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MICROBIOLOGICAL PROFILE OF LEAKING CHICKEN TABLE EGGS

Eman, F. Abdel-Latif & *Saad, M. F.
Cairo University, Faculty of Veterinary Medicine, Department of Food Hygiene & Control, Giza square, Egypt
*Corresponding author's email: drmeena2010@hotmail.com

ABSTRACT
Chicken table eggs are used in a wide variety of foods. A total of 50 composite samples of leaking shell eggs were randomly collected from different markets in Cairo, Egypt. The mean pH value was 8.68 ± 0.17. The mean value of total colony count was $5.36\times10^6 \pm 1.07\times10^5$cfu/gm, while the mean values of coliforms, Coagulase Positive Staphylococci and aerobic spore former counts were $2.61\times10^7 \pm 5.22\times10^5$ MPN/gm, $1.94\times10^7 \pm 3.88\times10^5$cfu/gm and $4.78\times10^5 \pm 9.56\times10^3$ cfu/gm respectively. Salmonellae failed to be detected in all examined samples. The public health and economical importance of isolated microorganisms were discussed.

KEY WORDS: Chicken table eggs, leaking shell eggs, Salmonellae, Coagulase Positive Staphylococci, public health.

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). As an egg is originally designed to create a chick, it has a complete life support system with many natural, built in barriers to prevent bacterial entrance and growth, protecting the developing embryo. The porous shell is not a full proof barrier due to the presence of bloom or cuticle. Other barriers to prevent contamination include the inner shell membranes and various layers of the white. Together, these layers fight bacteria in several ways. The inner shell membrane is believed to be made of protein fibers interwoven without any pores going straight through and it contains a high amount of lysozyme. Shell eggs without cracks have many natural, built-in chemical and physical properties that help prevent bacteria from entering and growing. These protect the egg on its way from the hen to the home (California Egg Commission, 1999). Government regulations in some countries require that eggs be carefully washed with special detergent and sanitizer to reduce the risk of egg spoiling and also food poisoning. Hen's original protective shell coating is generally replaced by a thin spray coating of a tasteless, odorless, harmless, natural mineral oil. However, eggs are susceptible to bacterial growth once the shell membranes are broken (Edema and Atayese, 2006). In underdeveloped countries, when the egg shell broken it's difficult to discard it and the content of more than one egg was packed in plastic bags to be sold at retail level in the grocery and public markets at about half the prices of whole eggs to be used for human consumption or used in catering. The contamination of the egg contents with microorganisms may lead to transmission of pathogens inducing cases of food-borne infection or intoxication to consumers constituting a public health hazards (American Egg Board, 2000). Owing to the continuous consumer demands for eggs, it is extremely necessary not only to increase egg production but also to safeguard consumers against health hazards, so the aim of this study to investigate microbiological profile of leaking chicken table eggs.

MATERIALS & METHODS

Collection of samples

This study was conducted on fifty random composite samples of leaking shell eggs (Composite samples resembling the content of three eggs). All samples were aseptically collected using sterile polyethylene bags in triplicate and taken to the laboratory for analysis in ice box with a minimum of delay to be immediately examined.

Determination of pH

The pH values of the raw eggs were determined by electronic type HANNA, HI 98/30 waterproof pH meter according to (AOAC, 1990).

Microbiological examination

Preparation of egg homogenate

Ten ml of egg sample was aseptically transferred with a sterile pipette to 90 ml of diluents 0.1% peptone water (Lab M, 104). The primary dilution was shaked for 10 seconds using mechanical agitator to obtain a 1/10 dilution, then 1ml of primary dilution was transferred to 9 ml of sterilized diluents to obtain tenth fold serial dilutions. The prepared dilutions were subjected to the following microbiological examinations.

Total Colony Count according to (ISO, 2003a)

One ml of the original sample and the previously prepared decimal dilutions were inoculated into duplicate plates of Standard Plate Count Agar (Oxoid, CM0463) and incubated at 30ºC for 72 hours.

