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Research Article
Bacterial Contamination of Table Eggs Sold in Jordanian Markets

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Abstract

Background and Objective: Microbial contamination of table eggs has important consequences to the poultry industry and illness from contaminated eggs is a serious worldwide public health problem. Contaminated eggs and their products increase the risk of illness in humans. The significance of these illnesses can vary from mild symptoms to life-threatening conditions. The purpose of this study was to investigate the foodborne pathogen contamination of table eggs sold in Jordanian markets. **Materials and Methods:** One hundred eggs were randomly purchased from packed eggs available in the markets, including free-range home eggs, eggs farmed just after cleaning and just before cleaning and delivered to the microbiology lab. The collected swabs were cultured on suitable media and standard microbiological tests were performed to identify the isolated organism. **Results:** The following bacterial species were isolated from egg shell surfaces: *Staphylococcus*, *Streptococcus* spp., *Pseudomonas* spp., *Proteus* spp., *Klebsiella* spp., *Escherichia coli*, *Bacillus* spp., *Listeria monocytogenes* and *Salmonella* spp. **Conclusion:** Although serious human pathogens were not prevalent in this study, effective control measurements should nevertheless be applied to prevent human illness.

Key words: Egg, *Escherichia coli*, *Campylobacter jejuni*, *Listeria monocytogenes*, *Salmonella* spp, food safety

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Egg shell quality is necessary for economical viability of the worldwide egg industry[1]. Poor egg shell quality can result in food poisoning. The majority of foodborne outbreaks is caused by microorganisms that have the capacity to reproduce in food. Foodborne disease is a worldwide public health concern that can lead to chronic illness, increased medical care costs, investigations, loss of personal productivity and even death.

Campylobacter and most *Salmonella* serovars are adapted animal pathogens that do not cause illness in animals but can cause harm when transferred to humans through eggs and meat. *Campylobacter* can contaminate eggs if manure from the hens comes into contact with eggs[2]. In the USA, *Campylobacter jejuni* is the most common cause of food-borne infections[3]. *Salmonella enteritidis* is a common strain that can contaminate eggs either by contact with the manure or by infecting the egg as it passes down the oviduct. An infection with *Salmonella* can cause diarrhoea, blood infection and typhoid fever. If typhoid fever is untreated, mortality in humans can reach 15%[3]. Another bacterium infecting food through contact with manure is *Escherichia coli*. This bacterium is found in the normal gut flora in humans and animals. However, there are some strains such as EHEC (0157:H7) which are pathogenic for humans[2].

Microbial contamination of eggs has important consequences to the poultry industry and illness from contaminated eggs is a serious worldwide public health problem. The significance of these illnesses in humans can vary from mild symptoms to life-threatening infections[4].

Today, eggs remain a staple food of the human diet, consumed by people throughout the world. They are very nutritious and consumed worldwide in various dishes as a cheap source of protein[5]. Although, eggs are considered as complete food source for growth and sustenance, studies indicated that microorganisms often contaminate eggs[6].

Freshly laid eggs are generally devoid of organisms. However, following exposure to environmental conditions (for example, soil, dust and dirty nesting materials), eggs become contaminated with different types of microorganisms[7]. Furthermore, these microorganisms may contaminate the egg contents either by penetration or withdrawal through pores of the shells[8] and also through the transovarian route[7]. Some other factors such as environmental temperature and humidity influence bacterial penetration and enhance infection and spoilage[9].

Foodborne diseases caused by microorganisms are a large and growing public health problem. Contamination of eggs and egg products with microorganisms can affect egg quality, which may lead to spoilage and pathogen transmission. This may induce cases of foodborne infection or intoxication to consumers, which constitute public health hazards. Several pathogenic microorganisms have been isolated from the surface of chicken egg shells and contents, including *Listeria monocytogenes*, *Yersinia enterocolitica*, *Escherichia coli* O157:H7, *Salmonella* and *Campylobacter* were detected[10,8].

This study was designed to isolate and identify foodborne pathogens that may contaminate egg shell surfaces in Jordanian markets.

MATERIALS AND METHODS

Sample collection: One hundred eggs were collected and divided into 4 groups (each group = 25 eggs) according to the source of the eggs. Chicken eggs from four different housing systems were used in this study. Group A was packed eggs available in the markets, group (B) was from free-range home eggs, group (C) was from the farm just after cleaning and group (D) was from the farm just before cleaning.

Bacterial isolation: Swabs were taken from each egg shell using sterile cotton swabs soaked in 0.1% peptone and the specimens were put in nutrient broth for bacterial growth and then cultured on the appropriate media, nutrient agar, MacConkey agar, blood agar, xylose-lysine deoxycholate agar (XLD), SS agar and mannitol salt agar or spread directly on agar media. All media were prepared by following the manufacturer's instructions and sterilized by autoclaving at 121°C for 20 min.

Identification of bacterial isolates: The bacterial isolates were identified by studying their cultural, morphological and biochemical characteristics according to previous specifications[11].

