

## Original Article

# Isolation and antibiogram of *Salmonella* spp. from duck and pigeon in Dinajpur, Bangladesh

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## ABSTRACT

**Objective:** This research work was conducted for isolation of *Salmonella* spp. from duck and pigeon and assessment of antibiotic sensitivity patterns of the isolated *Salmonella* spp.

**Materials and methods:** A total of 48 duck samples comprising of liver (n=16) spleen (n=16) and intestinal content (n=16) were collected from 16 ducks (8 sick and 8 dead). Similarly, 42 pigeon samples comprising of liver (n=14), spleen (n=14) and intestinal contents (n=14) were collected from 14 pigeons (7 sick and 7 dead). The samples were collected from the selected Duck and Pigeon farms at three *Upazillas* (sub-districts) in Dinajpur district, Bangladesh. The samples were subjected for isolation and identification of *Salmonella* spp. following standard bacteriological examinations such as cultural and morphological characteristics, biochemical properties and motility test. Commercially available antibiotic discs were used for the assessment of antibiotic resistant patterns by disc diffusion method.

**Results:** *Salmonella* spp. could be isolated from 39.58% (n=19/48) duck and 28.57% (n=12/42) pigeon samples. The growth of colony with different characteristics in Brilliant Green agar, Nutrient agar, Salmonella-Shigella agar and Mac Conkey agar indicated positive results. Dextrose and mannitol were fermented and in Triple Sugar Iron (TSI) slant media, the isolates produced red color, yellow in butt with slightly black color. Positive result was found in Indole and Methyl Red media. The isolated *Salmonella* organisms were found to be highly sensitive to Azithromycin, Ciprofloxacin and Levofloxacin.

**Conclusion:** Pigeons and ducks found in the study area may be the carrier of *Salmonella* spp. The farm should be checked periodically to know the status of Salmonella infection. Further study might be isolation and identification of Salmonella organism with detail genomic analysis and drug resistance.

## KEYWORDS

Antibiotic resistant, Duck, Isolation, Pigeon, *Salmonella* spp.

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## INTRODUCTION

A wide variety of *Salmonella* serovars in different hosts like duck, pigeon remain as significant problem with importance to public health all over the world ([Tabaraie et al., 1994](#)). All ages of poultry were infected with *Salmonella* spp. which produce considerable loss in poultry industry ([Habrun et al., 2006](#)). It may be a threat in poultry rearing farm and as a result, sound health of these birds will be affected producing low quality eggs and meat that will lead to poor market value ([Molla et al., 2006](#); [Yadav et al., 2006](#)).

One of the member of Enterobacteriaceae family is Salmonella and all the spp. are Gram-negative, non capsulated, small rod shaped, non spore forming, facultatively anaerobic and aerobic ([OIE, 2006](#); [Denise et al., 2015](#)). A large group of serologically and biologically related bacilli of genus *Salmonella* which are motile by means of peritrichous flagella with the exception of *Salmonella gallinarum* and *S. pullorum* ([Blood et al., 2003](#)). Only 10% *Salmonella* spp. have been isolated and over 2,300 serotypes were identified from poultry ([Gast, 1997](#)). Both *S. pullorum* and *S. gallinarum* were found in chicken which acts as natural host ([Snoeyenbos, 1991](#)). Pigeons might be the sources of salmonellosis for human. *S. enterica* subspecies serovar *typhimurium* variant Copenhagen ([Rabsch et al., 2002](#)) and *S. typhimurium* and *S. cerro* were isolated from Pigeon ([Tanaka et al., 2005](#)). Moreover, pigeons may transfer the infectious agents to outdoor domestic poultry and drinking water sources including agricultural crops ([Lillehaug et al., 2005](#)).

The greatest mortality of the ducklings occurs within the first week or 7-10 days. Generally different cultural, biochemical, serological and molecular studies are used to characterize the *Salmonella* organisms ([Begum et al., 2010](#)). Salmonellae giving rise to a large number of serotypes besides leading to variation in pathogenicity of the species and may cause serious confusion in diagnosis of the disease.

