

Characterization of Antibiotic Resistant *Salmonella spp* Isolated from Chicken Eggs of Dhaka City

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Abstract

In present study, the occurrence of *Salmonella* in local chicken eggs and their pattern of antibiotic resistance were determined. 100 egg samples collected from different locations of Dhaka city were analysed and *Salmonella spp* were found in 8% of the samples. Among all presumptive *Salmonella* isolates, 8 isolates were confirmed as *Salmonella enterica* subsp. *salamae* (4%), *Salmonella enterica* subsp. *indica* (1%), *Salmonella* Paratyphi-A (1%), *Salmonella bongori* (1%) and *Salmonella Choleraesuis* (1%) on the basis of serotyping and biochemical analysis. These isolates were subjected to susceptibility test against 10 antibiotic disks. All the isolates were found chloramphenicol sensitive. The highest percentage of resistance (87.5%) was found to amoxicillin and ampicillin. Resistance against erythromycin, cephalixin, doxycycline hydrochloride, ceftazidime, doxycycline and nalidixic acid was also found significant ranging from 25% to 62.5%. *Salmonella* isolated from egg shell surface were found more antibiotic resistant than that of egg yolk and white. The present study suggests that poultry eggs are potential reservoir of antibiotic resistant *Salmonellae*.

Keywords: Egg; *Salmonella*; Characterization; Antibiotic resistance.

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1. Introduction

Salmonella food poisoning is one of the most common and widely distributed diseases in the world [1,2] Outbreaks are usually associated with ingestion of contaminated food of animal origin like, poultry, meat and milk [3, 4]. Although the majority of infections results in asymptomatic or self-limited disease; however, in immuno-compromised patients, neonates and elderly, it requires antibiotic treatment [5, 6]. Recently multi-drug resistant (MDR) strains have emerged, presumably due to the extensive use of

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antimicrobial agents both in human and animals. In veterinary practice, antibiotics are used in livestock production, disease prevention and as growth-promoting feed additives [3, 7]. The use of antibiotics in animals disrupts normal flora of intestine, resulting in to emergence of antibiotic-resistant *Salmonellae* and their prolonged faecal shedding into the environment [8, 9]. The fatality rate in people infected with antibiotic-resistant *Salmonellae* is 21 times greater than that infected with non-antibiotic resistant *Salmonella* strains [10].

Egg-associated Salmonellosis is a public health problem. *Salmonellae* infect ovaries of healthy hen and contaminate eggs before shell is formed and if such eggs are consumed raw or undercooked, they cause illness. Imported birds and animals may act as source of *Salmonellae* to the local area leading to outbreaks [11-13]. Our present study aimed at isolation and characterization of different species of *Salmonellae* from chicken eggs of Dhaka city and investigation of their resistance pattern against different antibiotics.

2. Materials and Methods

Collection and enrichment of samples

Five local markets were selected from several places of Dhaka city i.e. New Market Kacha Bazar, Katabon Kacha Bazar, Kawran Bazar, Mohammadpur and Kazipara. Enrichment of egg samples was done according to Official Methods of Analysis of AOAC International (18th edition, 2005). Egg shells were washed with sterile Ringer's solution and enriched in lactose broth. Egg yolk and white was also enriched in lactose broth for 24 hours at 35°C. For selective enrichment, 1 ml from pre-enriched sample was transferred to 10 ml of selenite-cystine broth and additional 1 ml to 10 ml tetrathionate broth and incubated at 35°C for 24 hours.

Salmonella isolation

Selectively enriched samples from selenite cysteine and tetrathionate broths were streaked onto xylose lysine deoxycholate (XLD) agar, Salmonella-Shigella (SS) Agar, and Bismuth Sulfite Agar (BSA). These plates were incubated at 35-37°C for 24±2 hrs. After incubation, typical or suspicious *Salmonella* colonies were examined and further confirmed by biochemical tests including Triple Sugar Iron (TSI) agar, Lysine Iron Agar (LIA) slants and urease test. Only urease negative cultures were selected for characterization and identification by serotyping and biochemical tests including gram staining, IMViC pattern, sugar fermentation, motility, catalase and oxidase test.

