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Prevalence and multidrug-resistant pattern of *Salmonella* from the eggs and egg-storing trays of retail markets of Bangladesh

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Abstract

Aim: Salmonellosis is one of the most common and widely distributed foodborne illnesses in human, and multidrug-resistance of *Salmonella* spp. has increased in developing countries with the indiscriminate use of antibiotics in the poultry production system. A cross-sectional study was conducted on randomly selected retail markets of Chittagong City Corporation to determine the prevalence and antimicrobial resistance pattern of *Salmonella* isolated from commercial layer eggs, eggshell surface, and egg-storing trays.

Materials and Methods: Chicken eggs, egg surface, and egg-storing trays samples from the retail markets were collected for isolating *Salmonella* spp. (bacteriological culture methods) followed by antimicrobial susceptibility testing (disc diffusion method) against *Salmonella* isolates during the period from July to December 2013.

Results: Out of the 310 layer eggs, egg surface, and egg-storing trays samples, the highest prevalence of *Salmonella* spp. was found in eggs trays (57.15%) and the lowest (13.33%) in eggs and the prevalence differed significantly ($p<0.01$). On the other hand, the prevalence was higher (45%) in samples of Pahartali bazar and lower (31.43%) in samples of Bohderhat bazar but the variation among the sites was not varied significantly ($p>0.05$). Isolated *Salmonella* was tested for resistance to eight different antimicrobial agents, using disc diffusion method. Among eight antimicrobial tested (n=111), 100% resistance were found to ampicillin and amoxicillin followed by erythromycin (60-100%), tetracycline (72-93%), ciprofloxacin (22-66%), colistin (27-66%), enrofloxacin (42-54%), and pefloxacin 23.07% across the study sites. Ciprofloxacin remained sensitive in 40.9% cases and, pefloxacin and colistin appeared to be almost sensitive (61-72%) against *Salmonella* isolates at studied areas. *Salmonella* isolates showed multidrug-resistance pattern up to five of the eight antimicrobials tested.

Conclusion: It can be said that the rational use of antibiotics needs to be adopted in commercial poultry farming system of Bangladesh to prevent the emergence of drug-resistance *Salmonella* to protect the public health consequences.

Keywords: antimicrobial, public health, prevalence, resistance, *Salmonella.*

Introduction

Salmonella is one of the major bacterial agents that cause foodborne infections in humans world-wide [1]. The majority of salmonellosis outbreaks have been attributed to food such as eggs, chicken, beef, and fish to human carriers. The outbreaks involving eggs almost all have occurred in the food service sector and have been the result of inadequate refrigeration and insufficient cooking. Salmonellosis is a major problem in layer poultry in Bangladesh, and its prevalence ranged from 28% to 53.3% [2]. Among foodborne bacterial zoonoses salmonellosis causes huge economic losses in terms of massive morbidity and mortality [3]. Foodborne *Salmonella* is estimated to cause approximately 1.4 million illness, 15,000 hospitalization and 500 deaths per year in the United States [4]. The fatality rate in people infected with antibiotic-resistant *Salmonella* is 21 times greater than that infected with non-antibiotic-resistant *Salmonella* strains. Different serotypes of *Salmonella* including *Salmonella* Typhimurium and *Salmonella* Enteritidis are prevalent both in poultry and human and categorized as zoonotic pathogens [5]. *Salmonella* spp. contamination in egg producing farms and market outlets may arise at any production stage by horizontal or vertical transmission. One possible cause of *Salmonella* contamination in developing countries is reusable egg trays [6]. Outbreaks and sporadic cases of salmonellosis are frequently associated with the intake of infected hen eggs with *Salmonella* spp. The disease is endemic in many developing countries, particularly the Asian subcontinent and South and Central

America. Antibiotic used as a therapeutic, prophylactic, or growth promoter of poultry in many developing countries including Bangladesh [7] which deposit residues in meat [8] and eggs [9]. The emergence of antimicrobial-resistant *Salmonella* strains is of great concern and emerging antimicrobial resistance has become a public health issue worldwide. There are reports of high prevalence of resistance in *Salmonella* isolates from countries such as Bangladesh [10, 11], India [12], and France [13]. Similarly, there are various reports of multidrug-resistant *Salmonella* organisms isolated from chickens eggs in Bangladesh [14].

