

Original Research Article

Prevalence of *Salmonella* species from poultry eggs of local stores in Duhok

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ABSTRACT

Background: Salmonellosis is one of the foodborne illness acquired by consumption of infected raw or undercooked eggs and causes major public health problem. The aim of this study was isolation and identification of *Salmonella* spp. from the eggshells and the egg contents samples.

Methods: In this study, a total 350 eggs were randomly collected from five local stores in Duhok and Zakho city over a period of 6 months in summer of 2016. Eggs from each local store were collected and transferred to the microbiology laboratory. The conventional culture method used for detection of *Salmonella* spp.

Results: Out of the 350 eggs, seventeen (4.85%) samples of eggshells contaminated with *Salmonella* spp. and none of the egg content samples were contaminated with *Salmonella* genus. Out of 17 positive eggs, three different *Salmonella* serotypes were identified including; *Salmonella enteritidis* (10 strains), *Salmonella typhimurium* (5 strains), *Salmonella typhi* (2 strains).

Conclusions: The results of the present study provide the recent dataset of the prevalence of *Salmonella* spp. in eggs sold at local stores in the city. All isolates showed resistant to tetracycline, oxacillin and sulphadimethoxazole due to the indiscriminate use of these antibiotics in chicken at sub-therapeutic level or prophylactic doses which promotes selection of antimicrobial resistant strains and also increases the human health risks associated with consumption of contaminated quail eggs. To the best of our knowledge, this is the first study in Zakho- Duhok city, investigating the occurrence of *Salmonella* spp. in eggshell and content egg sold at local stores.

Keywords: Antibiotic susceptibility, Egg, *Salmonella*

INTRODUCTION

Salmonella is one of the most important foodborne pathogens.^{1,2} There are more than 2435 known serotypes of *Salmonella* and many of these serotypes are human pathogens.³ Foods with animal sources such as beef, poultry meat, egg and milk have been proven to carry *Salmonella* spp.¹ Although, eggs are nutritious foods and they form an important part of the human diet.

Consuming improperly handled egg and egg products can be source of food borne diseases. Several Salmonellosis outbreaks have been reported where eggs were the source of human infection in particular, undercooked or raw eggs are at high risk for humans.⁴⁻⁶

Salmonellae reside in the intestinal tract and are shed in the faeces of infected animals and humans as well. Many foods, particularly those of animal origin and those

subject to sewage pollution, have been identified as vehicles for transmitting these pathogens. Eggs may be contaminated with *Salmonella* by two routes; vertical transmission by transport *Salmonella* from infected reproductive tissues to egg prior to shell formation and horizontal transmission by contaminated the shell of egg with animal fecal and through environmental vectors such as farmers, pets and rodents. Improperly storage and shell damage may facilitate contamination with *Salmonella*.⁷ Both serovar of *Salmonella* enteric; *typhimurium* and *enteritidis* are the most frequently isolated serovar from foodborne outbreaks throughout the world and both serovar infect the reproductive tract in chicken.⁸ The present study was designed with an objective to know the status of Salmonellosis in poultry eggs in Zakho- Duhok city.

METHODS

Sample collection

Eggs were collected randomly from different local stores in Duhok-Zakho city over a period of 6 months in 2016. A total of 350 eggs were analyzed for the presence of *Salmonella* in eggshell and egg content. Eggs from each store were collected in sterile polythene bags and transferred by refrigerated containers to laboratory for bacteriological investigation.

Bacteriological analysis

A swab technique was used to sample the shell surface of the intact eggs. Sterile cotton swabs dipped in sterile buffered peptone water were used to swab the entire surface area of the eggshell. In order to collect the egg contents, eggs surface was sterilized by immersion in 70% alcohol for 2 min, air dried in a sterile chamber for 10 minutes and then cracked with a sterile knife.

For nonspecific pre-enrichment stage each egg's content was mixed thoroughly, and 25mL of mixed egg content was inoculated into 225mL of lactose broth medium and incubated at 37°C for 24 hours. For specific pre-enrichment stage, 1mL of the cultures of all sample types were transferred to 9mL of tetrathionate broth and to 9mL of selenite cystine broth and incubated at 37°C for 24 hours. The cultures were then streaked onto *Salmonella Shigella* agar and brilliant green agar plates, and incubated at 37°C for 24 hours. The plates were observed for typical *Salmonella*-like colonies, randomly, the suspected *Salmonella* colonies were subjected to biochemical screening such as; triple sugar iron agar, lysine iron agar, indole production in tryptone broth and urea splitting ability in Christensen's urea agar. Suspected colonies were then confirmed by serological method, slide agglutination test using somatic polyvalent *Salmonella* serum was performed for the tubes showing reactions typical of *Salmonella*.^{9,10} For serotyping, the isolated *Salmonella* strains were transferred to the Institute of Public Health, Baghdad.

Antibiotic susceptibility test

Antibiotic susceptibility test was performed by using Kirby-Bauer modified disc-diffusion technique as described by Clinical and Laboratory Standards Institute.¹¹ The antibiotic discs (Oxoid, UK) were evenly dispensed unto the surface of the inoculated agar plate using a disc dispenser and were gently pressed down to ensure complete contact with the agar surface. The plates were inverted and incubated at 37°C for 18 hours.

