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The Microbial Burden of *Pseudomonas* Species in Different Types of Table Eggs in Egypt

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Abstract: Microbial food safety of eggs has not been thoroughly investigated in Egypt. *Pseudomonas* species are among the most ubiquitous bacteria in the environment that may also contaminate eggs. To investigate the microbial burden of *Pseudomonas* species in eggs, one hundred composite samples (6 eggs in each) of table hen and duck eggs were prepares after they were collected from different shops and supermarkets in Dakahliya governorate, Egypt. *Pseudomonas* species were isolated and identified using microscopic examination, biochemical tests. *Pseudomonas* organisms were detected in 28, 24, 8 and 64% of egg contents and in 8, 44, 24 and 28% of egg shells of brown shell hen eggs white shell hen eggs, Baladi hen eggs and duck eggs, respectively. The mean viable count of *Pseudomonas* was higher in duck eggs and Baladi hen eggs that were home produced, than in white shell hen eggs, brown shell hen eggs that were commercially produced. The recovery rate of these bacteria was enhanced by using enrichment technique, resulting in higher recovery from commercially produced eggs than in home produced ones. This may by due to washing procedure applied on commercial eggs. *P. fluorescens*, *P. cepacia, P. putida* and *P. aeruginosa* were isolated from table hen and duck eggs, which indicate that these eggs and more prone to spoilage, especially considering their storage at room temperature in shops and supermarkets in Egypt. *P.aeruginosa* isolates were then verified by colony PCR method that showed high accuracy for detection of *P. aeruginosa* in table egg samples than conventional one, suggesting that the test is a more efficient tool for the quality control of egg production.

Key words: *Pseudomonas* species · *P. aeruginosa* · Table eggs · Colony PCR

INTRODUCTION

Fresh eggs are among the most important and nutritious foods in our daily diets. Eggs are included in the preparation of several food products and to serve various functions [1]. Eggs are an especially important part of children's diet [2]. Eggs are mainly considered a proteinaceous food moreover, they also contain all the vitamins and minerals needed in the human diet, except for vitamin C [3].

In Egypt, hen and duck eggs are used for human consumption as fresh eggs. These eggs directly consumed or used as an ingredient in many pastries and desserts. Some of those foods are not exposed to sufficient heat treatment or may not be heat-treated at all. Also, the consumption of raw domestic fowl eggs is a common practice among many people. For example, raw eggs have been consumed alone or mixed with milk or other drinks as a blood-building food.

Eggs and their shells are remarkable natural packages, nevertheless egg deterioration may occur due to the penetration of different microorganisms to their contents; poor treatment of freshly laid eggs results in the entrance of bacteria to the inside egg through the shell that results in eggs spoilage if bacteria are with sufficient numbers. Therefore, proper eggs handling and storage maintains their quality for long time.

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In Egypt, the majority of groceries store eggs at room temperature and do not maintain them at 4°C, which may lead to enhancing the growth and multiplication of bacteria.

Several species of the genus *Pseudomonas* are very often recognized as the principle causative agents of the spoilage of fresh foods stored aerobically. *Psudomonas aeruoginosa* recognized as a human pathogen and constitutes potential hazards to both human and animal health [4, 5]. Multi-drug resistant *P.aeruginosa* are highly disruptive to the intestinal epithelial barrier and can cause severe septicemia in immunocompromised hosts [6].

The present work was planned to study the prevalence of *Pseudomonas* organisms and enumerate them in content and shell of Egyptian market eggs and to specify the best technique for recovery of *Pseudomonas* species from eggs. Also, using Colony PCR to verify *Psudomonas areuginosa* isolated from different types of eggs.

