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CONTAMINATION OF TABLE EGG SHELL WITH COLIFORM BACTERIA IN MOSUL CITY MARKETS

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ABSTRACT

The study was designed to assess the hygienic condition of local and imported table egg-shell at Mosul markets. Two hundred local and imported eggs were randomly collected from the markets and transferred immediately to the scientific research unit in the college of Veterinary medicine / University of Mosul for bacteriological examination. Total counts using four types of medias were used: Standard Plate Count ager (SPC), Mac Conkey ager, Violet Red Bile Glucose ager (VRBG) and Tryptone Bile X-glucoronide (TBX) for *Escherichia coli* and fecal coliform bacteria. Results showed that the means of total viable coliform and *E. coli* bacterial count in the local egg-shell at different markets ranged from 0.3 to 3 $x10^7$ on SPC agar, 0.08-$10x10^7$ on Mac Conkey ager, 0.014-$3x10^5$ on (VRBG) and 0.3-$7x10^4$ on (TBX) compared with 0.032-$5x10^7$ on SPC agar, 0.03-$3.3x10^5$ on Mac Conkey ager, 0.014-$5.9x10^4$ on (VRBG) with no growth on (TBX) for coliform and *E. coli* in the imported table-egg shell at different markets. Biochemical tests for local and imported table egg-shell were performed. It was concluded that the rate of contamination in the local table eggs was more than the imported one and the isolates of *E. coli* bacteria were obtained only from local eggs on the TBX medium.

INTRODUCTION

Table egg production are regarded as a significant economic source in several countries of the world. However, it is estimated that annual global production of table egg is about 69 millions in the year of 2010 which exceeds its production percentage by 2.5% from the year 2000. Interestingly, China is considered as the most table egg producing country in the world, producing more than 28 million tons of egg per year,

followed by USA which produces 5.4 million tons, then India which produces 3.4 million tons and finally Japan which produces 2.5 million tons. Other important countries with regard to table egg production include Mexico, Brazil and Russia, which produces more than two million tons per year (1).

Concerning Iraq, table egg production has reached 46 thousand tons in the year 2010 with 8.1% decrease as compared to the year 2000. Annual egg production in the neighboring countries surrounding Iraq i.e. Turkey, Iran, Saudi Arabia, Jordan and Kuwait was 741, 740, 193, 163, 69 and 40 thousand tons, respectively (1).

Owing to the certain characters and traits of the egg, it is rapidly prone to perish and spoil, partially due to factors related to the ambient environment and its condition surrounding the eggs. However, fresh eggs lose its quality with lowering grades as well as shelf life alongside with prolonged storage (2). Keeping and storing of such eggs for long periods may alter the physical and chemical and hygienic qualities due to its exposure to droughts, high temperature, unpleasant odors and other contaminants (3).

Basically, the egg is affected through its formation in the ovary pre and post laying by several agents i.e. management, husbandry, feeding, the health status of the layers, practice of egg harvestmen, egg package and transportation, condition of storing and keeping quality, handling, manipulation, marketing and finally the type of its offering to the consumer (4). Shell contamination of the egg has received great care and attention of many workers which usually occurs by either pathogenic or non-pathogenic bacteria existed on the shell with subsequent invasion into the inner parts of the egg. The critical sources of such harmful bacteria is the litter and droppings of the layer itself (5).

Hence, such microorganisms invading egg content may cause food poisoning and spoilage inside the concerned egg causing serious health risk and hazards to the consumer (2,4,6).

Bacteria of Enterobacteriacae family are one of the pathogenic microorganisms which is the target of this study. So, the aim of the study is to elucidate the possible role of these bacteria causing food spoilage. The parameter of assessment includes total viable bacteria count as well as an attempt to isolate coliform and *Escherichia coli* present on the local and imported table egg-shell in the different Mosul city markets.

MATERIALS AND METHODS

Two hundred of local and imported table eggs were randomly collected from Mosul markets during the period from 1st till 20th March 2014. The collection involved 10 eggs from each region which were put in aseptic sterile packs. Bags were transferred to scientific research laboratory/ department of Veterinary Public health, College of Veterinary Medicine/ University of Mosul for bacteriological examination . Each egg sample was placed in sterile bag, then they were further washed with Ringer's solution in 100 ml glass beaker. Serial decimal dilution of the aliquots was performed from 10^2 to 10^7. Then 0.1 ml of the aliquots was transferred to four types of media; standard plate count ager (SPC), Mac Conkey ager, violet red bile glucose ager (VRBG) and finally using Tryptone bile X glucoronide (TBX), in duplicates. Media were incubated for 24 h. at $37c^0$. Dilutions show 30 to 300 colonies were counted for calculation $CFUml^{-1}$ of coliform and *E-coli* bacteria (7).