Coliforms count according to (APHA, 2004)

One ml from each of the original sample and the previously prepared decimal dilutions was inoculated into a series of three fermentation tubes containing Lauryl Sulphate Tryptose (LST) broth (Oxoid, CM0451) supplemented with inverted Durham's tubes. Inoculated

tubes, as well as, the control and original ones were incubated at 35ºC for 48 hours. One ml of positive LST broth tubes (gas production) were inoculated into Brilliant Green Lactose Bile broth 2% (Oxoid, CM0031) and incubated at 35ºC for 48 hours. From the results obtained, MPN/gm was computed. The completed test for Coliforms was applied by streaking a loopful from positive tubes on Eosin Methylene Blue agar plates (Oxoid, CM0069) before being incubated at 35ºC for 48 hours. Identification of the isolated Coliforms was done according to (BAM, 2001 and Collins *et al*., 2004).

Total Staphylococci Count according to (ISO, 2003b)

From the previously prepared decimal dilutions, 0.1 ml was transferred onto the dry surface of duplicate plates of Baird-Parker medium supplemented with egg yolk tellurite emulsion 3.5% (Lab M, LAB085) and spreaded with sterile bent glass spreader until the surface of the medium appears dry. The plates were incubated at 37ºC for 48 hours.

Coagulase test according to (ISO, 2003b)

Five colonies typical and atypical were selected from each plate. The selected colonies inoculated into 5ml Brain Heart Infusion broth. The tubes were incubated at 37ºC for 24 hours. From which 0.1 ml was transferred to tubes containing 0.3 ml of sterile citrated rabbit plasma. Inoculated tubes were incubated at 37ºC and examined for clot formation after 4 hours.

Thermo-stable Nuclease test according to (APHA, 2004)

Fresh brain heart infusion broth culture was placed in a boiling water bath for 15 minutes, then removed and cooled. Sufficient amount of heated culture was added to fill each well in the Toludine Blue DNA Agar plate. Inoculated plates were incubated in a moist chamber at 37ºC for 4 hours before being examined.

Enumeration and identification of Aerobic Spore Forming Organisms according to (Vos *et al*., 2009)

The egg homogenate was placed in water bath at 80ºC for 20 minutes, then sudden cooling. 0.1 ml of homogenate was spreaded on dextrose tryptone agar media and incubated at 30ºC for 72 hours.

Isolation of Salmonella spp. according to (ISO, 2002)

Twenty five ml of the original prepared sample was aseptically transferred to 225 ml of sterile buffered peptone water and incubated at 37ºC 16-20 hours. A loopful from each of the previously prepared pre-enriched broth inoculated into a sterile test tube containing 10 ml Rappaport Vassiliadis broth then incubated at 43ºC for 24 hours. A loopful from each Rappaport Vassiliadis enriched tube was streaked on the dried surface of Xylose Lysin Deoxycholate agar and MacConkey agar plates. Inoculated plates were incubated at 37ºC for 48 hours.

RESULTS

TABLE 1: Statistical analytical results of pH value in the examined samples

pH value	Min.	Max.	Mean ± SEM
	7.48	9.80	8.68 ± 0.17

TABLE 2: Statistical analytical results of microbial counts in the examined samples

Microbial counts	Total no. of examined samples	Positive samples		Min.	Max.	Mean ± SEM
		No	%			
TCC	50	50	100.00	1.0×10^3	1.0×10^7	$5.36\times10^6 \pm 1.07\times10^5$
CC	50	48	96.00	< 10	9.3×10^7	$2.61\times10^7 \pm 5.22\times10^5$
TSC	50	26	52.00	10	9.0×10^8	$2.17\times10^7 \pm 4.35\times10^5$
C +ve SC	50	26	52.00	10	8.0×10^8	$1.94\times10^7 \pm 3.88\times10^5$
TAC	50	27	54.00	50	1.5×10^7	$4.78\times10^5 \pm 9.56\times10^3$

TCC= Total Colony Count (cfu/gm), CC= Coliforms Count (MPN/gm), TSC= Total Staphylococci Count (cfu/gm), C +ve SC= Coagulase Positive Staphylococci Count (cfu/gm), TAC= Total Aerobic Spore Formers Count (cfu/gm).