Antibiotic sensitivity was performed by [Gyurov and Dyako \(2000\)](#) for the treatment of salmonellosis with effective antibacterial agents. However, due to the recent emergence of multi-drug resistant of *Salmonella* strains, the treatment of salmonellosis with antibiotic became difficult ([Mirza et al., 1996](#)). Result of extensive use of antibiotics in veterinary medicine, serious increase in the spreading of multiple antibiotic resistant *Salmonella* has occurred ([Cruchaga et al., 2001](#)). The present research work has been undertaken to isolate and identify *Salmonella* spp. with their antibacterial sensitivity from duck and pigeon.

## MATERIALS AND METHODS

**Ethical approval:** The samples were collected from the sick birds after sacrificing by following standard sample collection procedures from live bird.

**Sample collection:** In this study, total 90 field samples were comprised liver, spleen, feces and intestinal contents were aseptically collected from the selected Pigeon and Duck farms of three selective *Upazillas* (sub-districts) in Dinajpur district, Bangladesh for the characterization of salmonellae as per the procedure described in OIE Manual ([OIE, 2006](#)) and by [Douglas et al. \(1998\)](#). A set of sterile instruments were used for dissecting the samples after collection. For cultural examination swab samples containing test tubes were immediately brought to the laboratory.

**Isolation of *Salmonella* spp. by using bacteriological media:** After collection, all the samples were inoculated into each of the freshly prepared Selenite Broth (SB) and Nutrient Broth (NB) separately. Sample containing tubes were well marked then incubated at 37°C for 24 h aerobically in bacteriological incubator. Then examined for growth of bacteria and a loopful from all incubated tubes were streaked separately on to the Nutrient agar (NA) plates. For the growth of bacteria, the plates were incubated at 37°C for 24 h aerobically in bacteriological incubator. On to Brilliant Green (BG) agar, Eosin Methylene Blue (EMB) agar, Mac Conkey (MC) agar and Salmonella-Shigella (SS) agar plates, a loopful of bacterial culture from incubated tubes were streaked separately and incubated at 37°C for 24 h. To get a pure culture of *Salmonella* spp., characteristic colonies of Salmonellae from BG agar plates were selected for sub-culturing.

**Maintenance of stock culture:** Sterile buffered glycerin (20%) was performed by mixing 20 parts of pure glycerin and 80 parts of PBS. Then a loopful of thick bacterial culture was mixed with 20% sterile buffered glycerin in small vials and was preserved at -20°C ([Buxton and Fraser, 1977](#)).

**Morphological characterization by Gram stain:** According to the method stated by [Merchant and Packer \(1967\)](#), the smears were prepared from each of the cultural plates, fixed and stained by using the Gram's staining technique and examined under microscope at 100x magnifications.

**Specific biochemical tests for identification of isolated *Salmonella* spp.:** Triple Sugar Iron (TSI) agar slant reaction, carbohydrate fermentation tests, Methyl-Red-Voges-Proskauer (MR-VP), Indole and Motility

Indole Urease (MIU) reaction tests were performed for the identification of suspected *Salmonella spp.* For the detection of *Salmonella spp.*, all the isolates of different sources were tested by following the methods narrated by [Khan et al. \(2005\)](#) and in OIE Manual ([OIE, 2006](#)).

**Antibiogram study:** On SS agar media, the NB-cultured *Salmonella* isolates were poured uniformly. All antibacterial discs were applied aseptically and incubated at 37°C for 24 h in aerobic condition ([Detha and Datta, 2016](#)). Different antibacterial disc were used which were manufactured by Oxoid Ltd., Basingstoke, Hampshire, England (**Figure 1**).

## RESULTS

In this study, prevalence of *Salmonella spp.* in duck was 39.58% and in pigeon, the prevalence as 28.57% respectively (**Table 1**). After 24 h of incubation at 37°C aerobically found turbidity in NB. Growth of colony with different characteristics on NA, BG agar, SS agar and MC agar indicated positive results (**Table 2**). The organisms were found as Gram-negative with definite appearance as short rod in Gram's staining technique (**Table 2**). Acid and gas or only acid was produced due to fermentation of Dextrose and Mannitol by the isolates. Lactose and Sucrose were not fermented but slight fermentation occurred in Maltose. Red color turned to yellow indicated acid production. No urease production in the MIU medium was observed, and the test tubes remained clear

indicating non-motile nature of the organism. Further, the isolated *Salmonella spp.* produced an alkaline reaction (red) in slant and acid reaction (yellow) in the butt with slightly black color in TSI slant media due to H<sub>2</sub>S production. All the isolates were Indole, MR positive.

