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Original Article

Molecular detection of *Salmonella* spp. isolated from apparently healthy pigeon in Mymensingh, Bangladesh and their antibiotic resistance pattern

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ABSTRACT

Objectives: Here we determined the prevalence of *Salmonella* in cloacal swabs and pharyngeal swabs of apparently healthy pigeons sold in the live bird markets and villages in and around Bangladesh Agricultural University Campus, Mymensingh, Bangladesh.

Materials and methods: A total of 50 samples, comprised of cloacal swabs (n=24) and pharyngeal swabs (n=26) were collected. The samples were processed, and *Salmonella* was isolated through a series of conventional bacteriological techniques and biochemical tests followed by polymerase chain reaction (PCR).

Results: The prevalence rate of *Salmonella* was found to be 37.5% (n=9/24) in cloacal swabs and 30.77% (n=8/26) in pharyngeal swabs with an overall prevalence rate of 34% (n=17/50). The prevalence rate of *Salmonella* pigeon varied slightly among locations; 34.62% (n=9/26) in live bird markets, and 33.33% (n=8/24) in villages. Molecular detection of 17 *Salmonella* isolates obtained from biochemical test was performed by genus specific PCR, where all of them amplified a region of 496-bp segment of the *histidine transport operon* gene. Antibiogram study revealed multi-drug resistant traits in most of the isolates tested. The highest resistance was found against Ampicillin (88.23%) followed by Cephalexin (82.35%). The rate of sensitivity of the isolates to Ciprofloxacin was 100% followed by Azithromycin (82.35%), Gentamicin (76.47%) and Nalidixic acid (76.47%).

Conclusion: Our findings suggest that pigeons carry multi-drug resistant *Salmonella* that may transfer to the humans and animals.

KEYWORDS

Apparently Healthy, Antibiogram, PCR, Pigeons, *Salmonella* spp.

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INTRODUCTION

Pigeons (*Columbia livia*) are found in both rural and urban areas in Bangladesh, and the birds come into close contract with human in houses, live bird markets and farms. Pigeons and pigeon droppings are known to carry several communicable diseases (Weber, 1979). Pigeons are known to be the potential reservoir for several pathogenic microorganisms, including *Salmonella* spp., *E. coli*, *Chlamydia* spp. and *Cryptococcus* spp. (Tanaka et al., 2005). Feces of pigeon in yards and live bird markets largely contributed in spreading the infectious agent to the surrounding environment. Healthy pegions may carry *Salmonella* spp. that has zoonotic importance (Fallacara et al., 2001; Wahlstrom et al., 2003). Pigeons may act as a fecal contaminator of agricultural crops, drinking water sources, and may transfer the infectious agents to outdoor domestic poultry through direct contact (Lillehaug et al., 2005). During dressing, pigeon's meat might be contaminated with *Salmonella* due to lack of proper hygienic measures (Bryan and Doyle, 1995). The apparently healthy pigeons available in the farms and live birds markets are important sources of human salmonellosis (Hossain et al., 2012).

Nowadays, multidrug resistant *Salmonella* in food animals is an emerging issue all over the world. In developed world, zoonotic agents acquired antibiotic resistance in an animal host being entry to humans through the food chain (Molback et al., 2002; Threlfall, 2002). Resistance against an antibiotic may occur due to indiscriminate use of sub therapeutic doses of antibiotics. To the best of our knowledge, not much work has been carried out in Bangladesh on molecular detection of *Salmonella* spp. from pigeon. This study was therefore designed to detect *Salmonella* spp. from pigeon using polymerase chain reaction (PCR) based approach including their antibiogram.

MATERIALS AND METHODS

Ethical approval, sampling and preliminary isolation of *Salmonella* spp.: A total of 50 samples from cloacal swabs (n=24) and pharyngeal swabs (n=26) were collected from Bangladesh Agricultural University (BAU) market and surrounding areas over the period of January 2015 to May 2015. The samples were collected by following ethical guidelines without harming the brids. The collected swabs were inoculated into nutrient broth immediately after collection and incubated at 37^{0}C overnight for enrichment. The broth culture was then streaked onto Salmonella-Shigella (S-S) and MacConkey's agars. Suspected colonies were further analyzed by Gram's method and biochemical tests for preliminary detection of *Salmonella* spp. (Khan et al., 2005; Hasan et al., 2015; Parvej et al., 2016).

Molecular detection of *Salmonella* spp. by PCR: DNA was extracted from the isolated bacterial agents using boiling method (Queipo-Ortunet al., 2008). PCR was applied to detect *Salmonella* spp. using the genus specific primers targeting the *Histidine Transport Operon* gene, as described by Salauddin et al. (2015) with slight modification. The sequences of the oligonucleotide primers are mentioned in **Table 3**. PCR was carried out in a 25 µL reaction volume containing 1x*Taq* Polymerase PCR master mix and 10 pmol of each primer. Amplification was performed in a thermal cycler (Eppendorf, Germany). The cycling condition was as follows: initial denaturation 94°C for 3 min, followed by 30 cycles of denaturation at 94°C for 30 sec, annealing at 60°C for 30 sec, elongation at 72°C for 45 sec, and a final extension at 72°C for 10 min (Salauddin et al., 2015). Electrophoresis was run at 100-V on a 1.5% agarose gel after mixing PCR product with loading buffer along with a 100-bp DNA ladder (Promaga, USA). Agarose gel was staining with ethidium bromide and de-stained in distilled water in consequence. Then, the picture was documented and photographed under UV light.

