococcus* spp. with incidences of 24.0, 9.3, 5.3, 44.0, 8.0, 2.7, 1.3 and 12.0 %, respectively and coagulase-positive *S. aureus* with the incidences of 16.7, 14.3, 0.0, 18.2, 0.0, 0.0, 0.0 and 33.3 %, respectively. Enterotoxin profiling by PCR proved that two classical enterotoxin genes (Seb and Sed) were produced from three (42.86%) coagulase-positive *S. aureus* strains, as two Baladi hens’ eggshells produced Seb and one of the ducks’ egg contents produced Sed. The public health hazards of the isolated strains and enterotoxins had been discussed.

Keywords

Enterotoxins, PCR, *S. aureus*, Table Eggs

1. Introduction

Table eggs are devoured worldwide and play an important part of human diet for many reasons, which are the high quality of egg proteins with low cost and the fact that the interior of the egg is guarded by the shell. Also, eggs have high nutritive value, they supply the diet with several essential nutrients, such as zinc, selenium and retinol as well as many minerals and vitamins except vitamin C (Layman and Rodriguez, 2009).

The external shell contamination could be important for the shelf life and the food safety of eggs and egg products consumption. It is supposed that bacterial contamination of internal egg content could be the result of penetration of the shell by bacteria deposited on the surface of the egg after it has been laid (Smith et al., 2000).

*Staphylococcus* spp. are most common bacteria contaminating eggshells. Contamination is more likely associated with cracked egg, dirty shells, and contaminated storage. Also, it can be contaminated during formation and laying process (Abdullah, 2010).

*Staphylococcus* spp. are Gram’s positive bacteria that can tolerate dry, harsh and salty conditions and present in dust, soil and feces, which is the main reason of its presence on eggshells (De Reu et al., 2007). *S. aureus* is a type of bacteria found on human skin, infected cut, eczema, abscesses, wound infections, noses and throats, so it can be transmitted to eggs when handled by a person who has *S. aureus* infection. Also, eggs’ contents may be contaminated accidentally by *S. aureus* from shell or it might have originated from ova during egg formation, dust.
and from the surface of inanimate objects (California Egg Commission, 1999).

Food borne illness is a major public health problem. Staphylococcal food poisoning (SFP) is one of the most common food-borne diseases and results from the ingestion of staphylococcal enterotoxins (Ses) preformed in food by enterotoxigenic strains of *S. aureus*. It is ranked as the third among all foodborne diseases in the world (Boerema et al., 2006). Nearly, 50% of *S. aureus* produce enterotoxins which create food intoxication in consumers (Abdullah, 2010). Intoxication occurs when the food is contaminated with bacteria producing toxins, like the enterotoxins from *S. aureus*.

There are several types of staphylococcal enterotoxins; A-E, G, H, I and R-T, which are produced either singly or combined by most strains of *S. aureus* (Argudin et al., 2010).

The potential health hazards of *S. aureus* is ranged from a variety of self-limiting to life-threatening diseases. The bacteria are a major reason of food poisoning, resulting from the consumption of food contaminated with enterotoxins. Staphylococcal food poisoning (SFP) is characterized by rapid onset of nausea, vomiting, abdominal pain, cramps, and diarrhea. Scalded skin syndrome is caused by exfoliative toxins secreted on the epidermis and mostly affects neonates and young children. Moreover, staphylococcal exfoliative toxins cause other skin conditions such as blisters, pimples, furuncles, impetigo, folliculitis, abscesses and secondary infection (Fridkin et al., 2005; Eisenstein, 2008).

Therefore, this study was done to evaluate the potential risk of enterotoxigenic *S. aureus* in table eggs collected from groceries and supermarkets located in El-Fayoum city, Egypt.

2. Materials and Methods

2.1. Collection of Samples

250 table egg samples (75 Baladi hens’, 75 white farm hens’, 75 brown farm hens’ and 25 duck egg samples) were collected randomly from poultry farms, groceries, supermarkets and street vendors in El-Fayoum city, Egypt. Each Baladi hen’s egg sample was represented by five eggs, while each farm hen’s and duck egg sample was represented by three eggs. Each sample was placed in a sterile plastic bag and transferred to the laboratory without delay whereas they prepared and examined microbiologically.

