original article

Microbial quality and prevalence of *Salmonella* and *Listeria* in eggs

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ABSTRACT

Aims: This study was undertaken to determine the microbial quality and the prevalence of *Salmonella* and *Listeria* in table eggs in Isfahan, Iran.
Materials and Methods: A total of 525 samples were randomly collected from various shops in Isfahan, Iran. Microbial quality of eggs evaluated by coliform count and total bacterial viable counts. Also, detection of *Listeria* and *Salmonella* in egg contents and on eggs shells was performed.
Results: The mean of total viable bacteria and coliform counts in the egg contents were 3.95×10^4 CFU/g and 4.94×10^3 CFU/g, respectively. *Salmonella* and *Listeria* were not found on the shell or content of eggs. Enterobacteriaceae families were found in 357 of 525 (68.28%) and 276 of 525 (52.44%) of egg shell and egg content samples, respectively. Moreover, *Pseudomonas aeruginosa* was isolated from 175 (33.41%) and 144 (25.37%) of egg shell and egg content, respectively. The isolated Enterobacteriaceae were included: *Enterobacter aerogenes*, *Escherichia coli*, *Klebsiella pneumoniae*, *Buttiauxella agrestis*, *Cedecea lapagei*, *Cedecea davisae* and *Erwinia herbicola*.
Conclusion: The findings of the present study indicate although *Salmonella* and *Listeria* were not found in egg samples; however, there is an urgent need to improve the hygienic level of consumed eggs.

Key words: Coliform, egg, enterobacteriaceae, *listeria*, *salmonella*

INTRODUCTION

Eggs that constitute several dishes or foods consumed and are considered as good quality source of protein, have served as vehicles for numerous enteropathogens microorganisms.[1,2] The well-known enteric pathogens particularly *Salmonella*, *Escherichia coli*, *Campylobacter* spp. and *Listeria* spp. have been isolated from table eggs and their contents.[3-5] In addition, most of these bacteria have been responsible for numerous egg-borne epidemics globally.[6-9] Other members of the family of Enterobacteriaceae such as *Pseudomonas* spp., *Citrobacter* spp., *Alcaligenes* spp., and *Klebsiella* spp. have all been isolated from whole or cracked eggs with a potential to cause spoilage and enter the food chain through table eggs causing infection in consumers.[2,10-13]

Salmonellosis constitutes a major public health burden and represents a significant cost in many countries. *Salmonella* and in particular *Salmonella enteritidis* outbreaks in humans are very often linked to the consumption of contaminated eggs or food containing eggs.[14-16] The bacterium infects the

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eggs by either vertical transmission during development of the egg within the ovary or horizontal transmission through trans-shell contamination.[15,17-19] *Listeria monocytogenes* considered an important foodborne pathogen causes severe disease with high mortality in human.[20] The presence of *L. monocytogenes* has been reported from a wide variety of foods including eggs.[21,22] Also, many reports clearly show that eggs are a suitable substrate for the growth of *L. monocytogenes*.[23] The presence of *Listeria* and *Salmonella* in other foods in Iran has been reported. However, there are no data on occurrences of both pathogens in eggs.[24,25] There are also limited data available on microbial quality of table eggs in Iran. Therefore, the objective of this study was to determine the microbial quality and also the occurrence of *Listeria* and *Salmonella* in consumption eggs in Isfahan, Iran.

MATERIALS AND METHODS

Eggs samples

Overall, a total of 525 chicken eggs samples were randomly collected from various super markets in Isfahan, Iran over a period of one year (July 2009–July 2010). Each sample assessed for the total bacterial viable count and coliform count by pour plate method. Also, detection of *Listeria* and *Salmonella* were performed.

Swab sampling of eggs

A sterile swab soaked in saline was applied to the surface of each egg shell and swabs were then dipped into 10 mL of saline. The contents of the saline were mixed thoroughly using a vortex mixer and subsequently inoculated into appropriate bacteriological media for detection of *Salmonella* and *Listeria*.

Sampling of egg contents

Each egg sample was dipped in 90% ethanol for 5 min after which the pointed end of egg was famed for 5–10 s with a Bunsen burner. A sterile scalpel blade was used to make a small hole on the shell through which the contents were transferred into a stomacher bag. The egg contents (yolk and albumen) in each pool were then blended for 30 s after which the mixture was used to inoculate appropriate bacteriological media.