Antibiotic Sensitivity Assay: The isolated *Salmonella* spp. were subjected to antimicrobial sensitivity test against 6 commonly used antibiotics of different groups by disc diffusion method, as described by Khan et al. (2005). The antibiotics used were Ampicillin (25 µg/disc), Cephlexin (30 µg/disc), Ciprofloxacin (5 µg/disc), Gentamicin (10 µg/disc), Azithromycin (30 µg/disc) and Nalidixic acid (30 µg/disc). Comparing with 0.5 McFarland turbidity standard, inoculums were prepared and the test was done in freshly prepared Mueller Hinton agar (HiMedia, India). The results of the sensitivity test were expressed as either resistant or sensitive, as per the guidelines of Clinical and Laboratory Standard Institute (CLSI, 2012).

RESULTS AND DISCUSSION

Salmonella formed smooth, small, round and black centered colonies onto S-S agar, and colorless, smooth, transparent, raised in appearance on McConkey's agar. *Salmonella* showed Gram-negative (pink) rods, arranged in single or pair in Gram's method. Carbohydrate fermentation test showed that *Salmonella* fermented maltose, dextrose, and mannitol producing both acid and gas, but did not ferment sucrose and lactose. Methyl Red (MR) test was positive, whereas Voges–Proskauer (VP) and indole tests were negative.

Table 1. Prevalence of *Salmonella* in cloacal swabs & pharyngeal swabs of pigeons.

SL	Sources of samples	No. of samples	Positive for *Salmonella*	Prevalence (%)
01	Cloacal swabs	24	9	37.5
02	Pharyngeal swabs	26	8	30.77
Overall		50	17	34.0

Table 2. Prevalence of *Salmonella* in pigeons according to locations.

SL	Locations	No. of samples	Positive for salmonella	Prevalence (%)
1	Live bird market	26	9	34.62
2	Village	24	8	33.33

Table 3: *Salmonella* genus specific PCR primers with sequences.

Primer	Sequence	Target gene	Amplicon Size (bp)
Sal-G	F 5'-ACTGGCGTTATCCCTTTCTCTGGTG-3'	*Histidine Transport Operon*	496
	R 5'-ATGTTGTCCTGCCCCTGGTAAGAGA-3'		

Table 4: Resistance profile of *Salmonella* isolates of pigeons against ten antibiotics.

Sources of samples	No of isolates tested	GEN	AMP	CIP	CN	NA	AZM
Cloacal swabs	9	2	6	0	3	3	1
Pharyngeal swabs	8	1	5	0	3	2	0

AMP=Ampicillin, CIP=Ciprofloxacin, CN=Cephalexin, GEN=Gentamicin, NA=Nalidixic Acid, AZM=Azithromycin

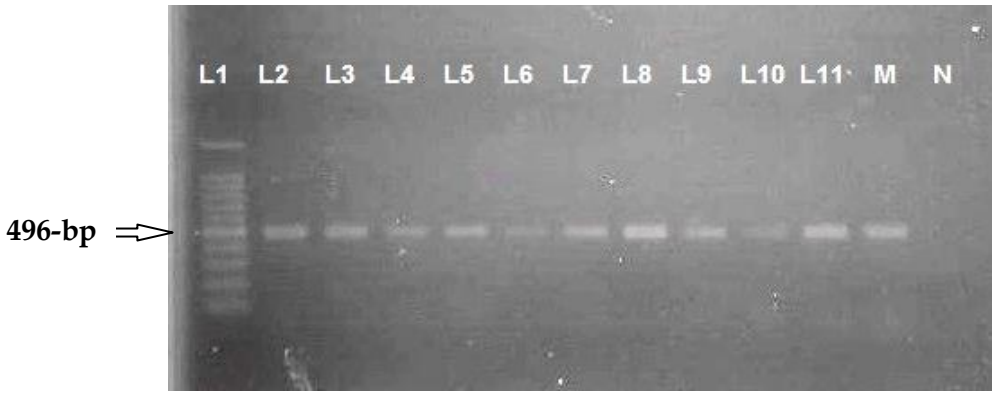

Figure 1. PCR assay to amplify *Histidine Transport Operon* gene of *E. coli* isolates recovered from apparently healthy pigeon. Lane 1: 100-bp DNA marker; Lane 2-11: representative *Salmonella* isolates from rectal swab; Lane M: Positive control, and Lane N: Negative control.