2.2. Preparation of the Samples

Egg samples were prepared according to (APHA, 1992). Eggshell was washed by a surface rinse method and the obtained rinse solution from the five or three eggs of each group was combined. The egg was prepared for evacuation of its content and the contents of each group sample were removed aseptically and received into a sterile mixer until the sample becomes homogenous. Also, the obtained egg content either from five or three eggs of each group was combined to represent one Egg content sample.

2.3. Isolation and Identification of Coagulase Positive *S. aureus* were done According to (ISO/AM 2010)

One ml of both rinsing solution and egg contents samples was transferred to Brain Heart Infusion broth (BHI) and incubated at 35 °C for 18-24 hours then a loopful from the inoculated BHI broth was spread onto dry surface of Baired Parker agar supplemented with egg yolk tellurite, then the inoculated plate was incubated at 37°C for 24 to 48 hrs. The suspected colonies were examined for the characteristic *S. aureus* colonial morphology which show circular, smooth, convex, moist, black, shiny with a narrow white margin colonies surrounded by a halo zone extended into the opaque medium. Biochemical tests such as Catalase test, Growth confirmation using Mannitol salt agar and Coagulate test were performed.

2.4. Molecular Identification of Coagulase Positive *S. aureus*

Molecular identification of the isolated strains was done through the detection of coa gene and staphylococcal enterotoxins (Sea, Seb and Sed) genes using PCR and implemented according to (Iyer and Kumosani, 2011; Mehrotra et al., 2000).

2.4.1. Extraction of DNA

DNA was extracted using QIAamp DNA Mini Kit. Briefly, 1.5 ml of an overnight broth culture of *S. aureus* grown in Brain Heart Infusion broth (BHI) at 37°C was centrifuged in a benchtop centrifuge at 8000 rpm for 5 min and the supernatant discarded. The cell pellet was re-suspended in PBS to a final volume of 200 ml. QIAGEN protease (20 ml) was pipetted into the bottom of a 1.5 ml micro centrifuge tube then 200 ml of the sample and 200 ml buffer AL were added and mixed by pulse vortexing for 15 seconds. After that, the mixture was incubated at 56°C for 10 min and centrifuged to remove drops from inside the lid. 200 ml ethanol (96%) were added to the sample and mixed again by pulse vortexing for 15 seconds. After mixing, centrifugation was used to re move drops from inside the lid. The mixture was carefully applied to the QIAamp Mini spin column (in ml collecting tube) for DNA extraction. The DNA concentration was measured using a spectrophotometer. An average of 10 mg of DNA was obtained.

2.4.2. Cycling Conditions of the Primers during PCR

The coa gene and staphylococcal enterotoxins (Sea, Seb and Sed) genes were amplified by a multiplex PCR as
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3. Results

3.1. Incidence of Staphylococcus spp. and Coagulase Positive S. aureus

Staphylococcus spp. and coagulase positive S. aureus were recovered from eggshells and contents of the examined Baladi, poultry farm (white and brown) and duck eggs samples were illustrated in Table (2). Out of 250 examined table egg samples, 52 Staphylococcus spp. isolates were recovered with an incidence rate 20.8%. Out of these isolates, 7 strains of S. aureus were identified, 3(16.7%) were isolated from Baladi hens’ eggs shells and 1(14.3%) was found in white poultry farms’ eggs shells, while 2(18.2%) and 1(33.3%) isolates were detected in the examined ducks’ egg shells and contents, respectively. PCR results for the coagulase positive S. aureus strains (coa gene) was represented in Fig. (1).

3.2. PCR for Detection of Staphylococcal Enterotoxin Genes

PCR results for detection of staphylococcal enterotoxins (Sea, Seb and Sed) genes in coagulase- positive S. aureus strains recovered from the examined Baladi, poultry farm (white and brown) and duck eggs samples were illustrated in Table (3) and Fig. (2).

