International Journal of One Health
Available at www.onehealthjournal.org/Vol.2/7.pdf

RESEARCH ARTICLE
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Prevalence and multidrug resistance pattern of *Salmonella* isolated from resident wild birds of Bangladesh

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Received: 05-08-2016, **Accepted:** 19-09-2016, **Published online:** 01-10-2016

doi: 10.14202/IJOH.2016.35-41 **How to cite this article:** Faruq AA, Hassan MM, Uddin MM, Rahman ML, Rakib TM, Alam M, Islam A. Prevalence and multidrug resistance pattern of *Salmonella* isolated from resident wild birds of Bangladesh. Int J One Health 2016;2:35-41.

Abstract

Aim: Salmonellosis is one of the most common zoonotic diseases, and the presence of antimicrobial resistant *Salmonella* in wild birds is global public health threat. Throughout the last decades, multidrug resistance of *Salmonella* spp. has increased, particularly in developing countries. Therefore, a cross-sectional study was conducted to investigate the prevalence of *Salmonella* spp. and antimicrobial resistance pattern against *Salmonella* spp. from two species of resident wild birds namely house crow (*Corvus splendens*) and Asian pied starling (*Gracupica contra*).

Materials and Methods: Samples were collected from cloacal swabs of house crows and Asian pied starling for isolating *Salmonella* spp. (bacteriological culture methods) followed by antimicrobial susceptibility testing (disk diffusion method) against *Salmonella* spp. isolates during March to December 2014.

Results: The prevalence of *Salmonella* in Asian pied starling and house crows were 67% and 65%, respectively. Within the category of samples from different species, the variation in prevalence was not varied significantly (p>0.05). Isolated *Salmonella* spp. was tested for resistance to six different antimicrobial agents. Among six antimicrobial tested, 100% resistance were found to penicillin, oxacillin, and clindamycin followed by erythromycin (50-93%), kanamycin (7-20%), and cephalothin (30-67%) from both species of birds. Kanamycin remained sensitive in (70-73%), cephalothin (26-70%), and erythromycin appeared to be (0-30%) sensitive against *Salmonella* spp. isolates. Isolated *Salmonella* spp. was multidrug resistant up to three of the six antimicrobials tested.

Conclusion: It can be said that the rational use of antimicrobials needs to be adopted in the treatment of disease for livestock, poultry, and human of Bangladesh to limit the emergence of drug resistance to *Salmonella* spp.

Keywords: antimicrobial, prevalence, resistance, resident wild birds, *Salmonella*.

Introduction

Salmonella has been renowned as a significant pathogen that can contaminate human and animals consequential in major morbidity and mortality [1]. *Salmonella* spp. was recognized as a major cause of food and waterborne illnesses in many countries that are closely associated with the presence of resident wild birds in the vicinity of domestic one where wild birds were a major carrier for this *Salmonella* spp. Most of the Salmonellosis is zoonotic in origin [2]. Salmonellosis has been found to be major infectious diseases of all ages of birds. This disease is endemic in many developing countries, particularly the Asian subcontinent and, South and Central America.

Salmonellosis is a major problem in poultry industries of Bangladesh and its prevalence ranged from 7% to 53.3% [3-5]. The probable cause for spreading of this disease can be *Salmonella* carrying wild birds, from them *Salmonella* spread into livestock and human. In recent years, problems related to *Salmonella* have increased significantly, both in terms of the incidence and severity of cases of human Salmonellosis. Heavy use of antibiotics for medical and veterinary purposes [6,7], as well as the domestic and agricultural use of pesticides and related compounds [7], caused significant antibiotic contamination of the natural environment and consequent development of resistance in communities.