MATERIALS AND METHODS

Sample Collection and Preparation: One hundred composite samples (25 each) prepared from chicken table-egg (brown shell, white shell and Baladi eggs) and duck egg samples, each composite (6 eggs) collected from different shops, homes and supermarkets. Samples were immediately transferred in sterile plastic bags to the laboratory for examination. 150 ml sterile buffered peptone water were poured into the plastic bags containing egg sample and thoroughly mixed, 25 ml of rinse buffered peptone water transferred to another sterile jar containing 225 ml of Tryptone Soya Broth (TSB) and mixed well. Egg shells were sterilized using ethyl alcohol 70% then were broken using a sterile blade then the contents transferred to a sterile beaker. Contents from 6 samples were pooled to form one sample. Transfer 25 ml of egg contents into sterile jar containing 225 ml of TSB, then homogenized for 30 s.

Total *Pseudomonas* organisms Count: Bacteria were counted as described by Roberts *et al.* [7]. Briefly, homogenized egg contents or egg shells were serially diluted aseptically in 0.1% peptone water up to 10^{-6}. Bacterial counts were determined by using surface method technique of enumeration on cetrimide agar containing glycerol (10 ml /L). The plates were incubated at 25-30°C /48 h followed by counting. The enrichment method was also used by culturing bacteria in TSB overnight at 25°C. A loopful of culture was then spread onto cetrimide agar containing glycerol (10 ml/L) and incubated at 25°C/ 48 h.

Isolation and Identification of Isolated Strains Using Conventional Method: On cetrimide agar plates containing Glycerol (10 ml/L), five presumptive *Pseudomonas* colonies show blue-green or fluorescence surrounding the colonies were picked from each selective agar plate and identified microscopically after Gram staining and biochemically according to Forbes *et al.* [8].

Detection of *Pseudomonas areuginosa* Using Colony PCR Method: Isolates were previously identified by conventional method as *P. aeruginosa* were subjected to colony PCR method. A single colony was used to grow on LB broth medium 37°C overnight. Variable amounts of culture (50-100 µl) were spread on cetrimide agar plates. The plates were incubated at 37°C for 16-18 hours. Subsequently, 2-4 colonies from each plate were randomly selected and collected using a sterile toothpick. Colonies were suspended in 50 µl distilled water and incubated at 95°C for 5 minutes. Following centrifugation at 13,000 rpm for 1 minute, direct colony PCR of the supernatant was performed with the Dream Taq Green PCR Master Mix (Fermentas). *P. aeruginosa* was identified by amplification of the 16S rDNA gene using primers PA-SS-F (5´ GGGGGATCTTCGGACCTCA-3') and PA-SS-R (5'-TCCTTAGAGTGCCCACCCG-3'). PCR cycling conditions were 95°C for 5 minutes, 35 cycles at 94°C for 20 seconds, 58°C for 30 seconds and 72°C for 50 seconds with a final extension at 72°C for 7 minutes. PCR products were visualized using ethidium bromide stained 1.2% agarose gel electrophoresis.

Statistical Analysis: The SPSS software (IBM, Armonk, NY, USA) was used for statistical data analysis. The ANOVA test was used for comparing sample means after log transformation of data to increase sample homogeneity.

RESULTS AND DISCUSSION

Prevalence of *Pseudomonas* Species in Table Egg Samples: In the present study, *Pseudomonas species* were detected by conventional method in 28, 24, 8 and 64%, also, in 8, 44, 24 and 28% of content and shell of brown shell, white shell, Baladi and duck eggs respectively (Table 1). Counts of *Pseudomonas* species in egg contents were $5.8x10^2$, $1.8x10^2$, $2.7x10^3$ and $5.0x10^2 log^{10}$ cfu/g in brown shell, white shell, Baladi and duck eggs, respectively. Counts in egg shells were $2.8x10^2$, 4.3x10, $2.0x10^3$ and $1.7x10^3 log^{10}$ cfu/g in brown shell, white shell, Baladi and duck eggs, respectively. The presence of *Pseudomonas* species in table eggs were reported by several investigators [9-12].

