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Original Research Article

Potential risk of some pathogens in table eggs

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ABSTRACT

This study was conducted to record the potential risk of some pathogens in table eggs in Beni-Suef city, Egypt. A total of 100 table eggs samples (Farm and Baladi eggs) were randomly collected from poultry farms, markets, supermarkets and groceries in Beni-Suef city, 50 farm eggs samples (each of 3 eggs) and 50 baladi eggs samples (each of 5 eggs) were examined for the presence of coliforms, *E.coli*, *Salmonella*, coagulase positive *Staphylococcus aureus* and Staphylococcal enterotoxins. Isolates were identified by biochemical, serological and molecular (PCR) methods. The obtained results in the present study revealed that (22%) of the examined samples were contaminated with Coliforms. The other identified genera were *Citrobacter freundii*, *Citrobacter diversus*, *Edwardsiella tarda*, *Enterobacter spp.*, *Morganella morganii*, *Klebsiella oxytoca*, *Providencia spp.*, *Serratia fonticola* and *Yersinia intermedia*. *E.coli* (atypical type) was detected in a percentage of 27.27%. True fecal *E. coli* and *Salmonella spp.* failed to be detected in any of the whole examined 100 eggs samples. Additionally, *Staphylococcus spp.* was detected with incidence rate of (13%), out of them, 8 (61.5%) isolates were accounted for coagulase positive *Staphylococcus aureus*. Enterotoxin profiling revealed that two classical enterotoxin genes (SEA and SED) were detected either singly or in combination. The potential health hazards and the suggested control measures of the isolated strains had been discussed.

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1. Introduction

Eggs are truly an inexpensive and highly nutritious food that can be considered a nutritious formula in the diet for people of all ages and at different stages of life. On average, the macronutrient content of eggs includes proteins, lipids and low carbohydrates (Papadopoulou et al., 1997; MAFF, 2000). Eggs supply the diet with several essential nutrients such as zinc, selenium, retinol, tocopherols and contain 18 vitamins and minerals. Most eggs have been found to be nearly sterile when laid, but they have the potential to become occasionally contaminated (Egg Nutrition Center (ENC), 2004; Egg safety center (ESC), 2010). Fresh egg contains a nature of physical barriers; the shell, cuticle and membranes that prevent microorganisms from gaining access to its contents. In addition, the albumen (egg white) contains substances that limit the growth of microorganisms (Jay et al., 2005, Gantois et al., 2009a). Human and hens can be a source of contamination of the eggs' shell (Gast and Holt, 2001, Ricke et al., 2001). The surface of an egg can be contaminated with any microorganisms before it is laid or after laying with the avian fecalmatter, nesting material, dust, feedstuff, shipping and storage containers (Al-Bahry et al., 2011) which is favored by high humidity and temperature leading to spoilage and economic losses or causing a public health hazard (Board and Fuller, 1994) The main isolated food-borne pathogens from table eggs and its contents are *Escherichia coli*, *Salmonella spp.* and *Staphylococcus aureus* (Adesiyun et al., 2005, Gole et al., 2013).

E. coli is considered an important pathogen of human diarrheal disease isolated from table eggs. Although most strains of *E. coli* inhabit the normal gut flora of humans and animals (Brooks et al., 1995), *E. coli* had been isolated from table eggs' shells and their contents (Hope et al., 2002, Adesiyun et al., 2005). *Salmonella*