In this study pattern of antibiotic sensitivity was performed after incubation plates were examined and diameters of the zones of inhibition of growth. From the antibacterial susceptibility we found *Salmonella spp.* from duck, were highly sensitive to Ciprofloxacin, Levofloxacin and Azithromycin (**Figure 1**). In addition moderately sensitive to Colistin sulphate; less sensitive to Doxycycline, Bacitracin, Erythromycin, Gentamicin and Cefotaxime. And resistance to Amoxicillin and Tetracycline. Among the isolates of pigeon highly sensitive to Azithromycin, Ciprofloxacin, Levofloxacin; moderately sensitive to Cefotaxim, Gentamicin and less sensitive to Bacitracin, Colistin Sulphate and Doxycycline (**Figure 1**).

## DISCUSSION

Present research work was conducted to isolate and identify *Salmonellae spp.* from pigeon and duck of different farms at Dinajpur district as well as antibiogram study of those isolated species. There was a great difficulty for the isolation of *Salmonella* in the present study due to the prevalence of other organisms like *Escherichia coli* and other unidentified organisms. To overcome this

**Table 1.** Result of isolation of *Salmonella* from liver, spleen and intestine of Duck and Pigeon

Species tested	Organs tested		Sample tested	Positive cases		Total <i>Salmonella</i> positive	Percentages of prevalence (%)	
	Sick	Dead		Sick	Dead			
Duck (N=16)	8	8	Liver (n=16)	48	2	5	19	39.58
			Spleen (n=16)		2	5		
			Intestine (n=16)		2	3		
Pigeon (N=14)	7	7	Liver (n=14)	42	3	2	12	28.57
			Spleen (n=14)		2	3		
			Intestine (n=14)		1	1		

**Table 2.** Morphology, cultural and staining characteristics of isolated *Salmonella spp.*

Isolates	Colony characteristics			Morphology (staining characters)
	SS agar	MC agar	BG agar	
Duck sample	Opaque translucent colorless smooth Round colonies.	Pale, colorless, smooth transparent raised colonies.	Pale, pink color colonies against a pinkish background	Pink color, short rod shaped and Singly arranged.
Pigeon sample	Opaque translucent colorless smooth round colonies	Pale, colorless smooth transparent raised colonies	Pale, pink color colonies against a pinkish background	Pink color, short rod shaped and Singly arranged.

**Table 3.** Carbohydrate fermentation and other biochemical tests of the isolated *Salmonella spp.*

CHO fermentation and other biochemical tests		Results	
		Duck	Pigeon
Dextrose fermentation		+	+
Lactose fermentation		-	-
Sucrose fermentation		-	-
Mannitol fermentation		+	+
Indole production		+	+
MR test		+	+
VP test		-	-
MIU medium	Motility	±	±
	Indole	+	+
	Urea	-	-

difficulties and confirmation of the isolates as *Salmonella spp.*, Gram's staining technique, cultures on specific media and biochemical tests were performed.

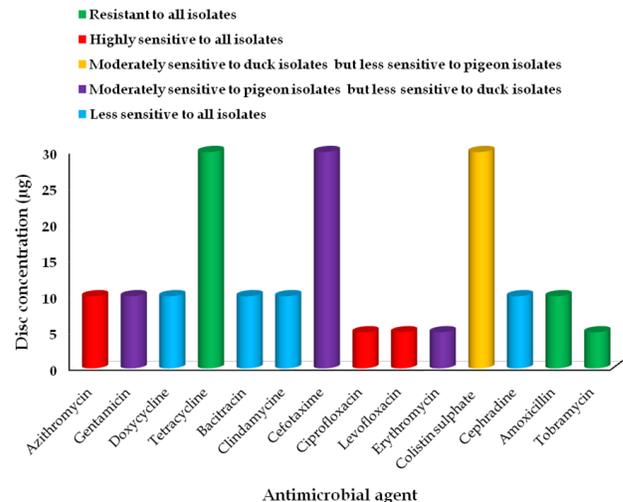
Infection by *Salmonella spp.* is a common cause of food poisoning in humans (Hobbs and Robert, 1993). Thus, this organism has great public health significance. Salmonellosis assumes to occur as one of the following forms- peracute septicemia, acute enteritis, chronic enteritis or a subclinical carrier state. Common clinical signs are- septicemia, fever, enteritis, diarrhea (Blood et al., 2003).