The following 11 antibiotic discs were used: chloramphenicol (C) 30µg, ciprofloxacin (CIP) 5µg, colistin 15µg, polymixin 25µg, tetracycline (TE) 30µg, cephalothin 15µg, cotrimazole 10µg, neomycin 30µg, sulphamethoxazole 25µg, oxacillin (OX) 5 µg, gentamycin (CN) 30µg, were applied in the test. The zone of inhibition was measured in millimeters with a ruler and compared with a zone interpretation chart By using McFarland scale the results were reported as sensitive, intermediate or resistant.¹²

Pure colonies of isolated strains were emulsified in normal saline and turbidity was matched with 0.5 McFarland turbidity standards. Selected antibiotic discs were placed on inoculated Mueller-Hinton agar medium. These plates were incubated at 37°C for 24 hours. Resistant and sensitive bacteria were defined according to CLSI guidelines.¹³

Statistical analysis

Data analysis was done by using t-test, ($p < 0.05$) considered significant.¹⁴

RESULTS

Out of the 350 eggs collected from different local stores of Duhok-Zakho city, seventeen (4.85%) samples of eggshells contaminated with *Salmonella spp.* and none of the egg content samples were contaminated with *Salmonella* genus. The overall prevalence of *Salmonella* in eggs was 4.85% (17/350) (Table 1). Out of 17 positive eggshell three different *Salmonella* serotypes were identified including; *Salmonella enteritidis* (10 strains), *Salmonella typhimurium* (5 strains), *Salmonella typhi* (2 strains).

Table 1: prevalence of *Salmonella* species in egg shell and content.

Source	No. of samples	No. of positive eggs	Prevalence (%)
Local store 1	63	2	0.57
Local store 2	70	4	1.14
Local store 3	65	3	0.86
Local store 4	71	4	1.14
Local store 5	81	4	1.14
Total	350	17	4.85

The antibiotic susceptibility profile of the *Salmonella* isolates from eggshell characterized in this study displayed resistance to one or more antibiotics (Table 2). The antibiotic sensitivity pattern of the isolates showed that all the seventeen isolates were found to be sensitive

to chloromphenicol, ciprofloxacin, colistin and polymixin followed by gentamycin in the decreasing order. However, all the isolates were resistant to tetracycline, cephalothin, cotrimazole, neomycin, sulphadimethoxazole and oxacillin (Table 2).

Table 2: Antibiotic sensitivity of *Salmonella* spp. isolated from shell eggs.

Antibiotic	<i>Salmonella enteritidis</i>	<i>Salmonella typhimurium</i>	<i>Salmonella typhi</i>
Chloromphenicol	S	S	S
Ciprofloxacin	S	S	S
Colistin	S	S	S
Polymixin	S	S	S
Tetracycline	R	R	R
Cephalothin	R	R	R
Cotrimazole	R	R	R
Neomycin	R	R	R
Sulphadimethoxazole	I	I	R
Oxacillin	R	R	R
Gentamycin	S	S	I

R: Resistant; S: Susceptible; I: Intermediate.

DISCUSSION

Out of 17 positive eggshells, three different *Salmonella* serotypes were identified including; *Salmonella enteritidis* (10 strains), *Salmonella typhimurium* (5 strains), *Salmonella typhi* (2 strains). Although, *Salmonella* was isolated from eggshells in this study, but none of the contents were contaminated by *Salmonella*. Our results were in agreement with similar studies conducted in Iran (1.6%) of eggshells and India (6.1%) of eggshells. Contamination of eggshells represents a serious risk for the consumers, as they can directly infect and cross-contaminate the egg contents or other foodstuffs.¹⁵

In USA (2010), Salmonellosis outbreak associated with contaminated eggshells with *Salmonella* showed the importance of shell contamination, which this outbreak caused illness in 1939 persons.¹⁶

The ability of *Salmonella enteritidis* to persist on the surface of the egg shell and contaminate the content of egg when eggs are broken for preparation of food, which could pose a potential health risk to the society. Therefore, removal of the quails wastes and disinfection can greatly reduce *Salmonella* contamination on the shell and the content.¹⁷ This indicates that the eggs were contaminated either during its lays through the contaminated cloacae or from direct contact with contaminated nest, litter, trays, and transport boxes after collection and delivered to markets. This finding means that the eggs were maybe not cleaned by brush. Although, fecal matter removed from eggshell in egg

trays; storage of eggs at room temperature help mesophilic bacteria grow and multiply fast if they get access to egg contents through cracks, which can develop during transportation and handling.

High sensitivity to ciprofloxacin, chloramphenicol and gentamycin adequately explain it increasingly and successfully used for treatment of septicemia Salmonellosis in humans.¹⁸ Antimicrobial use and misuse has been considered to be the most vital selecting force to antimicrobial resistance of bacteria development and spread in both veterinary and human medicine.¹⁹ All isolates showed resistant to tetracycline, oxacillin and sulphadimethoxazole. This may be due to the frequent and indiscriminate use of these antibiotics in chicken at sub-therapeutic level or prophylactic doses, which promotes selection of antimicrobial resistant strains and also increases the human health risks associated with consumption of contaminated quail eggs.

Poultry eggs were considered a vehicle for transmission of certain pathogens to human if such eggs are consumed raw. Bacteria on egg shell have been implicated as source of bacterial contamination of broken out eggs.^{20,21} The motility of *Salmonella* on eggshells may easily penetrate the shells to interior. The rate of penetration is influenced by humidity and storage temperature. At which the eggs were produced and stored.^{22,23}

CONCLUSION

From this study, it can be concluded that eggs may be a source of transmission of Salmonellosis. Early detection

and proper hygienic practice should be maintained in handling and marketing eggs by the farm handlers and retailers to prevent spread of infection.

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Ethical approval: The study was approved by the Institutional Ethics Committee

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