

# Frequency of drug resistant *Salmonella* spp. isolated from poultry samples in Bangladesh

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Present study was conducted to determine the levels of *Salmonella* spp. mediated contamination in samples collected from poultry and poultry environments in Bangladesh and to determine the antibiotic resistance trait in those pathogens. A total of 300 samples were collected from five different sources (cloacal swab, intestinal fluid, egg surface, hand wash of chicken handler and soil of chicken market) and 80 (26.6%) samples were found to be contaminated with *Salmonella* spp. One hundred and fifty *Salmonella* strains were isolated among which, 91 were detected as *S. enteritidis* and 59 as *S. typhimurium* through morphological, biochemical and serological tests. Susceptibility of the isolates against 13 antimicrobials was tested, and resistance against chloramphenicol (30 µg), erythromycin (15 µg), ampicillin (10 µg), rifampicin (5 µg), cephalixin (15 µg), cefixime (5 µg), penicillin (10 µg), tetracycline (30 µg), norfloxacin (10 µg), nalidixic acid (30 µg) and ciprofloxacin (10 µg) was found in 58%, 82%, 88%, 60%, 65%, 50%, 100%, 100%, 20%, 20% and 20% of the strains, consecutively. 76% isolates were found to be sensitive against gentamicin and 70% against streptomycin. Some of the isolates interestingly exhibited multiple-drug resistances against at least 6 to 10 antibiotics. The results indicated the higher resistance of *Salmonella* spp. against antibiotics which serving as a threat not only to poultry industry of Bangladesh but also possesses a serious threat to public and animal health.

**Key words:** *Salmonella* spp.; drug resistance; poultry environments

*Salmonella* serotypes are significant zoonotic pathogens in both humans and animals (1) and cause a wide range of human diseases such as enteric fever, gastroenteritis and bacteremia (2). The worldwide annual estimation of the incidence of nontyphoidal salmonellosis is 1.3 billion and annual death is estimated to 3 million cases (3). Poultry and poultry products are imperative elements within the human food chain but they are widely accepted as an important reservoir of intestinal and food-borne pathogen like *Salmonella* and recognized as vital sources of *Salmonella* infection in human (4, 5). Most cases of salmonellosis in human are the consequence of consuming contaminated poultry, pork, beef and eggs (6).

The exploitation of antimicrobial agents in any environment creates selective pressures that favor the endurance of antibiotic-resistant pathogens. The routine practice of antibiotic utilization to domestic animals as a means of preventing and treating diseases, as well as promoting growth, is an important factor in the

emergence of antibiotic-resistant bacteria that are consequently transferred to humans in the course of the food chain (7, 8).

In recent times, a significant increase in the occurrence of antimicrobial drug resistance in *Salmonella* strains is of great concern in both developed and developing countries (9-11). Furthermore, the wide spread use of antimicrobials in farm animals is often implicated in the emergence of multidrug-resistant strains of *Salmonella* (12). The bacterial resistance against antimicrobial agents is known to be driven by the interplay of several mechanistic and epidemiologic factors including the chromosomal defects, random mutation, plasmid exchange, and by the transfer of drug resistance genes by integron or transposon (13-16). In different parts of the world, multi drug resistant strains of *Salmonella* are ubiquitous in poultry and poultry environments (17-18). There are reports of high prevalence of antibiotic resistant *Salmonella* isolates in Bangladesh (19).

Contaminated poultry products are among the most important sources for food-borne outbreaks in humans and *Salmonella* are isolated more often from poultry and poultry products than from any other animal food products (20-23). Like in many other developing countries, the hygiene practice of raw poultry and poultry products, and

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epidemiological study of antimicrobial resistance are in their infancy in Bangladesh. In addition, the lack of rigorous controls on antimicrobial usage, particularly in farm animal production system increases the risk of pathogenic microbes harboring an array of resistance gene. Therefore, this study was focused on the determination of the level of *Salmonella* contamination in the poultry environments and their resistance against different antibiotics in Bangladesh.