In veterinary practice, antibiotics are used in livestock production, disease prevention, and as growth-promoting feed additives, and due to indiscriminate use of antimicrobials, the drug residues were deposited in the environment and also in the food animals tissues [8,9] which ultimately causes antimicrobial resistance. The use of antibiotics in animals disrupts normal flora of intestine, resulting into emergence of antibiotic-resistant *Salmonella* and their prolonged fecal shedding into the environment. Such practice has led to misuse of antibiotics with the associated high prevalence of antibiotic resistance among isolates from animal and food sources [10]. Effluents from environments (hospitals and slaughterhouses) are alarming risk for clinician to treat the patient with antibiotics due to harboring antibiotic resistance bacteria [11-13]. A growing body of evidence implicates environmental organisms as reservoirs of these resistance genes, antibiotic resistance, evolving and spreading among bacterial pathogens, poses a serious threat to human health. There are reports of a high prevalence of resistance in Salmonella isolates from countries such as Taiwan, India, and The Netherlands and resistant isolates from France, Canada, and Ethiopia. Similarly, there are various reports of multidrug resistant *Salmonella* organisms isolated in Bangladesh. Recently, antimicrobial resistance *Salmonella* spp. isolated from commercial layer eggs, eggshell surface, and egg-storing trays in Bangladesh [12]. However, resistance has also been found in the absence of antibiotic exposure, such as in bacteria from wildlife [14,15]. Most research on the epidemiology of antibiotic resistance spreading has paying attention on human and veterinary medicine, but there is a rising interest to recognize how bacterial resistance is transferred within reservoirs in natural environments [16]. Due to their diversity in migratory patterns and ecological niches, and their ease in picking up human/environmental bacteria, they act as mirrors of human activities. In addition, bird migration provides a possible mechanism for the establishment of new endemic foci of disease at great distances from where an infection was first acquired [17]. Besides, due to large range of migration and flying of wild birds close to the human settlement may cause drug resistance.

Some opportunistic and nonstructural studies had been conducted for *Salmonella* in commercial poultry in Bangladesh. Very few studies were conducted on isolation and drug resistance in *Salmonella* spp. throughout the world from wild birds but none in Bangladesh. This study, therefore, aimed to investigate the prevalence of *Salmonella* spp. in two species of wild birds (house crow and Asian pied starling) and antimicrobial resistance pattern against *Salmonella* isolates from those resident wild birds.

Materials and Methods

Ethical approval

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

Study design

A cross-sectional study was conducted in two areas of Chittagong. The current study was conducted at Pahartoli and Bakolia of Chittagong City Corporation (CCC) during March to December 2014.

Sample collection and preservation

A total of 100 cloacal samples were collected from the resident wild bird that were trapped using misnet and from which 40 samples were house crows and 60 were Asian pied starling. A sterile cotton swab stick was used to collect the cloacal sample and then put the stick immediately into a sterile vial containing 6 ml amines transport media (Oxoid) and kept in a cool box (4°C). The samples were then transferred to the Poultry Research and Training Center (PRTC) laboratory under sterile conditions and processed for the isolation of *Salmonella* spp.

Salmonella isolation and identification procedures

The study was conducted utilizing the conventional methods for the detection of *Salmonella* spp. following the standard guidelines. There were four definite sequential steps: (1) Nonselective preenrichment, (2) selective enrichment, (3) plating out and identification, and (4) confirmation using triple sugar iron (TSI) agar. Buffered peptone water was used as nonselective preenrichment broth. Xylose lysine deoxycholate (XLD) was used as selective enrichment broth. After incubation, a loop full of broth was streaked on XLD medium and incubated at 37°C for 24 h. Colonies with black centers were considered presumptive *Salmonella* spp., for plating out and identification *Salmonella-Shigella* (SS) agar was used. The depth of the agar in the Petri dishes were maintained approximately at 4 mm. The plates were incubated at 37°C±1°C for 24±3 h. The plates were incubated in an inverted position. After incubation, the plates were checked for the development of typical *Salmonella* colonies. Typical colonies of *Salmonella* on SS agar were blackish. TSI agar was used to see the typical colonies and biochemical reactions for the identification of *Salmonella* spp. typical colonies grown on the SS agar plates were transferred and inoculated in TSI agar slant and incubated at 37°C±1°C for 24±3 h. The TSI agar slant surface was streaked, and the butt was stabbed and incubated at 37°C±1°C for 24±3 h.