Biochemical test for the positive results isolates of both local and imported eggs were examined using Simmon's citrate, Motility, Gas formation and indole tests as described by (8).

RESULTS

The results of the study revealed bacterial contamination of the local egg-shell ranged from 0.3 to 3 $x10^7$ $CFUml^{-1}$ on SPC agar, 0.08-$10x10^7$ $CFUml^{-1}$ on MacConkey ager, 0.014-$3x10^5$ $CFUml^{-1}$ on (VRBG) and 0.3-$7x10^4$ $CFUml^{-1}$ on (TBX), comparable with 0.032-$5x10^7$ $CFUml^{-1}$ on SPC agar,0.03-$3.3x10^5$ $CFUml^{-1}$ on MacConkey ager and 0.014-$5.9x10^4$ $CFUml^{-1}$ on (VRBG) while no growth on (TBX) was detected for quantitative test and on and VRBG for biochemical tests as shown in table 1and 2.

Table (1): Means of total viable bacteria, coliform and *E. coli* counts in the local egg-shell in different markets.

Region	CFU/ml (SPC)	CFU/ml (Mac Conkey)	CFU/ml (VRBG)	CFU/ml (TBX)
1	$3.2x10^7$	$0.67 x10^5$	-	-
2	$0.7x10^7$	$8.7 x10^5$	$5.9x10^5$	$3 x10^4$
3	$0.87x10^7$	$7.3 x10^5$	-	-
4	$1.07x10^7$	$0.081 x10^5$	-	-
5	$0.36x10^7$	$0.71 x10^5$	-	-
6	$0.3x10^7$	$0.62 x10^5$	$0.034 x10^5$	$0.3x10^4$
7	$0.3x10^7$	$9.1 x10^5$	$0.3 x10^5$	$3.3 x10^4$
8	$0.46x10^7$	$1.0 x10^5$	$0.3 x10^5$	-
9	$0.42x10^7$	$0.95 x10^5$	$0.3 x10^5$	$0.3x 10^4$
10	$0.41x10^7$	$10.5 x10^5$	$0.014 x10^5$	$7 x10^4$

Table (2): Means of total viable bacteria, coliform and *E. coli* count in the imported table egg-shell in different markets.

Region	CFU/ml (SPC)	CFU/ml (Mac Conkey)	CFU/ml (VRBG)	CFU/ml (TBX)
1	$0.43x10^7$	-	$0.22x10^6$	-
2	$5x10^7$	$0.3x10^5$	-	-
3	$3x10^7$	$0.33x10^5$	-	-
4	$1.45x10^7$	$0.03x10^5$	-	-
5	$0.133x10^7$	$3.3x10^5$	-	-
6	$1.22x10^7$	-	-	-
7	$1.41x10^7$	-	-	-
8	$0.032x10^7$	-	-	-
9	$0.5x10^7$	-	$3 x10^6$	-
10	$0.081x10^7$	-	$0.35 x10^6$	-

Table (3), demonstrates the results of biochemical tests of *E. coli*, Klebsiella and non-Klebsiella isolates contaminated shells of local eggs.

Table (3): The results of biochemical tests of the cultured bacterial isolates of local table egg-shell.

genus	SIM			Simmon's citrate	No. of sample	No.of isolate	Total
	Motility	Indol	H_2S				
E-coli	+	+	-	-	6	12	10
Klebsiella	-	-	-	+	6	12	2
Non-Klebsiella	-	-	-	+	6	12	0

Also, table 4 states the biochemical tests of isolates of *E. coli*, Klebsiella and non-Klebsiella bacteria of the imported table egg shells cultured on different media.

Table (4): The results of biochemical tests of the cultured bacterial isolates of the imported table egg-shell.

genus	SIM			Simmon's citrate	No. of sample	No.of isolate	Total
	Motility	Indol	H_2S				
E-coli	+	+	-	-	10	20	0
Klebsiella	-	-	-	+	10	20	8
Non-Klebsiella	-	-	-	+	10	20	12

DISCUSSION

Due to the increased transportation and movement of both peoples and agricultural products among countries, foods began to be produced in countries to be consumed in others. This process witnesses acceleration and expansion in commercial relations among countries (3). However, table eggs represent one of the important staple with subsequent increase in the trade of such food due to proportional increase in human population. The frequent and urgent demand on the egg has led to increase their production reaching about 2 million egg / day (1).