## MATERIALS AND METHODS

**Sampling Area and Sample Collection.** The study was carried out during the period from October, 2008 to June, 2010. Three hundred samples from cloacal swabs of chicken ( $n = 80$ ), intestinal fluid of chicken ( $n=70$ ), egg surface ( $n = 60$ ), soil of chicken market ( $n=40$ ) and hand wash of chicken handlers ( $n=50$ ) were collected from various poultry shops of 4 City Corporation markets viz., Mohakhali, Dhaka New Market, Mirpur and Malibag of the Dhaka metropolitan city. The samples were collected following the procedure as stated by Akond et al., 2009 (24).

**Transportation of Sample.** All the samples were transported to the laboratory immediately after collection in an insulating foam box with ice maintaining the temperature ranging from 4 °C to 6 °C. In case of the cloacal swab samples, the test tubes containing selenite broth were incubated for 6 hours at 37 °C immediately after coming to the laboratory for the enrichment of the *Salmonella* spp.

**Isolation of *Salmonella* spp.** Serial dilutions up to  $10^{-3}$  were made for soil sample. 0.1 ml of sample from intestinal fluid, 0.2 ml of sample prepared from soil of chicken market (dilution up to  $10^{-3}$ ) and a loopfull of selective selenite broth from previously incubated cloacal swab sample were spread on the solid surface of *Salmonella-Shigella* (SS) agar medium (Hi-Media, India). A total of 1.0 ml sample from intestinal fluid and soil of chicken market was placed onto sterile plates which was then mixed with sterile medium poured into the plates after being cooled to about 42-45 °C. About 10-100 ml of water sample from egg surface and hand wash of chicken handlers was filtrated through the membrane filter (0.45 µm, Millipore) to isolate the organism. The membrane filter was then placed on the surface of *Salmonella-Shigella* (SS) agar medium. Replications of all samples using triplicate plates were tested for successful isolation of typical colonies by incubating them at 37 °C for 24 hours.

**Biochemical Identification and Serotyping of *Salmonella* spp.** A total of 150 strains were subjected for biochemical identification including Gram staining, urease test, catalase test, oxidase test, H<sub>2</sub>S production test, lactose utilization test, indole test, Voges-Proskauer test and citrate utilization test according to Buchanan and Gibbons, 1974 (25). Moreover, *Salmonella* serotyping was determined by latex agglutination tests using polyvalent antisera (Denka Seiken Co. Ltd, Tokyo, Japan), according to the manufacturer's instruction. The presumptive *Salmonella* isolates were mixed with the latex test reagent and was observed for the agglutination reaction to check the presence of *Salmonella*.

**Antimicrobial Susceptibility Test.** A total of 100 selected *Salmonella* strains, recovered from cloacal swab, intestinal fluid, handwash of chicken handler, egg surface and soil sample from chicken market, were tested for resistance against antimicrobial agents by Kirby-Bauer method (26) using Mueller-Hinton agar plates. The standard procedure of the Clinical and Laboratory Standards Institute (CLSI) (27) were strictly followed throughout the testing procedure. Quality control strain *Escherichia coli* ATCC 25922 was included in each run. All *Salmonella* isolates were subjected against 14 commercial antibiotic discs (Becton Dickinson Antibiotic Disc, U.S.A.) of streptomycin (10 µg), erythromycin (15 µg), chloramphenicol (30 µg), ciprofloxacin (10 µg), tetracycline (30 µg), penicillin (10 µg), norfloxacin (10 µg), cephalaxin (15 µg), rifampicin (5 µg), cefixime (5 µg), ampicillin (10 µg), nalidixic acid (30 µg) and gentamicin (10 µg). The isolates were categorized as susceptible, intermediate and resistant according to the zone diameter interpretative standards recommendations by CLSI (27).

## RESULTS

**Frequency of *Salmonella* isolates from different samples.** Out of 300 poultry samples, 80 (26.6%) were

found to be contaminated with *Salmonella* spp. as determined from several biochemical and serological tests (Tables 1 & 2). Higher proportion of *Salmonella* contamination were found in the intestinal fluid samples (60%). 23.3% of the cloacal swab samples found to harbor *Salmonella* spp. Relatively lower frequency of *Salmonella* contamination were found on the egg surface (13.3%), soil of chicken market (15%) and hand wash of chicken handler (6%) samples (Table 1).