TABLE 3: Incidence of isolated Coliform organisms in the examined samples

Type of isolates	No.	%
Escherichia coli	12	28.58
Citrobacter freundii	10	23.81
Enterobacter aerogenes	7	16.67
Klebsiella ozaenea	1	2.38
Klebsiella oxytoca	4	9.52
Enterobacter agglomerans	4	9.52
Enterobacter cloacae	3	7.14
Enterobacter sakazakii	1	2.38
Total no. of isolates	42	100.00

TABLE 4: Incidence of aerobic spore formers spp. in the examined samples

Type of isolates	No.	%
Bacillus lentus	13	36.10
Bacillus marisflavi	5	13.88
Bacillus badius	2	5.56
Bacillus papillae	2	5.56
Bacillus megaterium	2	5.56
Bacillus agaradhaerens	2	5.56
Sporosarcina ureae	8	22.22
Sporolactobacillus	2	5.56
Total no. of isolates	36	100.00

DISCUSSION

The data presented in Table (1) showed that the mean pH value of the examined samples was 8.68 ± 0.17 with 7.48 as a minimum and 9.80 as a maximum. The low pH can strongly affect as antimicrobial, in general, Gram negative bacteria are more sensitive to low pH than Gram positive bacteria, while yeasts and molds are the least sensitive. The explained high count of isolated gram negative bacteria compared the gram positive due to alkaline pH. Total Colony Count (TCC) is a prime consideration in examination of food. It gives numerical figure about the general hygienic quality of food. It reflects the sanitary measures adopted during production, handling and storage (ICMSF, 2009). Data recorded in Table (2) revealed the contamination level of TCC ranged from 1.0×10^3 cfu/gm to 1.0×10^7cfu/gm with a mean value of 5.36×10^6 ± 1.07×10^5 cfu/gm. At the time of laying, the eggs are sterile due to natural chemicals and physical defenses against microbial infection, but on exposure to environmental conditions as temperature and length of storage eggs were contaminated by different types of microorganisms which cause spoilage and public health problems (Abd Elhady and Emara, 1997). The shell of eggs must be fully developed and contain no breaks. Cracked eggs may be used if they are delivered directly to egg product manufacture, where they must be broken as soon as possible owing to their greater susceptibility to microbial penetration. The eggshell acts as a natural packing material for the egg contents, preventing the penetration of harmful bacteria. The quality of the shell strength, the absence of eggshell cracks is prerequisites for achieving egg product quality. During storage on farms or in the packing centers, as well as during transport, poor handling practices can lead to the disruption of the egg's physical barriers. The presence of cracks increases the risk of bacterial contamination of the broken egg and of other eggs if the cracked ones leak, affecting the quality of the shell and that of the egg contents (EFSA, 2014).