Salmonella detection

Samples were examined for the presence of Salmonella by the Iranian National Standards method No. 1810 recommended by the Institute of Standards and Industrial Research of Iran (ISIRI) for the isolation of Salmonella. Each egg's content was mixed thoroughly and then 25 mL of egg contents or one ml of saline containing swabs were added to 225 and 25 mL of Buffered Peptone Water (BPW, Merck, Germany) respectively and incubated at 37°C overnight as pre-enrichment. One milliliter of the cultures were transferred to 9 mL of selenite cystine broth (Merck; Germany) and incubated at 37°C for 24 h for selective enrichment. The cultures were then streaked onto xylose lysine deoxycholate (XLD, Merck; Germany) agar and incubated at 37°C for 24–48 h. The plates were observed for typical Salmonella-like colonies.[26]

Listeria detection

Samples were analyzed for the presence of *Listeria* spp. using selective enrichment and isolation protocol, recommended by United States Department of Agriculture (USDA).[27] Each egg's content was mixed thoroughly and then 25 mL of egg contents or one ml of saline containing swabs were added to 225 and 25 mL of University of Vermont Media (UVM) I respectively and incubated at 37°C overnight as pre-enrichment. Then, 1 mL of the cultures were transferred to 9 mL of UVM II (Fraser broth) and incubated at 37°C for 24–48 h for selective enrichment. The cultures were then streaked onto PALCAM (Merck; Germany) agar and supplemented with PALCAM Selective Supplement (HC784958 Merck; Germany) and incubated at 37°C for 24–48 h. The plates were observed for typical *Listeria*-like colonies.

Total bacterial viable and coliform count

Total bacterial viable count (TVC) and total coliform count (TCC) was performed using pour plate method. The egg samples were diluted by tenfold serial dilution in 0.1% sterile buffered peptone water (Oxoid; UK). From each dilution, one ml aliquot was added to Nutrient Agar (Merck; Germany) and Violet Red Bile Agar (VRBA, Merck; Germany) for TVC and TCC respectively. All plates were then incubated at 37°C for 72 h. Colonies were counted using a plate counter and the results expressed as CFU/mL of egg samples. The VRBA plates were examined for typical coliform colonies (violet colonies) and colorless Lactose-negative enterobacteria colonies. Then, at least five suspected colonies were subcultured on Trypton Soy Agar and incubated at 37°C for 24 h. All isolates were subjected to standard biochemical tests such as Gram staining, catalase test, Oxidase test, SIM test, TSI test, OF test, acid production from glucose, manitol, rhamnose, xylose, and MR/VP test. For further confirmations of *Psedumonas auroginosa* colonies other biochemical reactions, nitrate reduction, and growth on Cetrimide agar were performed according to the Bergey's Manual of Systematic Bacteriology.

RESULTS

As shown in Table 1, 213 (40.6%) of samples had a TVC of 0–10 CFU/g and TVC of 10^6–10^7 CFU/g found only in 1 (0.2%) of egg samples. TVC of 10, 10–10^2, 10^2–10^3, 10^3–10^4, 10^4–10^5, 10^5–10^6, and 10^6–10^7 CFU/g were in 213 (40.6%), 15 (2.9%), 27 (5.1%), 108 (20.6%), 116 (22.1%), 45 (8.6%), and 1(0.2%) of samples, respectively. Also in Table 1, the distribution of total coliform count (TCC) of egg samples is shown. TCC of 10, 10–10^2 , 10^2–10^3, 10^3–10^4, 10^4–10^5, 10^5–10^6 and 10^6–10^7 CFU/g were in 447 (85.14%), 24 (4.57%),

33 (6.29%), 12 (2.29%), 7 (1.33%), 0(0%), and 2(0.38%) of samples, respectively.

The prevalence of bacterial species isolated from the shell and content of consumed eggs are shown in Table 2. *Enterobacter erogenes*, *E. coli*, *Klebsiella pneumoniae*, *Buttiauxella agrestis*, *Cedecea lapagei*, *Cedecea davisae*, *Erwinia herbicola* and *Psedumonas aeruginosa* were the most common isolated species [Table 2]. *Pseudomonas* was the only non- Enterobacteriaceae member identified. In this research *P. aeruginosa* was the most frequently isolated species on egg shells and contents. This bacterium isolated from 175 (33.41%) and 144 (27.32%) of egg shells and contents, respectively.