TABLE 1. Frequency of *Salmonella* spp. in various poultry samples

Type of samples	No. of Samples Tested	No. of Samples Positive (%)	Total no. of isolated <i>Salmonella</i> strains	Type of <i>Salmonella</i> species (n = 150)	
				<i>S. enteritidis</i> (%)	<i>S. typhimurium</i> (%)
Cloacal swab	80	21 (26.3)	51	32 (62.8)	19 (37.2)
Intestinal fluid	70	42 (60)	74	43 (58.1)	31 (42.9)
Egg surface	60	8 (13.3)	12	8 (66.7)	4 (33.3)
Hand wash of chicken handler	50	3 (6)	5	3 (60)	2 (40)
Soil of chicken market	40	6 (15)	8	5 (62.5)	3 (37.5)
Total	300	80 (26.6)	150	91 (60.7)	59 (39.3)

TABLE 2. Morphological and biochemical identification of *Salmonella* strains

Test	<i>Salmonella</i> spp.	% <i>Salmonella</i> isolates
Gram Staining	Gram negative, Small	99
Urease	-	80
Indole production	-	75
Catalase	+	90
Voges-Proskauer	-	80
H <sub>2</sub> S production	+	90
Oxidase	+	90
Lactose fermentation	-	90
Citrate utilization	+	80

+ = Positive; - = Negative; BCTC = Black Centered Transparent Colony

Among 150 *Salmonella* isolates, 91 (60.7%) were identified as *S. enteritidis*. Rests of the isolates (39.3%) were detected as *S. typhimurium*. *S. enteritidis* was the predominant species in all the tested samples and it was found more frequently in the egg surface samples (66.7%). Whereas, intestinal fluid samples showed

TABLE 3. Antimicrobial drug resistance pattern of the *Salmonella* isolates

Antibiotic Discs	Antibiotic susceptibility/resistance pattern					
	Resistant		Intermediate		Sensitive	
	% isolates	Inhibition zone (mm)	% isolates	Inhibition zone (mm)	% isolates	Inhibition zone (mm)
Chloramphenicol (30 µg)	58	<12	25	13-17	17	>18
Erythromycin (15 µg)	82	<13	18	14-22	0	>23
Ampicillin (10 µg)	88	<13	12	14-16	0	>17
Gentamicin (10 µg)	0	<12	24	13-14	76	>15
Riphampicin (5 µg)	60	<16	24	17-19	16	>20
Cephalexin (15 µg)	65	<15	15	16-20	20	>21
Cefixine (5 µg)	50	<15	20	16-18	30	>19
Penicillin (10 µg)	100	<28	0	NA	0	>29
Tetracycline (30 µg)	100	<14	0	15-18	0	>19
Streptomycin (10 µg)	0	<13	30	14-17	70	>18
Norfloxacin (10 µg)	20	<12	25	13-16	55	>17
Ciprofloxacin (5 µg)	20	<15	15	16-20	65	>21
Nalidixic acid (30 µg)	20	<13	10	14-18	70	>19

relatively higher frequency (42.9%) for *S. typhimurium* than the other samples.

**Antibiogram of the isolates.** All the *Salmonella* isolates showed resistance against penicillin and tetracycline (Table 3). Higher resistance was observed against ampicillin (88%), erythromycin (82%), cephalixin (65%), riphampicin (60%), chloramphenicol (58%) and cefixine (50%). The isolates showed lower resistance (20%) against norfloxacin, ciprofloxacin and nalidixic acid. No isolate was found to be resistant against gentamicin and streptomycin (Table 3).