Bacteria were identified by culturing and incubation and their macroscopic morphology was characterized for size, shape, outline, colour and changes on various media. Bacteria were Gram stained and examined by light microscopy using a 100X oil immersion objective. Biochemical tests were conducted for correct diagnosis including coagulase, catalase, oxidase and IMVC.

Statistical analysis: Cross tabulation was performed to assess the association between type of bacterial growth and number of isolates in each group of the tested eggs.

Table 1: Association between bacterial growth and number of isolated bacterial species from each group of the tested eggs

	Number of isolates			
Bacteria	Group A*	Group B**	Group C***	Group D****
Staphylococcus aureus	2	1	3	2
Pseudomonas spp.	5	4	3	4
Klebsiella spp.	2	1	4	5
Escherichia coli	5	1	3	5
Bacillus spp.	2	1	2	5
Proteus spp.	1	4	3	3
Salmonella spp.	0	0	3	2
Streptococcus spp.	0	0	1	3
Listeria monocytogenes	0	0	0	1
Total isolates	17	12	22	30
p-value	0.285	0.306	0.293	0.304

* Packed eggs, **Free-range home, ***Farm after cleaning, ****Farm before cleaning

RESULTS

The results of the present study showed that table eggs available for consumers in the Jordanian markets were highly contaminated with different bacterial species. Many of the isolated bacteria are considered foodborne pathogens, which reflects negatively on public health in Jordan. It was obvious that free-range home eggs harboured the least number and the lowest pathogenic bacterial species indicating that this type of egg is the best table egg available in the Jordanian markets.

The type and percentages of Gram-negative and Gram-positive bacteria isolated from table eggs are shown in Table 1. *Staphylococcus* 8 (10%), *Streptococcus* spp. 4 (5%), *Pseudomonas* spp. 17 (21%), *Proteus* spp. 11(14%), *Klebsiella* spp. 12 (15%), *Escherichia coli*. 12 (15%), *Bacillus* spp. 10 (13%, *Listeria monocytogenes* 1 (1%) and *Salmonella* spp. 5 (6%) were isolated from the egg shell surfaces. *Escherichia coli* was isolated from all groups of eggs with a variable percentage. It was isolated from all eggs in group A and D, while it was isolated from one egg in group B and two eggs of group C. *Salmonella* species were isolated from group C (3) and group D (2) with a prevalence of 6%. *Pseudomonas* spp. was isolated from all groups with a percentage of 21%, which was the highest among the isolated bacterial species. *Proteus mirabilis* was isolated from all groups with a percentage of 14%. *Klebsiella* spp. 12 (15%) was isolated mainly from groups C and D with a single isolate from groups A and B. The tested egg shells in group D were heavily contaminated with *Bacillus* spp. 10 (13%). *Listeria monocytogenes* was isolated from 1 egg in group D.

The statistical analysis revealed non-significant differences between all groups although group B was the least contaminated one (12 isolates) and group D was the most contaminated group (30 isolates).

DISCUSSION

Escherichia coli is known to contaminate the surface of eggs, while mechanical processes can spread the bacteria through eggs and meat. Contamination with the pathogen while in the field occur through improperly decomposed manure, contaminated water and poor hygienic practices of the farm workers. *Escherichia coli* causes mastitis, urinary tract infection, meningitis, pneumonia and peritonitis[12]. *Escherichia coli* is considered one of the most common bacteria which causes diarrhoea especially in children. The elderly, infants and those with impaired immune systems may have a more severe illness. In these patients, the infection may spread from the intestines to the blood stream and then to other body sites and can cause death[4].

The low prevalence *of Salmonella* species is not consistent with reports by Yordanov[13] and Cox *et al*.[14]. This discrepancy might be due to the fact that eggs were received from farms free of salmonellosis where the owners of the farms follow good hygienic measures[15]. *Salmonella* has been recovered from shells of hatching eggs (2.48%) and hatching egg contents (0.35%) in Saudi Arabia[15]. However, *Salmonella* species can be isolated from dead embryos inside the shell[16,17]. The fluctuation in the prevalence of *Salmonella* species isolated from dead shell embryos may be due to the variable egg quality tested and the health status of parental flocks. In Taiwan, 13-29% of hatcheries were contaminated with *Salmonella* spp.[18]. Among the most common foodborne infections directly connected with egg consumption are *salmonella* infections[19].

Several species of the genus *Pseudomonas* are very often recognized as the principle causative agents of the spoilage of fresh foods stored aerobically. *Pseudomonas aeruginosa* is recognized as a human pathogen and constitutes potential hazards to both human and animal

health[20,21]. Multi-drug resistant *P. aeruginosa* is highly disruptive to the intestinal epithelial barrier and can cause severe septicemia in immunocompromised hosts[22].

Results of the current study are not consistent with those of Stepien-Pysniak[19], who stated that *Proteus mirabilis* was infrequently isolated, mainly from egg shell surfaces, irrespective of storage conditions or the source of eggs. *Klebsiella* spp. is usually isolated when both processing facilities were combined.