Table (1): Primers sequences used for detection of coa and staphylococcal enterotoxins genes.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primers sequences</th>
<th>Amplified product</th>
<th>Reference</th>
</tr>
</thead>
</table>
| Coa  | ATA GAG ATG CTG GTA CAG G  
ATA GAG ATG CTG GTA CAG G | Four different types of bands may be detected 350 bp  
430 bp  
570 bp  
630 bp | Iyer and Kumosani (2011) |
| Sea  | GGTTATCAATGTGGCGGGTGG  
CGGCACCTTTTTTCTCTCCGG | 102 bp | Mehrotra et al., (2000) |
| Seb  | GTATGGGGTGTGAACTGAGC  
CCAAATAGTGACGAGTTAGG | 164 bp | |
| Sed  | CCAATAATTGAGAAAATTTTAAAG  
ATTGGATTTTTTTTTCCGTTT | 278 bp | |

Table 2. Incidence of Staphylococcus spp. and coagulase positive S. aureus recovered from eggshells and contents of the examined Baladi, poultry farm (white and brown) and duck eggs samples.

<table>
<thead>
<tr>
<th>Examined samples</th>
<th>No. of examined samples</th>
<th>Staphylococcus spp.</th>
<th>Coagulase positive S. aureus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>Baladi hens’ eggshells</td>
<td>75</td>
<td>18</td>
<td>3</td>
</tr>
<tr>
<td>White poultry farms’ eggshells</td>
<td>75</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>Brown poultry farms’ eggshells</td>
<td>75</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Ducks’ eggshells</td>
<td>25</td>
<td>11</td>
<td>2</td>
</tr>
<tr>
<td>Baladi hens’ egg contents</td>
<td>75</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>White poultry farms’ egg contents</td>
<td>75</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Brown poultry farms’ egg contents</td>
<td>75</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Ducks’ egg contents</td>
<td>25</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>250</td>
<td>52</td>
<td>7</td>
</tr>
</tbody>
</table>

N.B. Eggshell and egg content are considered one sample.
Table 3. Occurrence of enterotoxins genes (Sea, Seb and Sed) in coagulate-positive S. aureus strains recovered from the examined Baladi, poultry farm (white and brown) and duck eggs samples.

<table>
<thead>
<tr>
<th>Tested samples</th>
<th>Sea</th>
<th>Seb</th>
<th>Sed</th>
</tr>
</thead>
<tbody>
<tr>
<td>One S. aureus isolate from Baladi hens’ eggshells</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Two S. aureus isolates from Baladi hens’ eggshells</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>One S. aureus isolate from white poultry farms’ eggshells</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Two S. aureus isolates from ducks’ eggshells</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>One S. aureus isolate from ducks’ egg contents</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Total</td>
<td>0</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>

Fig. 1. PCR result for the coagulase positive S. aureus strain (coa) gene (430bp). Lane L: DNA ladder, Lane Neg.: control negative, Lane Pos.: control positive, Lane 1, 2, 3, 4, 5, 6 and 7: S. aureus isolates.

4. Discussion
From data presented in Table (2), 18(24%) and 6(8%) Staphylococcus spp. isolates were detected in Baladi hens’ eggs shells and contents, respectively, while coagulase positive S. aureus was isolated from 3(16.7%) and 0(0%) of Baladi hens’ eggshells and contents, respectively. Higher incidences of S. aureus in shells and contents 48.6% - 17.1% and 40% - 13.3% were obtained by Refaat (2009) and Sadek et al., (2016) from Baladi hens’ egg shells and contents, respectively. Awny et al., (2018) could detect S. aureus in 16% of Baladi hens’ eggs contents. However, Abdel-Tawab (2020) found S. aureus in lower incidence (8%) in Baladi hens’ eggs shells and slightly higher result (2%) from contents.

Summarized results in Table (2) proved that Staphylococcus spp. were detected in 7(9.3%) and 4(5.3%) from white and brown poultry farms’ eggs shells respectively, where it was detected in egg contents in a percentage of 2.7 and 1.3, respectively, while 1(14.3%) of white poultry farms’ eggshells were contaminated with S. aureus. Although, S. aureus wasn’t detected in brown poultry farms’ shells, white and brown poultry farms’ eggs contents. Higher incidences in eggshells (33.3%) and contents (10%) of poultry farms’ eggs were reported by Sadek et al., (2016). Similar finding of S. aureus was reported by Refaat (2009) in poultry farms’ eggs shells (14.3%). On the other hand, lower result was detected by Abdel-Tawab (2020) in poultry farms’ eggs shells (4%), but Awny et al., (2018) detected S. aureus in 52% of poultry farms’ eggs contents.