## DISCUSSION

The present study demonstrated a considerable high frequency (26.6%) of contamination in poultry samples with *Salmonella* spp. Therefore, poultry seems to be one of the main reservoirs of *Salmonella* spp. in Bangladesh. The high level of contamination indicates an alarming situation, both for chicken farming and public health as well. A similar rate (25%) of *Salmonella* spp. contamination in the samples from poultry and poultry environments has been evident by Hassan, 2004 in Bangladesh (28). That study also reported that 25% of the cloacal swab samples and 50% of intestinal fluid samples were contaminated with *Salmonella* spp. In this study, we found *Salmonella* contamination in 26% and 60% of cloacal swab and intestinal fluid samples, respectively. The occurrence of *Salmonella* contamination in samples from poultry sources has also been reported from various parts of the world; 17%, 35%, 36%, 39%, and 53% in USA, Spain, Korea, Brazil and Vietnam, consecutively (29-33). The presence of *Salmonella* spp. in the hand wash samples

of chicken handler indicated a potential breakdown of personal hygiene at the stage of chicken handling and processing.

Studies have demonstrated that poultry feeds have been implicated as an important source of *Salmonella* spp. (34) and may therefore be the consequence of the subsequent contamination of eggs. However, egg surface might have been contaminated with *Salmonella* spp. with feces during lay in unhygienic condition or from infected poultry. Among the animal protein ingredients, a major ingredient of poultry feeds, locally processed cheap fish wastes were found to be a vital cause for bacterial contamination of poultry feeds (18). Moreover, *Salmonella* was reported as a common microflora in animal feedstuffs, raw feeding materials and poultry feeds (34). Careless and unhygienic handling process serves as a frequent source of contamination with *Salmonella* in pre-stuffed chickens in poultry shops. Poultry and poultry products like eggs and plastic-wrapped poultry meat found in various super shops and ready-to-eat foods become cross contaminated with *Salmonella* spp. and other pathogenic bacteria from food handlers with poor personal hygiene and may be from other raw poultry products.

In addition, our study confirmed a high incidence of antibiotic resistance with the frequency of 20% to 100% among *Salmonella* spp. isolated from poultry and poultry environments in Bangladesh. In present study, *Salmonella* strains isolated from poultry sources were commonly resistant against ampicillin, tetracycline and chloramphenicol and susceptible to nalidixic acid and gentamicin as found in several studies in Bangladesh (28, 32, 33, 35-37). However, resistance against gentamicin and streptomycin was absent in all strains and comparatively lower resistance was observed against

norfloxacin, nalidixic acid and ciprofloxacin. Resistance against penicillin, ampicillin, tetracycline and erythromycin was often observed due to low cost, ready availability and for drug abuse (33). Therefore, prudent use of antimicrobials in animal production system has been accepted worldwide as a means of preventing development of the antimicrobial resistance in pathogenic bacteria (38). Moreover, all the isolates exhibited multidrug resistance against more than five antibiotics. Similar findings on multidrug resistance among *Salmonella* strains have been reported from Bangladesh and various parts of the world (18, 28, 35-37).

The ability of bacteria to acquire antibiotic resistance gene and subsequently spread them to many different bacterial species is now well known. Integrins play an important part in the transfer of resistance among *Salmonella* serotypes to a variety of antimicrobial drugs (37, 39). Several surveys on antibiotic operation in Bangladesh have revealed that peoples are in the habit of consuming antibiotics familiarized in frequent uptake of antibiotics than necessary (28) and antibiotics can be bought here easily from drugstores without any prescription. It may facilitate the development of multi-drug resistant pathogens, as regular exploit of antimicrobials would put selective pressure for development and proliferation of resistance genes. In addition, low cost and available antimicrobials like ampicillin, penicillin, tetracycline and erythromycin are frequently used as growth promoters or feed additives or preservatives to the food producing animals and poultry flocks to assuage the escalating food requirements for the augmented population in Bangladesh.

The results of the study have illustrated the extent of antibiotic resistance in *Salmonella* serotypes from poultry sources in Bangladesh. The domestic and commercial handlers of poultry and poultry products in chicken shops and household and the peoples engaged in the poultry farms need more attention to strictly follow the rules and guidelines of hygiene to reduce or eliminate the risk of antibiotic resistant *Salmonella* and other pathogenic microbes. In addition, the use of antibiotics both for farming and for medication should be astute to minimize the chance for organisms to develop resistance.

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