Bacillus species are known to be a causative agent for foodborne gastroenteritis, emetic and diarrhoeal syndrome[23]. *Bacillus* spp. was isolated in group D where the eggs had been collected directly from the farm before cleaning and the eggs were very dirty. This may reflect poor hygiene in this particular farm. The *Staphylococcus* and *Streptococcus* isolated from the samples are often implicated with faecal contamination. These could be of great health concern given that species of these bacteria cause diarrhoea and fever in the hosts[24]. *Staphylococcus aureus*, which is considered an opportunistic pathogen causing mild food poisoning, was isolated from all groups with a percentage of 8 (10%). Groups A and B did not have *Streptococcus* spp., while group C had only one isolate and group D had three isolates. The overall percentage of *Streptococcus* spp. isolation was 5%.

Listeria monocytogenes can cause illness in pregnant women, babies and people with reduced immunity and febrile gastroenteritis in healthy people. The estimated annual incidence of listeriosis is quite low and poultry farms are not frequently examined for *L. monocytogenes*, however, a relatively high prevalence of contaminated raw chicken products has been reported. Thus, contaminated farms can be the source for contamination of the slaughter and processing environment[25].

The study was undertaken to evaluate the microbial quality of eggs that are sold for human consumption in local markets in Jordan. The major contaminants were Gram-negative bacteria namely *Pseudomonas* spp. 17 (21), *Proteus* spp. 11 (14%), *Klebsiella* spp. 12 (15%), *Escherichia coli* 12 (15%) and *Salmonella* spp. 5 (6%). In addition, *Staphylococcus* 8 (10%), *Bacillus* spp. 10 (13%) and *Streptococcus* spp. 4 (5%) were isolated in comparable percentages and only 1 (1%) of *Listeria monocytogenes* was isolated. These observations are consistent with those of previous studies[5].

Organisms belonging to the family Enterobacteriaceae are the most commonly isolated contaminant from all of the sources under study. *Escherichia coli*, *Proteus* spp. and *Klebsiella* spp. were isolated from each of the 4 groups. Other genera isolated from 2 of the 4 groups included *Streptococcus* spp. and Salmonella spp. The non-Enterobacteriaceae isolated and identified were *Pseudomonas* spp. 17 (21%) and *Bacillus* 10 (13%). The bacterial flora previously reported from eggs[5,26,27] were also isolated in the present study.

Coliforms, Enterobacteriaceae and *E. coli* populations can be used as a measure of food quality and sanitary processing conditions[28]. The prevalence of these bacteria in eggs isolated from various sites in the present study indicates poor sanitary conditions of handling eggs. *Pseudomonas* was reported to be associated with spoilage of packaged and processed food[29].

In the present study, *Pseudomonas* was recovered from all sample groups collected. In a heavily contaminated environment, the eggs become infected through trans shell contamination where the bacteria are translocated from the outer to the inner surface of the shell and bacteria grow quickly in the shell membrane because of the availability of iron[22]. This supports the high number of bacterial isolations from the egg shell and indicates poor hygienic conditions at the layer farms.

A few organisms isolated and identified in this study are considered to be foodborne pathogens (*Salmonella* spp., *Listeria* spp. and *E. coli*). Others may be opportunistic or rare human pathogens. The eggs from supermarkets were not stored at refrigerated temperatures and the level of contamination in eggs collected from these sites was considerable. Thus, we suggest that supermarkets should store eggs at refrigerated temperatures to control the contamination of table eggs. The results in this study also indicate that contamination may occur at the farm level rather than during handling. The data underscore the need for optimum hygienic conditions at the farm level to decrease the bacterial load in commercial chicken eggs in Jordan.

According to the results of the present study, the worst contamination with bacteria spp. was in group D (28 isolates, 35%) collected from the farm just before cleaning. The least contamination of eggs was observed in group B (12 isolates, 15%), which is free-range home eggs. These data suggest that free-range home eggs are considered the best choice for consumers to minimize the possibility of food poisoning upon eating table eggs.

The results of this work confirmed contamination of egg samples in Jordanian markets. While 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, several species isolated in this study have been associated with egg spoilage[30].

Findings from this study showed that eggs sold are highly contaminated partly due to handling by retailers, storage and environmental conditions. Consumers are at a high risk of infection because most of the organisms isolated are pathogenic to humans. Ensuring good hygienic standards at the various markets and farm houses in the metropolis is a shared responsibility between stakeholders, government, consumers and retailers. Retailers in particular should be impressed upon to endeavour to store and retail their eggs under refrigerated or good sanitary conditions to reduce microbial contaminations.

Despite the difference in the type of bacterial growth and different number of isolates in each group, this difference was statistically non-significant. This indicates that all types of table eggs in Jordan are not handled in healthy conditions.

SIGNIFICANCE STATEMENT

This study showed that foodborne pathogens comprise a major concern of table egg safety. Table eggs sold at Jordanian markets are highly contaminated, partly due to handling by retailers, storage and environmental conditions. The isolated bacteria are a health hazard in Jordan, which indicates that control is required at all levels of table egg processing. This study will help the national health agencies to apply food safety regulations to maintain the safety of this important food staple.

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