In recent years, antibiotic resistance in *Salmonella* has assumed alarming issue [15], and most of the *Salmonella* isolated from layer birds detected earlier as are resistant to at least one antimicrobial. Moreover, antibiotic treatment is considered the most important issue that promotes the emergence, selection, and spreading of antibiotic-resistant microorganisms in both veterinary and human medicine. Evidence indicated that antimicrobial resistance among human *Salmonella* isolates results from the presence of antimicrobials residues in the foods [2].

This study was designed with the aim to know the prevalence and identify antimicrobials resistance against *Salmonella* spp. in eggs and egg-storing trays to minimize a potential public health threat.

Materials and Methods

Ethical approval

The current study was approved by Animal Ethical Experimentation Committee (CVASU-AEEC) of Chittagong Veterinary and Animal Sciences University, Bangladesh.

Study area

Six retail markets (Pahartali bazar, Jhautola bazar, Colonelhat bazar, Reazuddin bazar, Bohderhat bazar, and Alonkar bazar) of Chittagong City Corporation (CCC) were randomly selected where a large number of layer eggs are sold every day in retail markets.

Study design

A cross-sectional study was conducted in different wet markets of Chittagong, Bangladesh, to investigate the prevalence and antimicrobial resistance pattern against *Salmonella* spp. in egg contents, eggshell surfaces, and egg-storing trays. The study was conducted for a period of 6 months from July to December 2013.

Source population and sampling frame

Commercial markets in CCC area was used as source population. All markets of the source population also included in the sampling frame. The smallest unit of the sampling frame had sales of at least 1000 eggs/market/day. The list of the markets and other details were retrieved from the database of the CCC.

Sample size calculation

Simple random sampling was used for the sample collection, and the sample size was estimated by the formula as described by Thrusfield [16]. Out of 310 samples, fresh egg samples were 120, eggshell surface were 120, and egg-storing tray samples were 70.

Collection and preservation of sample

Egg-storing trays and eggshell surface

A sterile cotton swab was used to wet in sterilized normal saline solution to swab on egg-storing tray and eggshell surface then the swab was put immediately into a sterile vial containing 6 ml Amines Transport Media (Oxoid). The individual sample in each vial was given unique identification number then immediately transferred to Poultry Research and Training Centre (PRTC) laboratory, Chittagong Veterinary and Animal Sciences University (CVASU) through ice box. Samples with transport media were stored temporarily in a refrigerator before laboratory evaluation.

Egg content

Egg samples were collected from egg trays from selected markets in CCC, live chicken, and eggs retailers shop according to FDA procedure. All samples were labeled and kept in an insulated box in the field and transported to the laboratory then stored in the refrigerator before laboratory evaluation. Eggshells were washed with normal saline solution then cracked with sterile knife and contents were taken into a jar and mixed well through stirrer.

Laboratory evaluation

A protocol followed by Hoque *et al*. [17] was used for the present study to perform a bacteriological culture of *Salmonella* spp. 1 ml of egg surface and the egg-storing suspension was transferred into 10 ml Mannitol Selenite Broth (Oxoid) and was incubated at 37°C for 18 h. After incubation, a loop full of broth was streaked on xylose lysine deoxycholate medium and incubated at 37°C for 24 h. Colonies with black centers were considered presumptive *Salmonella* spp. [17]. Presumptive colonies were sub-cultured in blood agar and subjected to some basic biochemical tests (urease, oxidase, and catalase) for confirmation of *Salmonella.*

Selection of antimicrobials

The most commonly used antimicrobial agents for either chemoprophylaxis or therapy for control of bacterial diseases in poultry in South Asia were included for antimicrobial susceptibilities such as ciprofloxacin, erythromycin, pefloxacin, tetracycline, amoxicillin, ampicillin, colistin, and enrofloxacin. These are also frequently used in Bangladesh.

Antimicrobial sensitivity test

Antimicrobial susceptibility testing against *Salmonella* isolates was performed using the antimicrobial disc (Oxoid) according to Kirby-Bauer antimicrobial disc diffusion techniques. Pure colonies of the *Salmonella* spp. isolates were inoculated in nutrient broth and incubated at 37°C for overnight. The isolates

Table-1: Prevalence of *Salmonella* in different samples and sampling sites.