A number of researchers were used specific enriched media and biochemical tests for the isolation and identification of the *Salmonella spp.* (Ruiz et al., 1992; Sharma and Katock 1996; Dhruba et al., 1999; Habrun et al., 2006). In this present research work, we also found the similar colony characteristics of *Salmonella spp.* from pigeon and duck on SS agar, BG agar and Mac Conkey agar (Table 3). Further, the morphology of the isolated *Salmonella spp.* exhibited Gram negative, small rod shaped, single or paired arrangement under microscope which were similar to the method described in OIE manual (OIE, 2006).

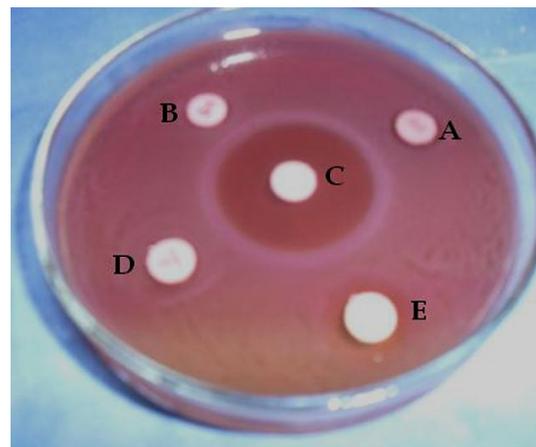
In biochemical tests, Dextrose, Maltose and Mannitol were fermented by the isolates but Lactose and Sucrose were not fermented (Table 3), as described by Buxton and Fraser (1977). All the isolates were positive to M-R test but negative to V-P test, as described by Parvej et al. (2016).

In this study, Levofloxacin, Azithromycin and Ciprofloxacin were found to be highly sensitive against the *Salmonella spp.*, which is supported by the findings of John et al. (2015). The duck isolates were moderately sensitive

to Colistin sulphate, whereas, the pigeon isolates were moderately sensitive to Gentamycin, Cefotaxime, Pefloxacin, Erythromycin and Carbinicillin. Doxycycline, Bacitracin, Erythromycin, Gentamicin and Cefotaxime were found to be less sensitive against the *Salmonella* isolates of ducks. The pigeon isolates were less sensitive to Bacitracin, Colistin Sulphate and Doxycycline. All the isolates were found to be resistant to Amoxicillin, Tetracycline and Tobramycin (Figure 2).



**Figure 1.** Sensitivity of *Salmonella* isolates to different antimicrobial agents with discs concentration.



**Figure 2.** Sensitivity of *Salmonella* isolates to (A) Gentamicin, (B) Amoxicillin, (C) Azithromycin, (D) Doxycycline, (E) Colistin sulphate.

In field condition, the antibacterial resistance was observed in the isolated *Salmonellae* due to indiscriminate use of antibacterial agents in the study areas, and the incorporation of resistant genes in the plasmid harboring in the isolates (Khan et al., 2005; Parvej et al., 2016). Careful drug choice by the veterinary

practitioners and physicians for the treatment of salmonellosis in human, animals and birds may reduce the economic loss, as described by [Cherry et al. \(2004\)](#), [Zhang et al. \(2006\)](#) and [Chowdhury et al. \(2011\)](#).

In this study, duck and pigeon isolates showed 100% resistance to Amoxycylin, and 78.57% to Gentamicin. These findings are in close agreement with the findings of [Saifullah et al. \(2016\)](#). It can be stated that, the isolates responsible for salmonellosis in the selected farms might be due to any one of the following agents namely *S. pullorum*, *S. gallinarum*, *S. anatum*, *S. enteritidis*, *S. Newport*, *S. Oregon*, *S. saintpaul*, *S. munhattan* or *S. manchester*. Specific identification and molecular studies including detail genomic analysis are suggested as future studies, which may give more specific data in terms of molecular basis of pathogenicity and drug resistance in Salmonellae.

## CONCLUSION

*Salmonella spp.* are persistently prevailing in the duck and pigeon farms at the study areas. The farms should be checked periodically to know the status of *Salmonella* infection and the positive reactors should be culled, and biosecurity plan of the farms should be improved accordingly. Ciprofloxacin, Levofloxacin and Azithromycin are the most effective antibiotics in the treatment of salmonellosis among the antimicrobial agents used in this present study.

## CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

## ACKNOWLEDGMENT

Nothing to declare.

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