Selection of antimicrobials for antimicrobial susceptibility testing

In the present research, the *Salmonella* isolates were tested with antimicrobial sensitivity testing to see whether they are resistant to antimicrobials (penicillin, erythromycin, clindamycin, oxacillin, kanamycin, and cephalothin) used in Bangladesh.

Antimicrobial sensitivity test

The antimicrobial sensitivity testing was done by the disk diffusion method as described by NCCLS

2000, now known as the Clinical and Laboratory Standards Institute [18]. Mueller-Hinton agar was used for this testing. The agar was prepared as per the instructions provided by the manufacturer. McFarland 0.5 turbidity standards were prepared as per the standard guidelines described by the CLSI. The predetermined battery of antimicrobial disks such as penicillin (10 units/disc), cephalothin (30 µg/disc), oxacillin (1 µg/disc), kanamycin (30 µg/disc), erythromycin (15 µg/disc), and clindamycin (2 µg/disc) were chosen and placed centrally onto the surface of the inoculated agar plate. The disk placed in the agar surface was not closer than 24 mm from center to center. A total of six disks were placed on one 150 mm plate. The plates were inverted and placed in an incubator set to 35°C within 15 min after the disks were applied. After 16-18 h of incubation, each plate was examined.

The resulting zone of inhibition was uniformly circular with a confluent lawn of growth. The diameters of the zones of complete inhibition were measured, including the diameter of the disk. Zones were measured to the nearest whole millimeter, using a suitable scale, which held on the back of the inverted Petri plate. The sizes of zones of inhibition were interpreted by referring to zone diameter interpretive standards from NCCLS 2000 [19], and the isolates were considered as sensitive, intermediately sensitive, or resistant to these agents tested according to the standard [18].

Data analysis

Field and laboratory data were stored and then cleaned in the MS Excel-2007 program before exporting to STATA/IC-13.0 for analysis. Descriptive analysis was performed to know the frequency and distribution of *Salmonella* and antibiotic resistance pattern.

Results

Salmonella spp. was isolated from cloacal sample on two species of wild birds such as crow and Asian pied starling at Pahartoli and Bakolia under CCC areas of Bangladesh to evaluate antimicrobial susceptibility to estimate the prevalence and pattern of antimicrobial resistance among *Salmonella* spp. isolates.

Table-1 shows the prevalence of *Salmonella* spp. within two species of resident wild birds. The prevalence of Asian pied starling and house crow was 67% and 65%, respectively. Within the category of samples from different species, the variation in prevalence was not varied significantly ($p>0.05$). The strength of association between the prevalence of *Salmonella* spp. within house crow and Asian pied starling were 1.4%, where the strength of association was 1.4% higher in Asian pied starling than house crows.

From house crow, resistance patterns of *Salmonella* were highest in penicillin, oxacillin, and clindamycin (100%) followed by erythromycin (50%), cephalothin (30%), and kanamycin (20%). It was revealed that no isolates were found sensitive to penicillin, oxacillin, and clindamycin. Kanamycin and cephalothin showed the highest level of sensitivity (70%) followed by erythromycin (30%). In the current research, all the isolates of *Salmonella* showed multiple antimicrobial resistances (Table-2).

Table-1: Prevalence of *Salmonella* spp. within two species of resident wild birds.

Species	Positive (%)	χ^2-value	p value
House crows (N=40)	26 (65)	0.014	0.9
Asian pied starling (N=60)	40 (67)		

The Asian pied starling resistance patterns of *Salmonella* were highest in penicillin, oxacillin, and clindamycin (100%) followed by erythromycin (93%), cephalothin (67%), and kanamycin (7%). It was revealed that no isolates were found sensitive to penicillin, oxacillin, and clindamycin. Kanamycin showed the highest level of sensitivity (73%) followed by cephalothin (26%). In the current research, all the isolates of *Salmonella* showed multiple antimicrobial resistances (Table-3).