species is considered the most important cause of food-related illness as they lead to more deaths than any other food-borne pathogen. *Salmonella* can cause illness on consumption of raw or undercooked eggs (FDA, 2010, USDA, 2011, CDC, 2017). Other members of Coliforms such as *Citrobacter spp.*, *Enterobacter spp.*, *Klebsiella spp.*, *Serratia spp.* and *Providencia spp.* have all the potential to cause spoilage of table eggs causing infection in consumers (Musgrove et al., 2004; Musgrove et al., 2008). *Staph. aureus* contaminates different kinds of food. On the other hand, coagulase positive *Staph. aureus* is considered the most important species of *Staphylococcus spp.* as it evokes pathogenic effect and produces enterotoxins which cause food toxication (Abeer, 1997). *Staph. aureus* is transmitted via people-to-food through improper handling and they are mainly found in restaurants or picnics as food is not properly refrigerated or stays out of the refrigerator too long (Songer and Post, 2005) and (Cha et al., 2006) The staphylococcal enterotoxins (SEs) are the products of *Staph. aureus* and are recognized as the causative agents of classical food poisoning in humans following the consumption of contaminated food (Ikeda et al., 2005). *Staph. aureus* enterotoxins are of several types; A–E, G, H, I and R–T, which are commonly produced either singly or combined by most strains of *Staph. aureus* (Argudín et al., 2010). Eggs are involved in outbreaks of Staphylococcal enterotoxiation (Yang et al., 2001, Shareef et al., 2009).

Therefore this study was executed to evaluate the potential risk of some pathogens in table eggs collected from groceries and supermarkets located in Beni suef city, Egypt.

2. Materials and Methods

2.1. Collection of samples A total of 100 table eggs samples (Farm and Baladi eggs) were randomly collected each from poultry farms, markets, supermarkets and groceries in Beni-Suef City, 50 farm eggs samples (each of 3 eggs) and 50 baladi eggs samples (each of 5 eggs). Each sample was placed in a sterile plastic bag and carried to the laboratory without delay where they were prepared and examined microbiologically.

2.2. Preparation of samples

Egg content: The eggs were prepared for evacuation of its content according to (APHA, 2004).

2.3. Microbiological examination

1- Isolation and identification of Coliforms and *E. coli* from egg

contents were carried out according to (Cheesbrough, 2006).

2- Isolation and identification of *Salmonella* from egg contents were performed according to (Cheesbrough, 2006).

3- Isolation and identification of coagulase positive *Staphylococcus aureus* were done according to (AM, 2003, ISO, 2003b).

4- Molecular identification of coagulase positive *S. aureus* gene (COA) and enterotoxin genes (A, B and D) by PCR was implemented according to (Mehrotra, 2000, Iyer and Kumosani, 2011).

3. Results

Table 1. Incidence of Coliforms in the examined table eggs samples

Type of egg samples	No. of the examined samples	No. of positive	%
Baladi	50	20	40
Poultry farm	50	2	4
Total	100	22	22

Table 2. Coliform isolates obtained from the examined table eggs samples

Bacterial isolates	Baladi egg samples		Poultry farm eggs samples	
	NO	%	No	%
<i>Citrobacter freundii</i>	2	10	-	-
<i>Citrobacter diversus</i>	1	5	-	-
Atypical E.coli	6	30	-	-
<i>Edwardsiella tarda</i>	2	10	-	-
<i>Enterobacter spp.</i>	-	-	2	100
<i>Morgonella morganii</i>	1	5	-	-
<i>Klebsiella oxytoca</i>	1	5	-	-
<i>Providencia spp.</i>	3	15	-	-
<i>Serratia fonticola</i>	2	10	-	-
<i>Yersinia intermedia</i>	2	10	-	-
Total	20	90.9	2	9.1