Table 1: Prevalence and total viable count of *Pseudomonas* species in examined egg samples

		Positive samples		Counts (log^{10} cfu/g)		
Type of egg	Type of samples	No.	%	Min.	Max.	Mean± SE
Brown shell hen egg	Content	7	28	$1.0x10^2$	$1.4x10^4$	$5.8x10^2$±0.20x10 [a]
	Shell	2	8	$2.0x10^2$	$4.0x10^2$	$2.8x10^2$±0.14x10 [a]
White shell hen egg	Content	6	24	$1.0x10^2$	$4.0x10^2$	$1.8x10^2$±0.12x10 [a]
	Shell	11	44	$1.0x10^2$	$4.4x10^4$	$4.3x10^2$±0.18x10 [a]
Baladi hen egg	Content	2	8	$3.0x10^2$	$2.5x10^4$	$2.7x10^3$±0.91x10 [a]
	Shell	6	24	$3.0x10^2$	$3.2x10^4$	$2.0x10^3$±0.20x10 [a]
Duck egg	Content	16	64	$1.0x10^2$	$3.3x10^3$	$5.0x10^2$±0.13x10 [a]
	Shell	7	28	$1.0x10^2$	$1.5x10^4$	$1.7x10^3$±0.21x10 [a]

[a]: No significant difference between the groups at $p< 0.05$.

Table 2: Frequency distribution of isolated *Pseudomonas* species from different types of eggs

			P. fluorescens		*P. cepacia*		*P. putida*		*P. aeruginosa*	
Type of egg	Type of samples	No. of isolates	No.	%	No.	%	No.	%	No.	%
Brown shell hen egg	Content	26	14	53.8	9	34.6	1	3.8	2	7.7
	Shell	17	5	29.4	2	11.8	1	5.9	9	52.9
White shell hen egg	Content	30	10	33.3	6	20.0	1	3.3	13	43.3
	Shell	40	14	35.0	9	22.5	2	5.0	15	37.5
Baladi hen egg	Content	5	5	100	0	-	0	-	0	-
	Shell	17	4	23.5	1	5.9	1	5.9	11	64.7
Duck egg	Content	30	14	46.7	2	6.7	0	-	14	46.7
	Shell	20	10	50	0	-	0	-	10	50
Total		185	76	41.1	29	15.7	6	3.2	74	40

By using the enrichment technique, *Psudomonas* species indicating a high prevalence rate (Fig. 2). White shell eggs showed a higher recovery rate of *Pseudomonas* species in both content (36%) and shell (28%), while duck eggs and Baladi eggs showed the lowest recovery rate from contents (12%, 0%) and shell (12%, 16%), respectively. These results indicate that the commercially produced eggs, such as white shell hen eggs and brown shell hen eggs, have high recovery rates of *Pseudomonas* species. The higher recovery rate in commercial eggs may have resulted from the cleaning and washing processing associated with these eggs. The process may have decreased initial bacterial counts, but introduced bacteria that further grew when eggs were stored at in appropriate temperatures.

Pseudomonas species are ubiquitous in the environment. Pathogenic species are important as they are known to be resistant multiple antibiotics and are capable in surviving in conditions that few other organisms can tolerate. These species are reported to be associated with food spoilage [13]. In our study, *Pseudomonas* species were recovered from all sample sources. Our findings also reveal that the shells had higher numbers of bacteria than egg contents, suggesting poor sanitization and hygienic conditions in the farm.

Isolated *Pseudomonas* species from table eggs were identified as *P. fluorescens*, (41.1%), *P. cepacia* (15.7%), *P. putida* (3.2%) and *P. aeruginosa* (40%) (Table 2). Obi and Igbokwe [11] demonstrated that occurrence of *P. aeruginosa* in hen eggs increased from 87.50% at zero time to 100% at 14 days. Milakovic-Novak and Prukner [9] isolated *P. aeruginosa* in 25.83% of examined egg while Edema and Atayese [21] couldn't isolate *P. aeruginosa* from shell or content of uncracked egg Callewaret *et al.* [14] and Deckers *et al.* [15] approved that *P. aeruginosa* was able to grow in egg white and not affected by the inhibitory effect of lysozyme. *P. aeruginosa* is of clinical significance as an opportunistic pathogen. Other species are significant in food spoilage, particularly in chilled foods. Levels higher than 10^7 cfu/g of food may result in off-flavors, off-odors and visual defects. In humans, *P. aeruginosa* is the most common pathogen, but Crohn's disease may result from *P. putida*, *P. fluorescens* or *P. cepacia* [16]. *P. aeruginosa* produces