The results obtained in Table (2) revealed that Staphylococcus spp. isolates were detected in 11(44%) of the examined Ducks’ eggshells samples and 3(12%) of ducks’ egg contents. S. aureus were detected in 2(18.2%) and 1(3.3%) of the examined ducks’ egg shells and contents, respectively. Higher results were obtained by Refaat (2009) who recorded that 51.4% and 40% of the examined ducks’ eggshells and contents were contaminated with S. aureus, respectively, while Awny et al., (2018) isolated S. aureus from 48% of the examined ducks’ egg contents.

The obtained results in our study revealed that the lowest incidence of S. aureus was observed in the examined samples of poultry farms hens’ eggs which may be attributed to good hygienic measures during production, handling, and storage at farms.

Molecular identification of isolated Coa positive S. aureus strains from the examined eggs samples in Fig. (1), revealed that all the examined coagulate positive Staphylococcus spp. were positive for S. aureus Coa gene. Enterotoxins profiling as illustrated in Table (3) and Fig. (2), cleared that three 42.86% of Coa positive S. aureus
strains were showing positive results for the enterotoxins genes. Results revealed that three classical enterotoxin genes (Sea, Seb and Sed) were detected in a percentage of 0.0, 28.6 and 14.3% in Baladi hens’ eggshells and ducks’ egg contents, respectively. Two isolates of coagulase positive S. aureus recovered from Baladi hens’ eggshells have Seb gene in percentage of 28.6% but don’t have other genes and one isolate was negative for all Ses genes, while one S. aureus isolate recovered from white poultry farms’ eggshells was negative for all Ses genes. Concerning to ducks’ eggs, one coagulase positive S. aureus isolate recovered from ducks’ egg contents has Sed gene in percentage of 14.3% but 2 isolates recovered from ducks’ eggshell were negative for all Ses genes.

Our results agreed with Fueyo et al., (2001) who recorded that the detected S. aureus isolates by PCR in Spanish eggs were accompanied with production and detection of classical enterotoxin genes. Slightly higher result was detected by Abdel-Tawab (2020) who found that Ses were produced by 50% of S. aureus isolates, but lower results were obtained by Kitai et al., (2005) and Naffa et al., (2006) who recorded that Ses were produced by 21.7 and 23% of S. aureus isolates, respectively. Moreover, Yang et al., (2001) reported the highest levels of Sea (≥64 ng/mL) and Seb (≥64 ng/mL) which were produced by S. aureus isolates, while Rasoul et al., (2015) found Seb in 4.1% of S. aureus. Concerning to the Egyptian Organization for Standardization and Quality Control (2007) which mentioned that S. aureus must not be found in the egg content. It was noticed that 7 samples failed to achieve the Egyptian Standard levels with incidences of 16.7, 14.3, 18.2 and 33.3% from Baladi hens’ eggshells, white poultry farms’ eggshells, ducks’ eggshells and ducks’ egg contents, respectively as recorded in Table (2).

Staphylococcal enterotoxins (Ses) constitute a group of biologically and structurally related toxins. The Ses are the major reason of many outbreaks of food borne diseases and staphylococcal enterotoxin h(Seb) is the most common toxin associated with classic food poisoning (Lamaita et al., 2005).

5. Conclusion
This study revealed that the Baladi hens’ and ducks’ eggs have higher incidences of Staphylococcus spp. and coagulase positive S. aureus than poultry farm hens’ eggs. We recommend that the strict hygienic measures should be adopted in the farms and during production, handling and processing of eggs to protect eggs from being contaminated with pathogenic bacteria causing food poisoning.

6. Authors Contributions
All authors contributed equally to study design methodology, interpretation of results and preparing of the manuscript.

7. Conflict of interest
The authors declare no conflict of interest.

8. References
ISO/AM (2010). Enumeration of coagulase and thermos nuclease-positive Staphylococci (Staphylococcus aureus and other species) in raw milk and fresh soft cheese: An evaluation of Bairded-Park agar, rabbit plasma fibrinogen...