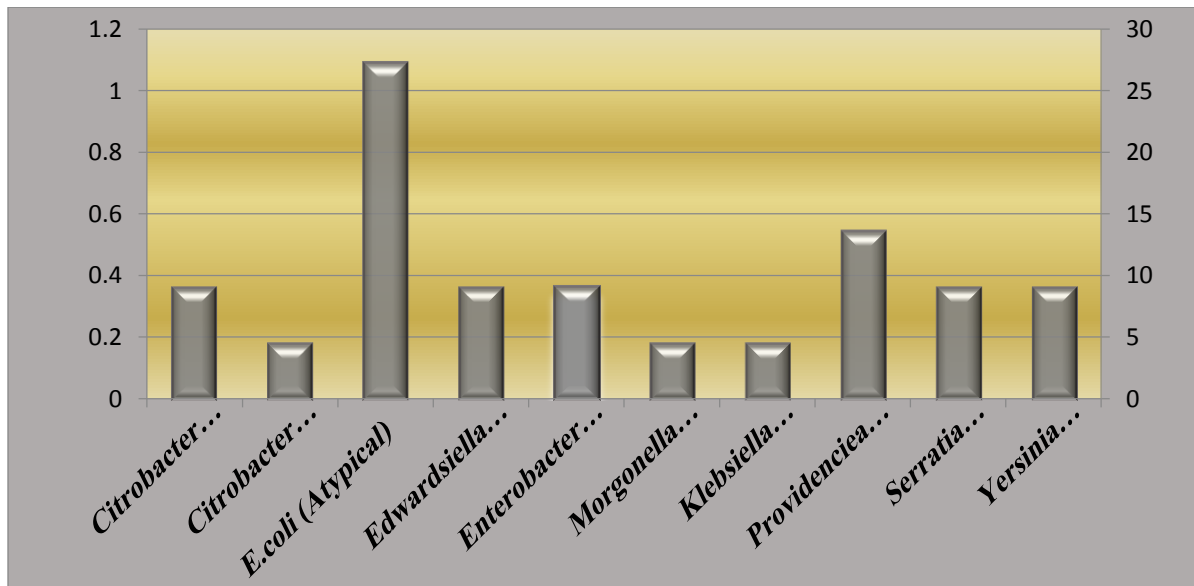


Fig. 1 Frequency of Coliform Isolates in table eggs samples

Table 3. Incidence of *Staphylococcus spp.* in the examined table egg samples

Type of eggs	No. of examined eggs	No. of positive samples		No. of Coagulase positive samples		No. of Coagulase negative samples	
		No	%	NO	%	NO	%
Baladi	50	7	14	5	71.4	2	28.6
Poultry farm	50	6	12	3	50	3	50
Total	100	13	13	8	61.5	5	38.5

Table 4. Molecular identification of coagulase (CoA) gene positive *Staph. aureus* strains recovered from the examined egg samples

<i>No of tested Isolates</i>	<i>No of identified CoA S.aureus strains</i>	<i>%</i>
8	8	100

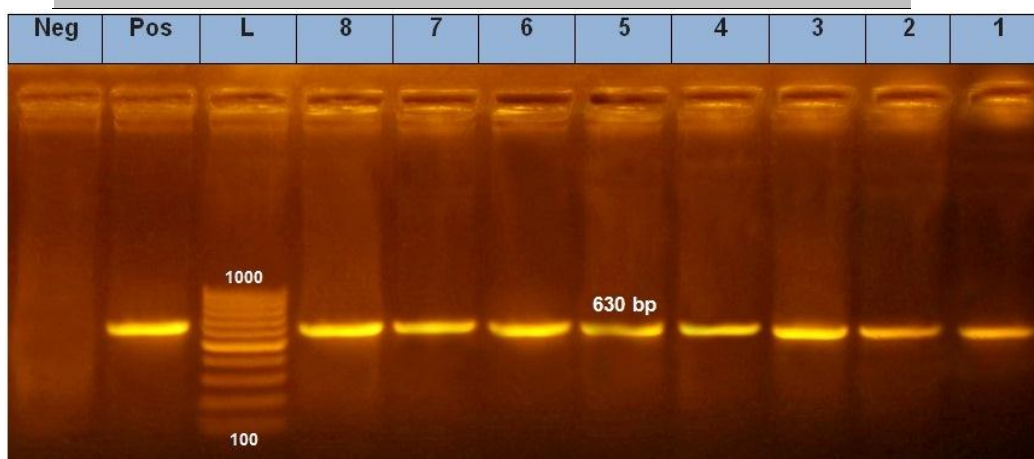


Fig. 2 PCR result for the coagulase positive *Staph. aureus* strain (CoA gene) (630bp). Lane L: DNA ladder, Lane Neg.; control -ve, Lane Pos.; control +ve, Lane 1, 2, 3, 4, 5, 6, 7 and 8: CoA *S. aureus* isolates