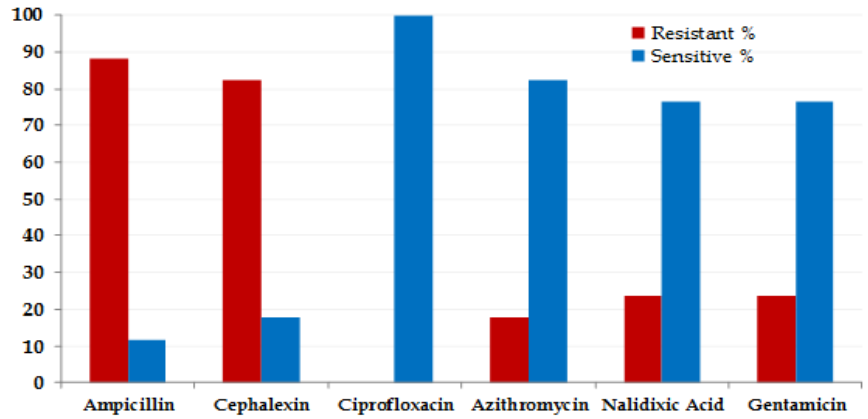

Figure 2. Antibiogram profile of *Salmonella* isolated from pigeons against ampicillin (AMP), cephalexin (CN), ciprofloxacin (CIP), azithromycin (AZM), nalidixic acid (NA) and gentamicin (GEN).

A total of 17 isolates were confirmed as *Salmonella* spp. by genus specific polymerase chain reaction (PCR) (**Figure 1**). Prevalence of *Salmonella* found as 37.5% (n=9/24) in cloacal swabs and 30.77% (n=8/26) in pharyngeal swabs with an overall prevalence rate of 34% (n=17/50) (**Table 1**). The prevalence rate of *Salmonella* in pigeon slightly varied among locations; 34.62% (n=9/26) in live bird market, and 33.33% (n=8/24) in villages (**Table 2**).

Antibiogram study of 17 *Salmonella* positive isolates revealed multi-drug resistance *Salmonella*. The highest resistance was found against Ampicillin (88.23%) followed by Cephalexin (82.35%). The rate of sensitivity of the isolates was higher to Ciprofloxacin (100%) followed by Azithromycin (82.35%) Gentamicin (76.47%) and Nalidixic acid (76.47%). The resistance patterns of the isolated *Salmonella* isolates against antibiotics are given in **Table 4**.

DISCUSSION

The aim of the present research work was to isolation and characterization of *Salmonella* spp. from apparently healthy pigeons from different live bird markets and village areas in and around BAU Campus, Mymensingh, Bangladesh using PCR based approach.

In this study, among the 50 samples analyzed 17 (34%) were found to be positive for *Salmonella* spp. The sugar fermentation test revealed all the isolates as fermenter of dextrose, maltose and mannitol and produced acid, and non-fermenter of sucrose and lactose, as stated by Buxton and Fraser (1977). The isolates were also found positive for MR test and negative for VP and to indole test, as reported by Merchant and Packer (1967) and Parvej et al. (2016).

The prevalence of *Salmonella* spp. in cloacal swabs and pharyngeal swabs were higher as compared to the prevalence rate reported by Hosain et al. (2012) who found a 22.22% prevalence of *Salmonella* in cloacal swab. This higher prevalence of *Salmonella* may be due to lack of proper hygiene and sanitary practices, cross contamination with other species of birds and overcrowding. Data of this study underscore the need for strict hygienic and sanitary practices at the live bird markets and in rearing house of village.

The significance of occurrence of antibiotic resistance in food-borne pathogens has increased sharply and probably linked with the extensive use of antimicrobial agents in veterinary medicine and human (Bronzwaer et al., 2002). Several species of *Salmonella* are known to carry multi drug resistant genes (Gebreyes and Altier, 2002) which have been a matter of concern. This study recorded the presence of multidrug resistance *Salmonella* in pigeons in Mymensingh area. The resistance and sensitivity profile of *Salmonella* isolates of pigeons against 6 antibiotics recorded in this study satisfied partly the findings of some authors (Rahman et al., 2011; Hosain et al., 2012). In this study, the rate of sensitivity of the isolates to Ciprofloxacin was 100% followed by Azithromycin (82.35%), Gentamicin (76.47%) and Nalidixic acid (76.47%). The highest resistance was found against Ampicillin 88.23% followed by Cephalexin 82.35%. This data was almost similar to the findings of Jahantigh and Nili (2010).

CONCLUSION

Salmonella spp. has been successfully isolated from cloacal swabs and pharyngeal swabs of apparently healthy pigeons sold at live bird markets and villages in and around BAU, Mymensingh. The isolates are confirmed as *Salmonella* by PCR. The overall prevalence rate is 34% (n=17/50). Antibiogram study reveals the presence of multi-drug resistance *Salmonella* spp. in most of the isolates. The highest resistance has been found against Ampicillin (88.23%) followed by Cephalexin (82.35%). On the other hand, the rate of sensitivity of the isolates to Ciprofloxacin was 100% followed by Azithromycin (82.35%). Present findings suggest that apparently healthy pigeons found in live market and villages in the study area maintain multi-drug resistant *Salmonella* which may be transferred to the humans and animals.

CONFLICT OF INTEREST

The authors declare that they have no competing interest.

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