Among the six tested antimicrobials resistance pattern against *Salmonella* isolates penicillin, oxacillin, and clindamycin turned out as the highest level of resistance (100%) followed by erythromycin (50-93%), cephalothin (30-67%), and kanamycin (7-20%) from house crows and Asian pied starling. The rate of sensitivity to individual antibiotics against *Salmonella* isolates from two different species of birds was highest in kanamycin ranged (70-73%) followed by cephalothin (26-70%) and erythromycin (0-30%). Penicillin, oxacillin, and clindamycin showed no variation of resistance level; it was (100%) resistance in two species of birds and no isolates were found to sensitive against these three antibiotics. In case of erythromycin, 93% of *Salmonella* isolates from Asian pied starling and 50% from house crows were resistance to erythromycin. Antimicrobial resistance of kanamycin was found in crows (20%) and Asian pied starling (7%). The resistance level of cephalothin was higher in Asian pied starling (67%) and lower in house crows (30%). The sensitivity of kanamycin and cephalothin to isolated *Salmonella* spp. was better within two species of birds among all used antibiotics. Highest resistance pattern for intermediate type was in kanamycin ranged (10-20%) followed by erythromycin (7-20%), cephalothin (0-7%), and (0%) for other three antibiotics to isolated *Salmonella* for both species of birds. Highest intermediate type resistance was found (20%) to isolated *Salmonella* spp. from Asian pied starling in both kanamycin and erythromycin (Table-4).

Discussion

House crow (*Corvus splendens*) and Asian pied starling (*Gracupica contra*) are very much common in our country, especially in the major city areas. They

Table-2: Antimicrobial resistance pattern of *Salmonella* isolates from house crows.

Antibiotics	Number of positive samples tested	Pattern (%)		
		Resistance	Intermediate	Sensitive
Penicillin	20	100	0	0
Erythromycin	20	50	20	30
Oxacillin	20	100	0	0
Kanamycin	20	20	10	70
Clindamycin	20	100	0	0
Cephalothin	20	30	0	70

Table-3: Antimicrobial resistance pattern of *Salmonella* isolates from Asian pied starling.

Antibiotics	Number of positive samples tested	Pattern(%)		
		Resistance	Intermediate	Sensitive
Penicillin	25	100	0	0
Erythromycin	25	93	7	0
Oxacillin	25	100	0	0
Kanamycin	25	7	20	73
Clindamycin	25	100	0	0
Cephalothin	25	67	7	26

Table-4: Patterns of multidrug resistance in isolates of *Salmonella* from two different species.

Antimicrobials	Pattern	House crows (N=20) (%)	Asian pied starling (N=25) (%)	Range (%)
Penicillin	R	100	100	100
	I	0	0	0
	S	0	0	0
Erythromycin	R	50	93	50-93
	I	20	7	7-20
	S	30	0	0-30
Oxacillin	R	100	100	100
	I	0	0	0
	S	0	0	0
Kanamycin	R	20	7	7-20
	I	10	20	10-20
	S	70	73	70-73
Clindamycin	R	100	100	100
	I	0	0	0
	S	0	0	0
Cephalothin	R	30	67	30-67
	I	0	7	0-7
	S	70	26	26-70

R=Resistance, I=Intermediate, S=Sensitive

are usually carrion eaters and take their foods from the garbage of city areas. Nowadays, for the easy and frequent access of these two species in rural and urban area where different antibiotics are used regularly for human and livestock treatment, house crow and Asian pied starling got some antibiotic residue or antibiotic resistant bacteria from those human and livestock source anyway and they developed antibiotic resistance against some bacterial pathogens, such as Salmonellosis, which have important public health significance.