Table 5. Occurrence of enterotoxin genes (A+D) in coagulase positive *Staph. aureus* strains recovered from the examined table egg samples

Tested samples	CoA gene	SE A	SE D	SE A+D
1	+	-	-	-
2	+	+	-	-
3	+	+	+	+
4	+	+	-	-
5	+	-	-	-
6	+	-	+	-
7	+	-	-	-
8	+	-	-	-
Total	8	3	2	1

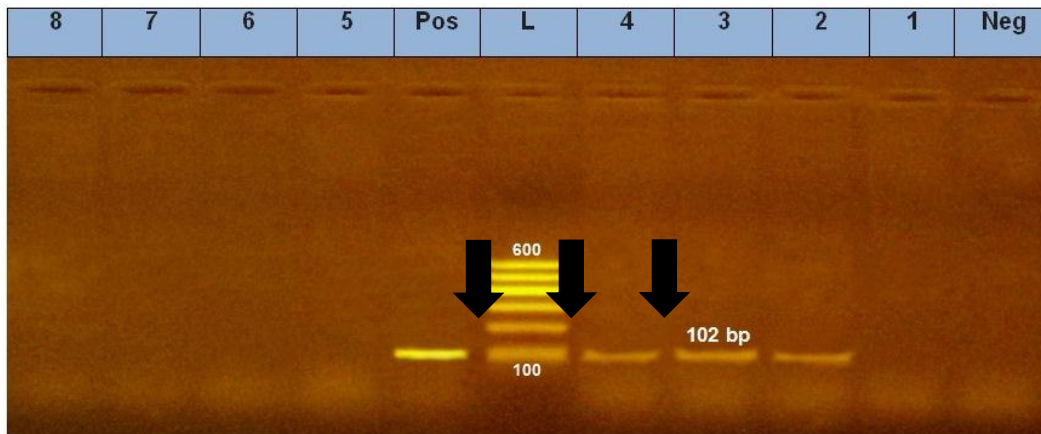


Fig. 3 PCR result for enterotoxin gene SEA (102 bp) among *Staph. aureus* Lane L: ladder, lane Pos: control positive, lane Neg: control negative, lane 2, 3 and 4 (+ve SEA), lane 1, 5, 6, 7 and 8(-ve SEA).

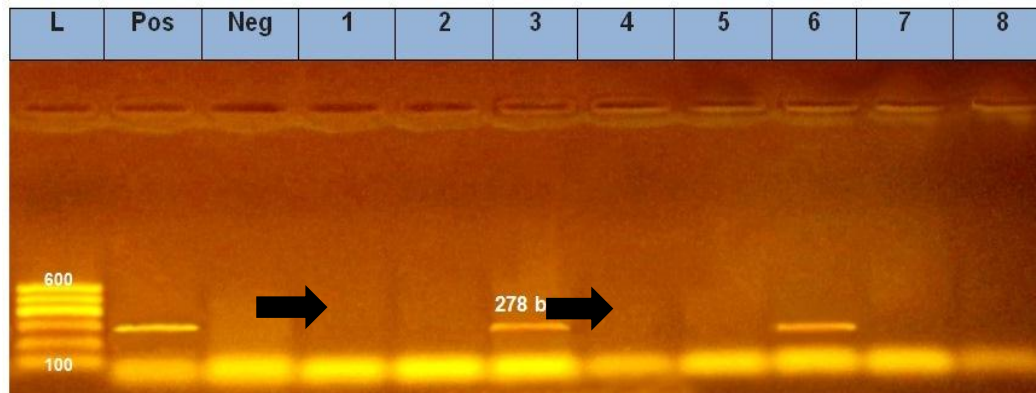


Fig. 4 PCR result for enterotoxin gene SED (278 bp) among *Staph. aureus*, Lane L: ladder, Lane Pos.: control positive, Lane Neg.: control negative, Lane 3 and 6 (+ve SED), Lane 1, 2, 4, 5, 7 and 8(-ve SED)

4. Discussion

According to the results presented in Table (1), it was recorded that 20 (40%) Baladi eggs samples were contaminated with Coliforms, while only two (4%) of the Poultry farm eggs samples were contaminated with Coliforms.