Coliforms count is the traditional indicator of possible fecal contamination, microbial quality, wholesomeness and reflect the hygienic standards adopted in the food operation. One of the essential quality characteristics of food is its sanitary quality, *i.e*. which is produced, processed and handled under strict sanitary conditions. Lack of good sanitary practices may result in loss of quality, spoilage or in some cases create a health hazard (Vanderzant and Splittstoesser, 2005). Results recorded in Table (2) proved that coliform organisms were present in the examined samples with a mean value of 2.61×10^7± 5.22×10^5 MPN/gm. The biochemical identification of coliform organisms in the examined samples in Table (3) revealed that *Escherichia coli* was the most common (28.58%). Adesiyun *et al.* (2006) analyzed the frequency of coliforms in eggs and noted the presence of genera *Enterobacter* spp. and *Klebsiella* spp. The bacteria most frequently isolated from eggs are Gram-negative bacteria such as *Enterobacter*, *E. coli* and *Klebsiella* species (Papadopoulou *et al*., 1997 and Musgrove *et al*., 2008). The prevalence of such contaminants may be attributed to the poor hygiene in the resulting areas; consequently such eggs with high coliforms constitute an economic and public health importance (Sabreen, 2001). *Klebsiella pneumoniae* 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 frequent responsible for pneumonia and necrotic damage of the lungs, while Enterobacter species were incriminated in urinary tract infection and septicemia (Bernabe *et al*., 1998). Citrobacter can cause a wide spectrum of infections in humans, such as infections in the urinary tract, respiratory tract, wounds, bone, peritoneum, endocardium, meninges, and bloodstream. Among the various sites of infection, the urinary tract is the most common, followed by the respiratory tract, and skin/ soft tissues (Pavani, 2012). Table (1) illustrated that Staphylococci were present in 52.00% of the examined samples. The presence of Staphylococci in high number of samples is probably as a result of the dominance of the genus on parts of the human body such as hands, nose, skin and clothing (Nwagu and Amadi, 2010). The minimum count of Coagulase Positive Staphylococci was 10; the maximum was 8.0×10^8 with a mean value of 1.94×10^7 ± 3.88×10^5 cfu/gm. Staphylococci can be divided into two groups according to the production of Coagulase enzyme, which is capable of coagulating blood plasma. The synthesis of this enzyme is restricted to some species in the genus, among which *Staphylococcus aureus*. The other Staphylococci that do not synthesize coagulase are referred to as Coagulase Negative Staphylococci (Kloos & Bannerman, 1995 and Koneman, 1997). Stepien-Pysniak *et al.* (2009) found a high degree of contamination of table eggs with *Staphylococcus* bacteria. They added that twelve species of staphylococci were isolated from the egg tested, including both coagulase-positive strains (*Staphylococcus aureus*) and coagulase-negative strains. Regarding to the public health hazards, *Staphylococcus aureus* food poisoning is one of the most common types of food borne diseases worldwide, which caused by an intoxication resulting from the ingestion of food containing Staphylococcal enterotoxins, which is emetic, pyogenic

and mitogenic, suppresses immunoglobulin secretion and enhances toxic shock (Stewart *et al*., 2002). On the other hand (Abeer, 1997) mentioned that Coagulase positive *Staphylococcus aureus* is considered the most important species of Staphylococci due to its pathogenicity and enterotoxin production which cause food intoxication. Evencio-Luz *et al*. (2012) examined the chicken eggs microbiologically and found that Coagulase Positive Staphylococci in samples. The authors revealed a significant risk of intoxications from consuming these foods. Data depicted in Table (2) revealed that 54.00% of the examined samples were contaminated with aerobic spore formers with a mean value of $4.78\times10^5 \pm 9.56\times10^3$ cfu/gm. Biochemical identification of isolates of aerobic spore formers revealed that *Bacillus lentus* was the most frequent one with a percentage of 36.10 (Table 4). Presence of aerobic spore formers in a food article proved to be of great importance, as some of them having a public health hazards such as *B. cereus* and *B. megaterium* which have been implicated in cases of food poisoning (De Jonghe *et al*., 2010). Salmonellae failed to be detected from egg content of the examined samples. Similar results were recorded by (El-Leboudy *et al*., 2011). It is concluded that the shell pores are filled with fibrous materials act as a wick to draw in moisture also the presence of lysozymes are known by its ability to prevent infection (American Egg Board, 2000). The percentage of unacceptable samples based on TCC and pH according to maximum limits of (Egyptian Standards, 3169/2007) was 56.00% and 38.00% respectively. The percentage of acceptable samples was 100.00% according to the presence of *Salmonella*, while the higher incidence (52.00%) of the unacceptable samples was associated with coagulase positive staphylococci.

CONCLUSION

The examined leaking table egg samples did not met the minimum requirements for human consumption. Guidance on legislation covering the marketing of chicken table eggs is considerably required and providing consumer with awareness to use intact good quality eggs.

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