Serotyping of Salmonella isolates

Salmonella positive isolates were serologically confirmed by slide agglutination test using polyvalent somatic (O) antisera kit (Remel Europe Ltd, UK). One drop of the polyvalent

O antiserum was placed on the glass slide and one drop of saline suspension of 18 hours old culture was mixed with it. The slide was tilted in a back-and-forth motion for one minute and any degree of agglutination was taken as a positive reaction [14]

Antibiotic susceptibility test

The antibiotic susceptibility test was performed using agar disc diffusion assay as described by Clinical and Laboratory Standards Institute (CLSI, 2000). Antibiotics used were amoxycillin 10 µg, ampicillin 10 µg, cephalixin 30 µg, ceftazidime 30 µg, chloramphenicol 30 µg, doxycycline 30 µg, erythromycin 15 µg, kanamycin 30 µg, nalidixic acid 30 µg and doxycycline hydrochloride 30 µg. Pure colonies of isolated *Salmonellae* were emulsified in normal saline and turbidity was matched with 0.5 McFarland turbidity standards. Selected antibiotic discs were placed on Mueller Hinton Agar plates seeded with bacteria. These plates were incubated at 37°C for 24 hours. The organisms were observed for antibiotic sensitivity based on diameters of zones of inhibition on petridishes. Susceptible and resistant isolates were defined according to the criteria suggested by the CLSI.

3. Results and Discussion

In this investigation, out of 100 samples analyzed, 8 were found positive (8% occurrence). Of the 8 isolates obtained, 6 were from eggshell and 2 from egg yolk & white. On the basis of their biochemical characteristics, these isolates were identified as *Salmonella enterica* subsp. *salamae* (12Y), *S. enterica* subsp. *salamae* (17W), *S. enterica* subsp. *salamae* (19W), *S. enterica* subsp. *salamae* (41W), *S. bongori* (33Y), *S. enterica* subsp. *indica* (13W), *S. Paratyphi-A* (15W) and *S. Choleraesuis* (28W). One *S. enterica* subsp. *salmae* and one *S. bongori* were isolated from egg yolk and white. Three *S. enterica* subsp. *salamae*, one *S. enterica* subsp. *indica*, one *S. Paratyphi-A* (15w) and one *S. Choleraesuis* were found from egg shell. Percentage of *Salmonellae* occurrence on egg shell (6%) was found higher than that of egg yolk and white (2%) (Fig. 1).

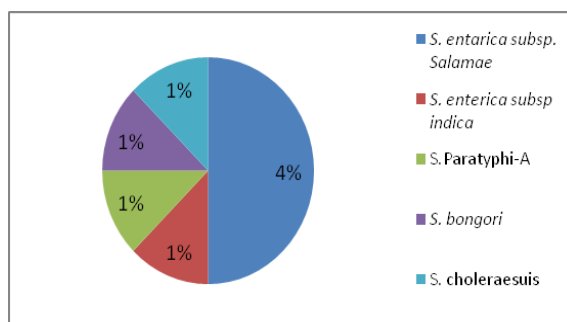


Fig. 1. Occurrence of *Salmonellae* in egg shell and egg yolk.

Approaches to prevent and control Salmonellosis in the food/animal industry by various means such as improved bio-security, vaccination, competitive exclusion and immuno-potential additionally demand antimicrobial chemotherapy. But extensive use of antibiotics in particular has led to emergence of resistant bacteria. In the present study, all the isolates showed significant resistance to 10 commercial antibiotics.

In this study, the size of zone of inhibition of every antibiotic disc was measured in millimeter and while those zones of inhibition compared with zone diameter interpretive standards from CLSI (2000) (Table 1). Among all the antibiotics tested, amoxicillin and ampicillin showed highest resistance followed by cephalixin, erythromycin and doxycycline hydrochloride. Chloramphenicol was the only antibiotic which was found sensitive to all the isolates tested.

Table 1. Antibiotic resistance patterns of isolates.