Our results were nearly similar to those evoked by (Adesiyun et al., 2005, Abdullah 2010, Ghasemian Safaei et al., 2011, AL-Ashmawy 2013) while it was lower than those reported by (El-Kholy et al., 2014, Salihu et al., 2015) and higher than the results obtained by (Awany et al., 2018).

The results recorded in Table (2) revealed that the identified genera of Coliforms from the examined eggs samples were *Citrobacter freundii*, *Citrobacter diversus*, Atypical *E.coli*, *Edwardsiella tarda*, *Mogonella morgani*, *Klebsiella oxytoca*, *Providencia spp.*, *Serratia fonticola* and *Yersinia intermedia* with the incidences of (10%), (10%), (5%), (30%), (10%), (5%), (5%), (15%), (5%) and (5%) from Baladi eggs samples, respectively. While only two (100%) *Enterobacter species* were isolated from examined farm eggs samples.

The presence of different members of Coliforms in eggs was recorded by different authors as (Papadopoulou et al., 1997, Jones et al., 2004, Musgrove et al., 2004, Adesiyun et al., 2005, Musgrove et al., 2005, Adesiyun et al., 2006, Obi and Igbokwe, 2007, Musgrove et al., 2008, Al-khalaf et al., 2009, Ansah et al., 2009; Abdullah, 2010, Stępień-Pyśniak et al., 2010, Gole et al., 2013, Awany et al., 2018).

Our study cleared that *E.coli* true fecal type and *Salmonella spp.* could not be detected in any of the examined eggs samples.

Many authors reported that *Salmonella* failed to be detected which agreed with findings of this study as (El-Leboudy et al., 2011, Ghasemian Safaei et al., 2011, Mahdavi et al., 2012, El-Kholy et al., 2014, Abdel-Latif and Saad, 2015, Lee et al., 2016). Higher percentages of *Salmonella* were illustrated by (Adesiyun et al., 2005, Evêncio-Luz et al., 2012, Ghazi and Amer, 2015, Amin, 2017, Awany et al., 2018, Al Momani et al., 2018). Failure to detect fecal *E. coli* in our results agreed with (Bahobail et al., 2012, Siriporn et al., 2015), while True fecal *E. coli* were shown with high incidence rate in the results of (Adesiyun et al., 2005, El-Kholy et al., 2014, Eid et al., 2015, Mansour et al., 2015, Elafify et al., 2016, Lee et al., 2016, Momani et al., 2018).

Regarding the increasing consumption of egg and its products, it is necessary to investigate egg contamination. The egg shell contamination occurs as a result of deposition of fecal matter on the shell, ovarian or oviduct and gut flora, debris material, egg crates, packing and storage, clothes and hands of poultry workers, dust and weather conditions or during transportation and marketing (Al-Bahry et al., 2012). The prevalence of Coliforms may be attributed to the poor hygiene; consequently, such eggs with high coliforms constitute an economic and public health importance (Sabreen, 2001), while the lower contamination rate of poultry farm eggs may be due to its hygienic laying, handling and the cleaning process of eggs before marketing. Coliforms have been used as indicator microorganisms which indicate the possibility of fecal contamination (Mahdavi et al., 2012) and the microbial quality and safety of the food and also reflect the hygienic standards adopted in the food operations (Roberts et al., 1995). *E. coli* is one of the major problems in chicken production influencing heavier losses and severe drop in egg production, about 5.5 % mortality and 10-20% drop in eggs was observed with *E. coli* infections (Qu et al., 1997). *E. coli* can bring about urinary tracts infections, pneumonia, meningitis, peritonitis and induce profuse watery diarrhea in humans (Schiaivoni and Vergora, 2000). *Citrobacter spp.* can cause a wide spectrum of infections in humans. Among the various sites of infection, the urinary tract is the most common, followed by the respiratory tract, and skin/soft tissues (Pavani, 2012). *Enterobacter spp.* were incriminated in urinary tract infection and septicemia, while *Klebsiella spp.* is a world wide spread bacteria that can be responsible for arthritis, meningitis, appendicitis, cystitis and septicemia outbreaks in kids and newborns, but is more frequently responsible for pneumonia and necrotic damage of the lungs (Bernabe et al., 1998). In our study