Other factors contributing the increase of egg production is the improvement of modern mechanical gathering and conveying of eggs as well as improvement of washing, cleansing, grading, packaging and other operations which will lead to notable improved view of the consumer (9).

Owing to these processes, the eggs can be exposed to different bacterial contamination through different stages of processing causing a significant decrease in quality and shelf-life and storage of the eggs as well as occurrence of food spoilage(2) .

The increased probability for further contamination of the eggs can cause putrefaction and spoilage when the eggs are stored for prolonged period to avoid such

adverse effect on the stored eggs, many countries enacts and legislate laws and directions to flush the raw eggs once in order to supply intact and clean eggs. This practice is aimed to supply safe eggs to the consumer (10).

Flushing of eggs is carried out using water containing diluted concentrations of antiseptic and disinfectants with rapid drying of the eggs. Although this practice is commonly followed by several obstacles impede the provision of sale eggs to consumers. Hence, such obstacles are contributing in the existence of coliform bacteria with further growth and multiplication on egg-shell which was recorded in the study were in accordance with (4,11,12).

The study focused on the concentration and their counts of table egg-shell (local and imported) with bacteria of Entrobacteriacea and the findings revealed high counts of bacterial contamination in the local table egg-shells as compared with imported table eggs concerning both qualitative and quantitative assessment. As mentioned earlier, bacterial contamination is present in local table egg, but absent in imported table eggs as shown in table 1 and 2. According to the findings shown in tables 1 and 2, the numbers of growing bacterial colonies cultured on VRBG media one more than those growing on MacConkey agar for both types of table eggs (local and imported). The excessive growth of bacteria on VRBG agar can be described to the fact that this medium is selective for coliform entero-bacteria (13). Identification and description of these bacteria were preliminary fulfilled as shown in tables 3 and 4, based on the results of biochemical test as mentioned by (14), The differences in the bacterial counts between local and imported table eggs may be due to several factors such as system of rearing and type of handling the eggs pas laying. It is known that egg laying is taking place on the litter or on the nests in the local rearing of the hens and layers. On the contrary, layers of imported eggs one often reared by the modern technology (batteries), hence egg gathering and harvesting is taking place mechanically with great decrease of the change of bacterial contaminating as compared with those of bedding, litter or nests (12).

The numbers and counts of the obtained entero-bacteria in this work exceed those recorded as permissible and the maximum acceptable limit for enterobacteriaecae counts present on the egg-shell according to the European specification is 10^2 CFU/gm (15).

The excessive counts in the numbers of coliform entero-bacteria may be in part related to unhygienic conditions in local poultry forms. However, techniques and methods followed for identification and diagnosis of coliform entero-bacteria play on important role in reflecting the real total bacterial counts for both domestic and imported eggs. Application of different techniques used for culturing and identification of bacteria and their subsequent variations in the results is the subject of several studies. However, the recent techniques used for identification of bacteria is the application of molecular biology mentioned that the upper total count of bacteria is 10^2 CFU/gm. This figure is less than those obtained in the cement study (16).

Undoubtedly, relative humidity of the air has great role in the growth and multiplication of microorganisms existing on the surface of egg-shell. The moisture is related to several factors such as degree of nest cleanliness, frequency of egg gathering and finally with the period and type of egg storing in the poultry form (16,17).

Contamination of egg-shell is proportionally related to the bacterial load of air and environment of layer forms and such condition may lead to microbial contamination since the ambient environment is regarded as a crucial agent, which increases bacterial counts. The results of the study ascertained these findings mentioned by several researchers who are in agreement with (12).

The high level of egg contamination of external environment source greatly affect the suitability of eggs and its nutritional value (18).

The microorganism in the bacterial counts present on egg surface with frequent increase can cause spoilage of the egg due to their increase inside the egg content (19).

This alteration in egg quality may lead to lessen the nutritional value and hygienic quality of the eggs with serious reflection on consumer's health (20).

CONCLUSIONS

Existence of bacterial contamination of the local and imported eggshell. The rate of contamination in local table egg exceeded the rate of imported table egg

contamination, and isolates of *E. coli* bacteria were obtained only from local eggs on the TBX medium.

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