Variables	Categories	Positive (%)	Chi-square value	p value
Sample	Egg-storing trays	40 (57.2)	45.47	0.00
	Eggshell surface	55 (45.8)		
	Egg content	16 (13.3)		
Sampling site	Jhautola bazar	13 (32.5)	3.57	0.61
	Pahartali bazar	18 (45.0)		
	Reazuddin bazar	24 (32.0)		
	Colonelhat bazar	19 (42.2)		
	Alonker bazar	15 (37.5)		
	Bohderhat bazar	22 (31.4)		

were streaked thoroughly on the Mueller-Hinton agar using sterile glass rod (60°cone-shaped) and antimicrobial discs; ampicillin (30 mcg/disc), amoxicillin (30 mcg/disc), ciprofloxacin (5 mcg/disc), colistin (25 mcg/disc), erythromycin (15 mcg/disc), enrofloxacin (30mcg/disc), pefloxacin (5 mcg/disc), and tetracycline (30 mcg/disc) were placed centrally using antimicrobial disc dispenser (Oxoid). The petridishes were incubated at 37°C for 24 h. The plates were observed for antimicrobial susceptibility pattern by measuring the zone of inhibition according to CLSI [18], and the isolates were considered as sensitive, intermediately sensitive or resistance.

Data analysis

Descriptive analysis was performed to know the frequency and distribution of *Salmonella* and antibiotic resistance pattern using STATA/IC-11.0.

Results

Table-1 shows the prevalence of *Salmonella* spp. in different samples such as egg contents, egg-storing trays, and eggshell surfaces. The highest prevalence was found in egg-storing trays (57.2%) and lowest (13.3%) in egg contents. Among the category of samples, the variation in prevalence differs significantly ($p<0.01$). On the other hand, the prevalence was higher (45.0%) in samples of Pahartali bazar and lower (31.4%) among the samples of Bohderhat bazar, but there were observed insignificant ($p>0.05$) variation of prevalence among the study sites.

The prevalence and pattern of antimicrobial resistance of *Salmonella* isolates from egg-storing trays have been outlined in Table-2. Resistance patterns of *Salmonella* were the highest in amoxicillin (100%) followed by ampicillin (97.5%), erythromycin (90%), tetracycline (82.5%), enrofloxacin (60%), colistin (52.5%), ciprofloxacin (27.5%), and pefloxacin (5%). In the current research, all the isolates of *Salmonella* showed multiple antimicrobial resistances.

The results of antimicrobial resistance pattern against *Salmonella* isolated from the eggshell surface are shown in Table-2. The results revealed that the isolate from eggs shell surface was the highest resistance to ampicillin (100%) followed by amoxicillin (98.2%), tetracycline (94.5%), erythromycin (83.6%), and others (0-50.9%). The increasing rates of resistance to ampicillin, amoxicillin, tetracycline, and erythromycin among the isolates might attribute to the emergence of multi-resistance *Salmonella* spp.

Table-2: Antimicrobial resistance pattern of *Salmonella* isolates from egg-storing trays, eggshell surface, and egg contents.

Antibiotics	Resistance pattern (%)		
	Egg-storing trays (n)	Eggshell surface (n)	Egg contents (n)
Enrofloxacin	60.0 (40)	47.3 (55)	31.3 (16)
Amoxicillin	100 (40)	98.2 (55)	93.8 (16)
Colistin	52.5 (40)	50.9 (55)	60.0 (16)
Erythromycin	90.0 (40)	83.6 (55)	87.5 (16)
Tetracycline	82.5 (40)	94.5 (55)	62.5 (16)
Ampicillin	97.5 (40)	100 (55)	87.5 (16)
Pefloxacin	5.0 (40)	9.1 (55)	12.5 (16)
Ciprofloxacin	27.5 (40)	49.1 (55)	31.3 (16)

In this study, all the egg contents isolates were tested for the susceptibility against eight different commercially available antimicrobial discs which are showed in the Table-2. Resistance spectrum of *Salmonella* for eight antibiotics tested were enrofloxacin (31.3%), amoxicillin (93.8%), colistin (43.8%), erythromycin (87.5%), tetracycline (62.5%), ampicillin (87.5%), pefloxacin (12.5%), and ciprofloxacin (31.3%).