DISCUSSION

The mean total viable bacteria and coliform counts of tested eggs were 4.31×10^4 and 1.46×10^3 CFU/g, respectively. 163 (30.9%) of egg samples had TVC more than FDA standard. These numbers are more than the FDA total and coliform standard count of eggs that is 5×10^3 CFU/g for total count and 1 CFU/g for coliform count.[28] Since the TCC of more than 1 CFU/g is unacceptable, 79 (15%) of the samples are considered above the standard limits. The presence of coliform indicates the possibility of fecal contamination. Coliform has been used as an indicator microorganism to determine the microbial quality and safety of the food. They are defined as rod-shaped Gram-negative nonspore forming bacteria that universally present in large numbers in the faeces of warm-blooded animals. While coliforms are themselves not normally causes of serious illness, they are easy to culture and their presence is used to indicate that other pathogenic organisms of faecal origin may be present.

The presence and population of bacteria on shell and content eggs is an important factor for evaluating the efficacy of washing and packaging as well as the quality and safety of the final product. Enterobacteriaceae, a bacterial family that includes the important human pathogens of *Salmonella* and *Shigella*, are known to contaminate the shell egg processing environment. High levels of these bacteria in the processing plant can signal inadequate sanitation. However, little is known about the number of genera and species that contribute to contamination of eggs.[29,30]

Recently Michael Musgrove and Deana Jones (2009) found 100% prevalence for Enterobacteriaceae on nests run carts at one plant and 80% at the other. They were also reported the presence of *Escherichia*, *Enterobacter*, *Klebsiella*, *Salmonella*, and *Pseudomonas*. Knowing bacterial species are vital pieces of information in developing strategies to reduce and remove bacterial contamination. This work demonstrated that nest run egg carts serve as reservoirs for Enterobacteriaceae in the shell egg processing environment. These findings helped egg industry and regulators agents to encourage development of better sanitation procedures or the use of more easily cleaned shelving materials.

P. aeruginosa is an opportunistic, nosocomial human pathogen of immunocompromised individuals and typically infects the pulmonary tract, urinary tract, burns, wounds, and also causes other blood infections in humans.[31] In addition, bacteria of the genus *Pseudomonas* have been shown to more readily penetrate into whole eggs of poor shell quality.[32] Not surprising, at the present study, *P. aeruginosa* were prevalent on both egg content and egg shell. Since the *Pseudomonas* spoilage is the most frequent spoilage in stored eggs, this can raise a concern on safety and quality of eggs in Iran. Different researchers reported the role of penetration of bacteria through the egg shell with associated membranes and on whole egg contamination. In our research, as expected, contamination of egg shell is higher than egg content. This finding indicated the shorter shelf life for eggs especially in poor storage condition.

Musgrove and Jones (2009) reported *Escherichia* and *Enterobacter* were present most often compared to other member of Enterobacteriaceae in swab samples from the cart shelves. Furthermore, Adesiyun *et al*. (2006) reported 71 (38.6%) of 184 composite eggs (shells, yolk/albumen or both) samples were positive for enteric microbes, other than *E. coli*, *Salmonella*, *Campylobacter* spp., and *Listeria* spp. *Enterobacter* spp. and *Klebsiella* spp. were isolated from 15 (8.2%) and 14 (7.6%), respectively, of pooled egg shells alone and from 6 (3.3%) and 3 (1.6%), respectively, of egg content samples alone.[33] In this work, *Enterobacter aeruginosa*, *E. coli*, and *Klebsiella pneumoniae* were also found in 132 (25.12%), 32 (6.1%), and 13 (2.44%) of swab samples, respectively and from 123 (23.41%), 9 (1.71%) and none of egg contents, respectively [Table 2]. Chousalkar *et al*. (2010) studied the prevalence of *Salmonella* and *E. coli* from the surface of egg shells, egg shell membranes or pores, and internal contents from unwashed eggs collected from commercial caged layer farms in Australia.[34] Similar to our findings *Salmonella* spp. was not detected either in internal egg contents or egg shell surface. Seven percent *E. coli* were isolated from the egg shell surface. Two percent *E. coli* strains

Table 1: Distribution of total bacterial viable count (TVC) and total coliform count (TCC) of egg samples