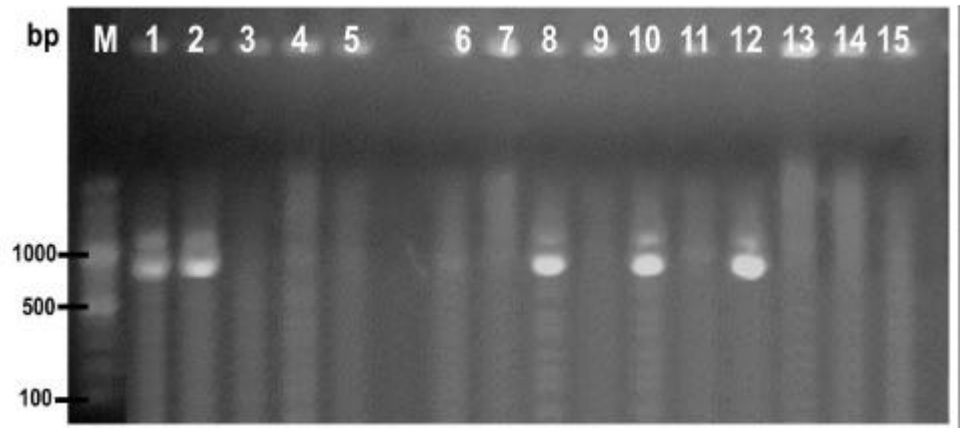

Fig. 1: Agarose gel electrophoresis of PCR reactions. Lanes; 1: Ladder, 2-14 samples, 15 C-ve

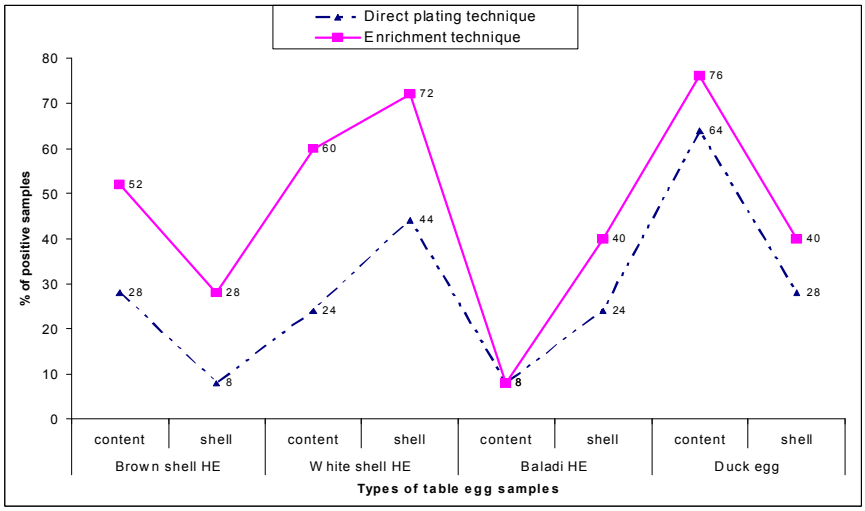

Fig. 2: Prevalence and recovery rate of *Pseudomonas* species using direct plating and enrichment technique. HE: hen eggs

a variety of pathogenicity factors to assist in its adhesion to and penetration of intestinal epithelial cells Zaborina *et al.* [17] and Madi *et al.* [18] have also investigated adhesion and cytotoxicity by *P. fluorescens* and compared its pathogenicity to the better studied opportunistic pathogen *P. aeruginosa*. Green rot in eggs arises from invasion of the shell contents by strains of *P. fluorescens* that multiply in the albumen and produce the characteristic green pigment. The early stages of rotting cannot be detected during routine candling by white light. The yolk is also invaded and eventually the entire contents break down into a semi-liquid mass with a characteristic putrid odor. Organisms on the egg shell surface are capable of penetrating the pores into the interior of the egg and contaminating the components. *P. aeruginosa* are motile Gram-negative bacteria and can more easily penetrate the egg components [19].