Salmonella spp.	Name of antibiotic									
	Amoxicillin	Ampicillin	Cephalexin	Ceftazidime	Chloramphenicol	Doxycycline	Erythromycin	Kanamycin	Nalidixic acid	Doxycycline hydrochloride
<i>S. enterica</i> subsp. <i>salamae</i> (12Y)	R	R	R	S	S	I	R	I	R	I
<i>S. enterica</i> subsp. <i>salamae</i> (17W)	R	R	I	R	S	S	I	S	I	S
<i>S. enterica</i> subsp. <i>salamae</i> (19W)	R	R	I	S	S	R	R	S	I	R
<i>S. enterica</i> subsp. <i>salamae</i> (41W)	R	R	S	R	S	R	R	I	S	R
<i>S. bongori</i> (33Y)	R	R	R	R	S	S	R	I	I	S
<i>S. enterica</i> subsp. <i>indica</i> (13W)	R	R	R	S	S	R	I	S	S	R
<i>S. Paratyphi-A</i> (15W)	R	R	R	I	S	S	R	S	I	R
<i>S. Choleraesuis</i> (28W)	I	I	I	S	S	I	I	I	R	I

R- Resistant, I-Intermediate, S-Sensitive

About 87.5% isolates were found resistant to amoxicillin and ampicillin followed by erythromycin (62.5%), cephalixin (50%), doxycycline hydrochloride (50%), ceftazidime (37.5%), doxycycline (37.5%) and nalidixic acid (25%). While, 50% of isolates were intermediately to kanamycin and nalidixic acid followed by 37.5% to erythromycin and cephalixin, 25% to doxycycline and doxycycline hydrochlorid, and 12.5% to amoxicillin, ampicillin and ceftazidime. Kanamycin was sensitive in 50% of the isolates, ceftazidime and doxycycline were sensitive in 50%, nalidixic acid and doxycycline hydrochlorid were sensitive in 25% and cephalixin was sensitive in 12.5% of the isolates. Chloramphenicol sensitivity was 100% in all the isolates (Fig. 2).

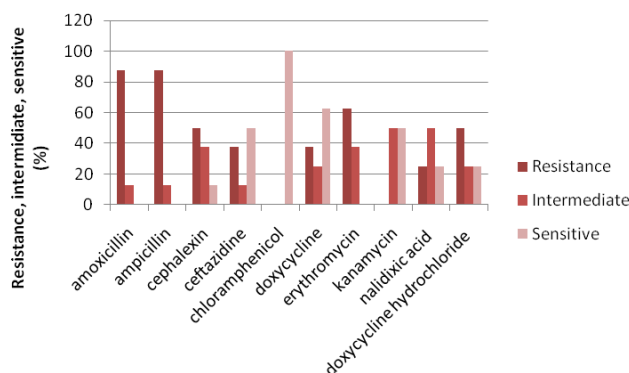


Fig. 2. Percentage of resistant, intermediate and sensitive isolates against commercial antibiotics.

The *Salmonella* isolates from India between 1996-99 and 2001 were reported to be 100% sensitive to chloramphenicol (15). Also, high prevalence of nalidixic acid resistance among poultry isolates (89%) has been reported from France in the year 2000 [16]. Resistance to cephalixin was found lower than reported earlier in India [17].

The level of resistance of *Salmonellae* to antibiotics should be alarming to the food processing, distribution and handling of food product [18, 19]. Therefore, it is necessary to inform people involved in the food industry as well as distributors to take care in handling the food products. Because of the widespread use of antibiotics in poultry, emergence of drug resistant *Salmonellae* has become a matter of concern in Bangladesh. Infection by such multi-drug resistant *Salmonellae* may no longer be treated by conventional therapeutic agents. In order to control *Salmonella* infection of poultry in Bangladesh detailed epidemiological investigation and strain identification are prerequisites [20].

The results indicate the presence of antibiotic resistant *Salmonellae* in chicken eggs of Dhaka city. However, further investigations with bigger sample size are needed to identify the source and cause of drug resistance.

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