Total counts	CFU/g						
	>10	10–10^2	10^2–10^3	10^3–10^4	10^4–10^5	10^5–10^6	10^6–10^7
TVC (%)	40.6	2.9	5.1	20.6	22.1	8.6	0.2
TVC (No)	213	15	27	108	116	45	1
TCC (%)	85.14	4.57	6.29	2.29	1.33	0	0.38
TCC (No)	447	24	33	12	7	0	2

Table 2: The prevalence of bacterial species from the shell and content of consumed eggs

Isolated bacteria	Shell (No)	Shell (%)	Content (No)	Content (%)
Enterobacter aerogenes	132	25.12	123	23.41
Escherichia coli	32	6.1	9	1.71
Klebsiella pneumoniae	13	2.44	0	0
Cedecea lapagei	1	0.24	0	0
Cedecea davisae	1	0.24	0	0
Buttiauxella agrestis	1	0.24	0	0
Erwinia herbicola	2	0.49	0	0
Psedumonas auroginosa	175	33.41	144	27.32
Total	357	68.28	276	52.44

were also isolated from shell crush. However, unlike our study, the internal contents of eggs appeared to be sterile.

The results of this work are confirmed contamination of Isfahan egg samples, while there were not found *Salmonella* spp. Salmonellosis is one of the most common causes of foodborne diarrheal disease worldwide and remains a major public health problem in many parts of the world.[35] *Salmonella enterica* subspecies *enterica* serovar *enteritidis* (*S. enteritidis*) is the world-leading cause of salmonellosis and is often implicated in over 60% of cases of human salmonellosis in Europe.[36] The majority of human cases are caused by only a few nontyphoidal serovars. For instance, approximately 60% of human cases reported to the CDC in 1995 were caused by four serovars, including *Salmonella enteritidis* (24.7%), *S. typhimurium* (23.5%), *S. newport* (6.2%), and *S. heidelberg* (5.1%).[37] In spite of the fact that food poisoning has often been found after consuming eggs in different parts of the world, little research has been done on quantitative determination of *Salmonella* in naturally contaminated eggs so far. Investigations have showed that the concentration of *Salmonella* spp. in most cases is very low, from one to ten cells in one egg despite keeping the eggs at the room temperature for 7 days.[38]

Based on investigation of 525 eggs purchased from Isfahan shops, *Salmonella* spp. was not found on the shell or inside the eggs. The findings give the impression that eggs are relatively rarely contaminated by *Salmonella* spp. on the surface. Research carried out in USA and Great Britain showed a very low level of egg contamination even when the eggs came from flocks infected with *Salmonella* spp. In contrast to our results, the Public Health Laboratory Service in Great Britain has investigated eggs coming from farms whose eggs had earlier caused food poisoning and demonstrated that approximately 0.1% of contents were positive for *Salmonella*.[38] Radkowski (1990) examined 700 eggs from Poland, and in 400 eggs coming from state farms, *Salmonella* spp. was found on the shell of only one egg (0.25%) and in 300 eggs coming from individual farms it was found on the shells of three eggs (1%).[39]

Listeria monocytogenes has been recognized as a human pathogen for decades and is known to be an important foodborne pathogen. There have been no documented foodborne *L. monocytogenes* illnesses due to the consumption of eggs or egg products, even though the bacterium has been isolated from faeces, body fluid, and oviducts of asymptomatic laying hens.[22] In contrast to this study, Rivoal *et al*. 2010, was detected *L. monocytogenes* in 25 of the 144 raw egg samples collected in France. In addition they proposed contamination of raw egg products appeared to be season dependant and was higher during summer and winter than during autumn. In Japan, Miho *et al*. (2009) was also isolated *L. monocytogenes* from 2 of 487 (0.4%) unpasteurized liquid whole egg samples in 1993 and 1994, and 2 of 316 (0.6%) unpasteurized liquid whole egg samples. Sayed *et al*. (2009) in Egypt in contrast to present study reported that egg shells were contaminated by *Listeria* up to7% while similar to our finding, none of egg contents were contaminated, concluding that egg shell was more subjected to contamination with *L. monocytogenes* than egg content.[40]

The absence of *Listeria* and *Salmonella* in eggs investigated in this study is in agreement with the finding of Ghasemian Safaei *et al*. (2011) who recently reported no contamination of *Salmonella* spp. and *Listeria* spp. in 100 eggs.[41] In spite of the fact that we have reported *Listeria* spp. and *Salmonella* spp. contamination of various foods in the same region it is not clear that why eggs are not contaminated by *Listeria* spp. and *Salmonella* spp.[24,25]

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