Handling, storage temperature and storage humidity are factors that may increase bacterial infection in eggs, causing contamination and spoilage. In many cases, eggs are held at room temperature (25°C) until they are transported to retail stores, where may be further stored under non-refrigerated conditions. It was reported that low temperature and humidity are important factors for the survival of *Pseudomonas* species on the egg shell [20].

Detection of *P. aeruginosa* Using Colony PCR Method: *P. aeruginosa* threaten human health results in opportunistic infections and what worsen the situation, P. aeruginosa recorded a high rate of resistance against most common antibacterial agents. We tried to measure accurate prevalence rates in different types of table eggs sold in Mansoura city. In our study, colony PCR was used for detection of 16S rDNA gene specific for

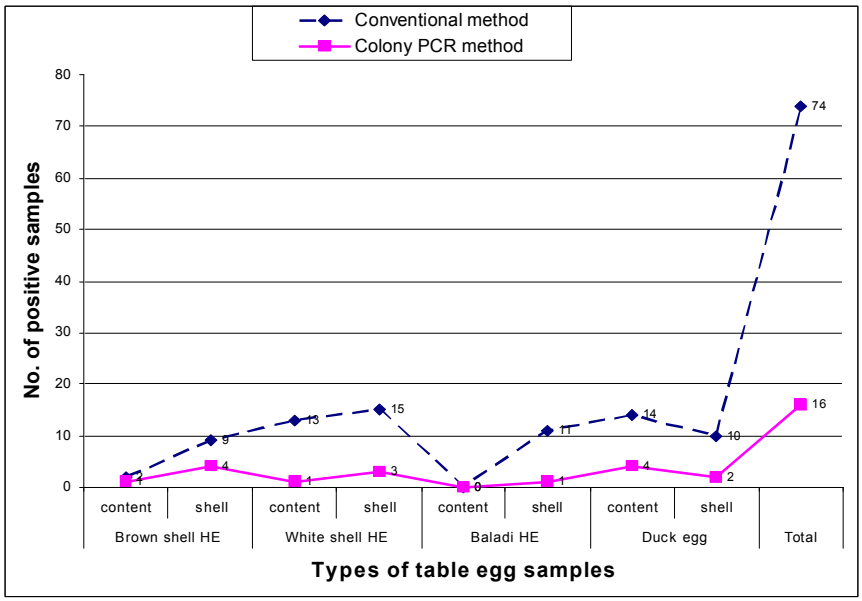

Fig. 3: Frequency distribution of *P. aeruginosa* by conventional method and colony PCR methods HE: hen eggs

P. aeruginosa indicated that only 16 (8.6%) of *P. aeruginosa* strains identified by conventional method were positive for such gene (Fig. 3). The amplified gene was detected at the expected molecular size of 618 bp (Fig. 1). Therefore, colony PCR method showed high accuracy for detection of *P. aeruginosa* in table egg samples than conventional one, as it ignored all the false positive results obtained by conventional one, suggesting that the test is a more efficient tool for the quality control of egg production.

We strongly recommended that the government set quality control standards in the storage conditions of market eggs. Furthermore, cross-contamination of freshly laid sterile eggs by contaminated poultry feeds and wash-water may be a factor in increase eggs contamination. Therefore, eggs handling and processing must be monitored and the practice of using water for washing eggs must be stopped entirely in order to prevent microbial migration into the eggs and subsequent spoilages. As with other foods from animal origin, eggs shouldn't be consumed raw and must be heat treated or processed before human consumption.

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