



## **Prevalence and Antibiotic Susceptibility of *Salmonella* from Chicken Eggs in Naogaon District of Bangladesh**

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### **Authors' contributions**

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

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### **ABSTRACT**

*Salmonella* is one of the most common zoonotic bacteria that cause foodborne illness in humans. An investigation was conducted to determine the prevalence and antibiotic susceptibility patterns of *Salmonella* isolates from chicken eggs in the Naogaon district, Bangladesh. *Salmonella* was isolated from cultures on different selective-differential media and further identified by biochemical tests. Antibiogram study was done by the disk diffusion method. The overall prevalence of *Salmonella* was recorded as 7.78%, whereas 5.56% was on eggshell surfaces and 2.22% was in egg contents. The *Salmonella* prevalence was 8.33%, 13.33% and 1.67% in chicken eggs from layer farms, whole sellers and retailers, respectively. *Salmonella* isolates were found 50.0% to 85.71% sensitive to chloramphenicol, gentamycin, ciprofloxacin, and ceftriaxone. Resistance against gentamycin, chloramphenicol, and ampicillin was found significant ranging from 21.43% to 71.42%. The highest resistance was found in amoxicillin (92.86%). The present study proposes that chicken eggs are a potential reservoir of multidrug-resistant *Salmonella*. Antibiotic-resistant *Salmonella* will pose a problem to treat *Salmonella* infection in humans. Thus, the aim of this study is to assess the risk of *Salmonella* resistance in chicken eggs.

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## 1. INTRODUCTION

Chicken eggs are rich in nutrients and perfect foodstuffs for a human diet containing high-quality proteins, amino acids, and vitamins [1]. Though eggs are rich in high-quality nutrients, it makes itself vulnerable to the growth of many pathogenic bacteria especially *Salmonella*. *Salmonella* contaminants are more likely to be associated with crack eggs, dirty shell eggs and eggs stored in *Salmonella* contaminated environment. Bacterial contamination can take place at three main parts of egg these are egg yolk, egg albumen, and eggshell [2]. When the contaminated eggshell is broken, it increases the chance of egg contents contamination [3].

*Salmonella* is one of the most prevalent pathogenic bacteria that cause foodborne illness in humans [4,5]. Chicken eggs are a major reservoir of *Salmonella* that causes egg-associated salmonellosis in humans, which has major public health significance [6,7]. In humans, *Salmonella* causes several clinical illnesses such as enteric fever, gastroenteritis, enterocolitis, septicemia and systemic infections [8].

Globally, antimicrobial resistance is increasing and becoming a major public health hazard [9]. Antibiotics are widely used as feed additives as well as infectious disease preventers. This overuse of antibiotics in the chicken disturbs normal micro-flora of intestine resulting in the development of antibiotic-resistant *Salmonella* sp. [10]. There are many investigations about the prevalence and antibiotic resistance of *Salmonella* in eggshells and egg contents around the world [11,12]. However, data about the prevalence as well as antimicrobial resistance of *Salmonella* in contaminated chicken eggs are rare in Bangladesh. Therefore, the aim of the present study was to evaluate the prevalence as well as the antimicrobial susceptibility pattern of *Salmonella* from chicken eggs.

## 2. MATERIALS AND METHODS

### 2.1 The Study Area and Sample Collection

The chicken eggs were collected from randomly selected commercial layer farms and different sale outlets in the Naogaon district of Bangladesh, during the period from January to December 2017. A number of 180 egg samples were collected in which 60 from farms, 60 from

retailers and 60 from whole sellers. In this study, 60 white shell eggs, 60 brown shell eggs, and 60 indigenous chicken eggs were collected and examined.

### 2.2 Egg Samples Preparation

For eggshell preparation, one sterile swab stick was soaked with sterile saline solution and was rubbed on the egg surface. Then the swab stick was submerged in 1 ml of sterile saline solution as 'eggshell wash'. For egg content preparation, eggshells were sterilized by washed with 70% ethyl alcohol, flamed and broken with sterile forceps. Then the egg contents (yolk and albumen) thoroughly mixed for nearly one minute using a blender. The blender was thoroughly sterilized before using for each egg sample following the standard method.