Salmonella was not detected in any egg samples and this may be the result of strict control measures applied against these bacteria and may be attributed to the fact that poultry farmers practice strict medication and care as directed by the International Commission on the Microbiological Specification for Food (ICMSF, 2009). Regarding to the Egyptian Organization for Standardization and Quality Control; (E.O.S.Q.C, 2007) fresh table eggs must be free from *Salmonellae spp.* in their contents (Nil), so all examined eggs samples satisfy the standards in balady and farm hen eggs.

The summarized results in Table (3) revealed that the incidence of *Staphylococcus spp.* in the examined table eggs samples was (13%) out of them, 8 (61.5%) were coagulase positive *Staphylococcus aureus* (Table 4). The PCR result of the COA gene was enough to confirm the virulence of the *Staphylococci* isolates as it is considered a marker for its virulence.

Results of this study were lower than those reported by Abdullah, (2010) and Salihu et al., (2015), while higher than results illustrated by (Stepień-Pyśniak et al., 2009, Pyzik et al., 2014, Abdel-latif, 2015, Eid et al., 2015, Fardows et al., 2016, Rodríguez-Lázaro et al., 2017, El-Nagar et al., 2017).

The results recorded in Table (5) revealed that enterotoxins were found in 4 out of 8 (50%) of *Staph. aureus* strains and these strains showed positive results for the presence of enterotoxin genes singly and in combination. The enterotoxin profiling recorded tow classical enterotoxin genes (SEA and SED) which were recovered from three and two *Staph. aureus* strains and detected at 37.5 % and 25%, respectively, while only one *Staph. aureus* strain was positive for both SEA and SED genes (12.5%).

Our data agreed with (Fueyo et al., 2001, Yang et al., 2001, Çepoğlu et al., 2010).

Regarding to the Egyptian Organization for Standardization and Quality Control (E.O.S.Q.C, 2007) which stated that *Staphylococcus aureus* must not be found in egg content (Nil), it was noticed that there were 8 samples that failed to achieve the Egyptian Standard levels with incidences of 71.4% and 50% of Balady and Poultry farm eggs samples, respectively Table (3). Among the pathogenic food poisoning organisms that affect human, *Staph. aureus* organisms have a serious concern to public health (Wyah, 1992) causing Staphylococcal food-borne disease (SFD), which can be transmitted to eggs when handled by persons who have *Staph. aureus* infection. Also, eggs' contents may be contaminated accidentally by *Staph. aureus* from the egg shell as it might have originated from ova during egg formation, dust and from the surface of inanimate objects (California Egg Commission, 1999) Staphylococcal enterotoxins (SE) constitute a family of biologically and structurally related toxins. The SEs are the main cause of many outbreaks of food borne diseases (Lamaita et al., 2005) as well as the classical food poisoning in humans (Balaban et al., 2000, Dinges et al., 2000).

5. Conclusion

We can recommend that strict hygienic measures to safeguard eggs from being contaminated with pathogenic bacteria causing food poisoning should be adopted in the farms and during production, handling and processing of eggs.

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