Discussion

The eggshell surface contamination can occur through egg contact with fecal material, and feed or even during transportation, storage or handling. The results of the present study indicated that the prevalence of *Salmonella* in eggshell surface was significantly higher, and a similar finding was reported by Davies and Breslin [19].The prevalence of *Salmonella* was reported 40% in eggshells in a previous study carried out in Pakistan [20] and 6.1% in India [21]. One possible cause of *Salmonella* contamination in developing countries is repeated use of same egg-storing trays [6]. Egg-storing trays contamination might be due to chicken fecal material or due to the environment [22]. The results of *Salmonella* incidence in commercial egg-storing trays were 7.5% [21] in India. Prevalence was also recorded 43.93% in Pakistan [20] that was almost similar to current research. *Salmonella* prevalence is higher in egg-storing plastic trays due to the use of plastic trays for a long duration without washing

properly or disinfecting. In this study, *Salmonella* was isolated about 13.33% from egg contents, which is higher than in egg contents from Dhaka city (8%), reported earlier by Ahmed *et al*.[14]. In this investigation, the occurrence of *Salmonella* spp. among market eggs is lower than the prevalence of *Salmonella* spp. in egg content reported as 15.07% in Pakistan [20], 5% in North India [23], 7.7% in Southern India [21], and 3.33% in Nigeria [24]. Research findings suggest that in 2002 the prevalence of *Salmonella* spp. in table eggs in some overseas countries were below 1% such as in Denmark, Germany, and the Netherlands were 0.06%, 0.62%, and 0.01%, respectively, whereas the prevalence of *Salmonella* spp. was above 1% among the table eggs in some European countries such as in Austria, Greece, Italy, and Spain were 1.1%, 3.8%, 3.1%, and 8.1%, respectively [25]. The major *Salmonella* spp. isolated during outbreaks was transferred through egg contents all over the world including Bangladesh [14].

Ciprofloxacin is extensively used against *Salmonella* infection in Bangladesh. The results of resistance for ciprofloxacin in this study were more or less similar with other investigations conducted in different places in Bangladesh [2, 26] and other parts of the world [7, 27]. This might be due to ciprofloxacin is naturally less resistance drug against *Salmonella* spp. [28]. Pefloxacin and colistin appeared to be less resistance against *Salmonella* spp. isolated from egg samples. This is might be due to the fact that these drugs are newly introduced and not commonly used against poultry diseases in Bangladesh. The use of antimicrobials in food animals has resulted in the development of antimicrobial resistance, through mutation and acquisition of resistance encoding genes [29]. The situation in developing countries like Bangladesh may be exaggerated by easy accessibility of antimicrobials at a cheaper price and their extensive use in poultry production system [30]. Another major setback might be the quality and potency of locally produced antimicrobial drugs; for example, there are over 80 different brands of the fluoroquinolones in Bangladesh. Thus, there is widespread availability and uncontrolled use of antibiotics poses the antimicrobial resistance in food animals and their products which are the actual threat of public health. These threats correspond to the many non-epidemiological and opportunistic earlier studies in Bangladesh [14] and India [21]. *Salmonella* isolate from the chicken egg and its environment showed resistance to 10 antimicrobials in the US, but the isolates of *Salmonella* in this study were found resistant to ampicillin, amoxicillin, erythromycin, and tetracycline. Multidrug-resistant *Salmonella* Typhimurium was reported in the past few decades and is frequently reported from the Indian subcontinent. Ongoing infection with *Salmonella* organism and use of medication at breeder level could considerably increase the prevalence of multiple resistant *Salmonella* in poultry rearing environment in Bangladesh. Therefore, the present study demonstrated that the *Salmonella* organisms were present in poultry egg and its environment and showed different antibiotic resistance pattern which may cause a serious public health problem in our country.

Conclusion

The results of the present study indicate that *Salmonella*-contaminated eggs are common in the retail markets of Chittagong, Bangladesh. The poor storage and handling practices of eggs at the site of sale could be a source of contamination. From the findings of this study, it is avowed that there are many resistant isolates of *Salmonella* against ampicillin, amoxicillin, tetracycline, enrofloxacin, and erythromycin. The excess utilization of antibiotics in the poultry farms might be the cause of increased resistance. Rational use of antibiotics in animal production and more prudent use of drugs in humans are needed. It is important to take concerted action to improve antibiotic resistance surveillance worldwide with a view to monitoring the emerging resistance genes and their transfer in both animal and human.

Authors' Contributions

TM and MMH conducted the research and actively prepared the manuscript. TM, MMH, and MA designed the work and provided the information. MMK, MSB, and AI participated in the manuscript preparation and advice during the research work. All the authors read and approved the final manuscript.

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Competing Interests

The authors declare that they have no competing interests.

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