### 2.3 Isolation and Identification of *Salmonella*

For isolation of *Salmonella*, eggshell wash and egg content samples were enriched in buffered peptone water (Hi-Media, India) at 37°C for 18-24 hours. For selective enrichment, 0.1 ml of pre-enriched culture was inoculated into 10 ml of tetrathionate broth (Hi-Media, India) and incubated at 37°C for 24 hours. After that, cultured samples were streaked onto selective media such as Xylose Lysine Deoxycholate (XLD) (Hi-Media, India) and *Salmonella*-*Shigella* (SS) agar (Hi-Media, India) plates and incubated. After that, presumptive *Salmonella* colonies were confirmed by a series of biochemical tests such as sugar fermentation test, catalase test, Indole test, Methyl red test, Voges-Proskauer test, Simmon's citrate agar, and Triple Sugar Iron (TSI) agar test according to Cheesbrough [13] and Özkalp [14]. All the *Salmonella* isolates were catalase, Simmon's citrate agar and Methylene red positive but Voges-Proskauer and indole negative. All isolates fermented dextrose, mannitol, and maltose with acid and gas production but did not ferment lactose and sucrose. On TSI agar slant, isolates producing alkaline slant (red color) and acid butt (yellow color) with black color due to H<sub>2</sub>S production.

### 2.4 Antibiogram Study of Isolated *Salmonella*

The disk diffusion method described by Jorgensen and Turnidge [15]; CLSI [16] were used to test the antibiogram study of the 14

*Salmonella* isolates. Briefly, pure *Salmonella* isolates were enriched in nutrient broth (Hi-Media, India) at 37°C for 18-24 hours. Then enriched broth culture was taken onto Mueller Hinton agar (Hi-Media, India) plate and spread uniformly. After that, the antimicrobial discs were dispensed onto the surface of the agar plates and incubated at 37°C for 18-24 hours. The susceptibility zones were measured and interpreted according to CLSI standards or guidelines [16].

### 3. RESULTS

The overall prevalence of *Salmonella* in chicken eggs was 7.78%, where 5.56% was on eggshells only and 2.22% was in egg contents only (Table 1). The *Salmonella* prevalence was 8.33%, 13.33%, and 1.67% in eggs collected from selected farms, whole sellers and retail shops (Table 2).

The results of antibiotic sensitivity patterns of isolated *Salmonella* showed sensitive to amoxicillin (7.14%), ampicillin (14.29%), chloramphenicol (50%), gentamycin (57.14%),

ciprofloxacin (78.57%) and ceftriaxone (85.71%). *Salmonella* was found 14.29% intermediate sensitive to ampicillin and ciprofloxacin further 21.43% intermediate resistant to gentamycin, and chloramphenicol. Similarly, the resistant pattern of the isolates showed resistant to ciprofloxacin (7.14%), ceftriaxone (14.29%), gentamycin (21.43%), chloramphenicol (28.57%), ampicillin (71.42%) and amoxicillin (92.86%) (Table3).

### 4. DISCUSSION

In the present study, the overall prevalence of *Salmonella* was 7.78%, which were similar or in agreement with reports by Samanta et al. [17]; Islam et al. [18] and Xie et al. [19]. Samanta et al. [17] identified 10% of *Salmonella* from backyard poultry eggs in West Bengal, India. Islam et al. [18] observed the *Salmonella* prevalence in eggs as 20.71% in Dhaka city, Bangladesh. Xie et al. [19] reported that 5.40% of *Salmonella* isolated from eggs in Guangdong, China. Previous workers, who found 2.98% [20] and 3.5% [21] *Salmonella* prevalence in Ethiopia and Paraguay, reported lower *Salmonella* isolation rates.

**Table 1. Prevalence of *Salmonella* in different types of chicken eggs**

Type of chicken eggs	No. of samples tested	Prevalence of <i>Salmonella</i> on eggshells only (%)	Prevalence of <i>Salmonella</i> in egg contents only (%)	The overall prevalence of <i>Salmonella</i> (%)
Indigenous chicken eggs	60	4 (6.67%)	2 (3.33%)	14 (7.78%)
Brown shell chicken eggs	60	2 (3.33%)	1 (1.67%)	
White shell chicken eggs	60	4 (6.67%)	1 (1.67%)	
Total	180	10 (5.56%)	4 (2.22%)	

**Table 2. Prevalence of *Salmonella* from different sources of sample**

Sources of sample	No. of samples tested	Prevalence of <i>Salmonella</i> on eggshells only (%)	Prevalence of <i>Salmonella</i> in egg contents only (%)	The overall prevalence of <i>Salmonella</i> (%)
Farms	60	3 (5%)	2 (3.33%)	5 (8.33%)
Whole sellers	60	6 (10%)	2 (3.33%)	8 (13.33%)
Retailers	60	1 (1.67%)	0%	1 (1.67%)
Total	180	10 (5.56%)	4 (2.22%)	14 (7.78%)

**Table 3. Antibiotic susceptibility patterns of *Salmonella***

Name of antibiotic disks used (µg/disk)	Susceptibility patterns (n=14)		
	Sensitive (%)	Intermediate sensitive (%)	Resistant (%)
Ampicillin (25 µg)	14.29%	14.29%	71.42%
Amoxicillin (30 µg)	7.14%	0%	92.86%
Gentamycin (10 µg)	57.14%	21.43%	21.43%
Ciprofloxacin (5 µg)	78.57%	14.29%	7.14%
Chloramphenicol (30 µg)	50.00%	21.43%	28.57%
Ceftriaxone (30 µg)	85.71%	0%	14.29%

In this study, *Salmonella* prevalence was recorded 8.33%, 13.33%, and 1.67% in farms, whole sellers and retailers, respectively that agreed with Singh et al. [22]. Singh et al. [22] found 3.84%, 4%, and 7.4% chicken eggs contaminated with *Salmonella* in the farm, wholesale and retail market, respectively. The higher rate of *Salmonella* in the farms and whole seller's chicken egg may be due to egg surface contamination when collected from farms, handling, transport, and storage of eggs.

The prevalence of *Salmonella* on eggshell only recorded as 5.56%, which was lower than the findings of Harsha et al. [23] and Islam et al. [18]. Harsha et al. [23] stated that 4% to 20.66% of chicken eggshells were *Salmonella* contaminated in Kerala, India. Islam et al. [18] reported that 13.57% of eggshells contaminated with *Salmonella* in Dhaka city, Bangladesh. However, El-Kholy et al. [24] and Karimiazar et al. [25] found no contamination of *Salmonella* in examined eggshell samples.

The prevalence of *Salmonella* in egg contents only was 2.22% which was similar to Taddese et al. [20] stated that farms egg contents, and market egg contents with 2.4%, and 3.6% *Salmonella* was prevalent in Jimma Town, Ethiopia. However, the present study was higher than the findings of Karimiazar et al. [25] reported that 1.66% of industrial and local egg contents contaminated with *Salmonella* in Zanjan province, Iran. Islam et al. [18] stated that 7.14% of *Salmonella* prevalent in egg contents in Dhaka city, Bangladesh.

In the current study, antibiotic sensitivity and resistance patterns of *Salmonella* to different antimicrobials are in harmony with previous reports by Islam et al. [18]; Ugwu et al. [26] and Xie et al. [19]. Islam et al. [18] revealed sensitivity rates against ciprofloxacin (88.89%), ceftriaxone (83.33%), gentamicin (66.67%), chloramphenicol (50%), ampicillin (16.67%) and amoxicillin (5.56%) whereas resistance patterns were amoxicillin (94.44%), ampicillin (77.78%), chloramphenicol (38.89%), and gentamicin (22.22%). Ugwu et al. [26] stated that *Salmonella* was 26.79% and 53.37% sensitive to gentamicin and ceftriaxone although 60.71% and 33.93% *Salmonella* resistant to gentamicin and ceftriaxone. Xie et al. [19] found that *Salmonella* isolates were sensitive to ampicillin (35.18%), gentamicin (72.22%) and ciprofloxacin (87.04%), as well as isolates, were resistant to ciprofloxacin (9.26%), gentamicin (18.52%) and ampicillin

(59.26%). The antimicrobial resistant pattern of *Salmonella* varies depending on *Salmonella* serotypes, region, the particular farm, broilers versus layers, and the antimicrobial agents [27]. It was reported that high levels of antibiotics used as food additives in farms or indiscriminating use rather than recommended doses have correlations with the development of multidrug resistance *Salmonella* [28].

## 5. CONCLUSION

The present study showed that the prevalence of *Salmonella* was higher on eggshells when compared with egg content. We anticipate that eggs available for sale must be free from faces, soil, and dirt. The *Salmonella* prevalence in chicken eggs and their multidrug resistance is obviously significant. It could be established that the antibiotic-resistant *Salmonella* from chicken eggs might pose a public health hazard to consumers. Therefore, *Salmonella* has both risks of public health through disease-causing and antibiotic resistance across Bangladesh.

## 6. LIMITATION

The *Salmonella* isolates were not molecularly characterized due to a lack of funding and resource.

## ETHICAL APPROVAL

As per international standard or university standard ethical approval has been collected and preserved by the author(s).

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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