The prevalence of *Salmonella* in crow was 65% which was very much higher than the result of previous research isolated from crows cloaca [20]. The prevalence of *Salmonella* in Asian pied starling was 67% which was also very much higher than the previous studies [21,22] on European starlings. Research conducted by isolation of *S. enterica* from gastrointestinal tract samples of European starling showed 2.5% prevalence [23] which is much lower than the current study. The higher prevalence rate of the present study may be due to overpopulated environment of Bangladesh and more frequent presence of house crow and Asian pied starling as a store house for microorganisms including *Salmonella* spp. [11,13]. The favorable climatic condition and substandard hygienic condition of Bangladesh may also contribute to a higher prevalence rate of *Salmonella*.

Approaches to prevent and control Salmonellosis in the food animal industry by extensive use of antibiotics [24] has led to emergence of resistant bacteria [25] through mutation and acquisition of resistance encoding genes [26]. The situation in developing countries such as Bangladesh may be exaggerated by easy accessibility of antimicrobials at a lower price and their extensive use in poultry production system [27]. The current study recorded multiple antimicrobial resistances against *Salmonella* spp. (up to six) and in most cases, estimated 100% resistance to 2-4 antimicrobials across the study sites. These threatening correspond to the many nonepidemiological and opportunistic earlier studies in Bangladesh [28,29], India [30], Nepal, and Bhutan. The results of present study revealed that all the *Salmonella* isolates from cloacal swab of crows and Asian pied starling were resistance to penicillin which has the similarity with earlier studies [31-33]. Several previous researches [32,34,35] found 100 resistance of oxacillin against *Salmonella* both in house crow and Asian pied starling. Erythromycin was resistance more against *Salmonella* spp. isolated from Asian pied starling (93%) than the resistance against *Salmonella* spp. isolated from house crows (50%). This results varied with many other previous studies where they said erythromycin were totally resistant to *Salmonella* spp. [32,36,37]. The lower resistance of kanamycin found in the current study has more or less similarity with the result of different scientist where kanamycin resistance to *Salmonella* spp. was 18-60% [38-41], and it is good news for Bangladesh because it may be used effectively against *Salmonella*. Clindamycin showed 100% resistance against *Salmonella*, which found the similarity with the result of others similar researches [42-45]. Cephalothin was resistance more against *Salmonella* spp. isolated from Asian pied starling (67%) than the resistance against *Salmonella* spp. isolated from house crows (30%). This result was more or less similar with many other previous studies where they said cephalothin was slightly resistant to *Salmonella* spp. [46].

Penicillin, oxacillin, erythromycin, kanamycin, clindamycin, and cephalothin antibiotics are commonly used in livestock, poultry, and human treatment in Bangladesh. Therefore, residues of these antibiotics could have been passed through different environmental sources to the birds or they found antibiotic resistant *Salmonella* spp. from those sources during their migration. The high resistance of antibiotic to *Salmonella* spp. from cloacal swabs of house crow and Asian pied starling of the present study might be due to indiscriminate use of these antibiotics to human as well as in livestock. That's why wild birds got the antibiotic resistant *Salmonella* spp. or residues of those antibiotics by taking the dung or stool or other wastage of those livestock, poultry, or human. In addition, sometimes farmers select drugs by their own or rely on neighbor experienced farmers or nonveterinarians and these malpractices may not always ensure proper drugs doses, frequency of drug administration and complete course of drug treatment those can be a major cause for the establishment of antibiotic resistant *Salmonella* spp.

Conclusion

Salmonellosis is a leading, zoonotic, and widely distributed disease throughout the world. The level of resistance of *Salmonella* to antibiotics is an alarming issue to the human and livestock. Therefore, it is necessary to inform people about the future alarming condition of the antibiotic resistance. There is need for more rational use of antibiotics in animal production and more careful use in humans. It is important to take concerted action to improve antibiotic resistance surveillance capacity worldwide with a view to monitoring the emerging resistance genes and their transfer in both animal and human. In addition, alternatives to antibiotics should be explored such as the application of probiotics in poultry for production of safe edible products. To control *Salmonella* infection of wild birds in Bangladesh detailed epidemiological investigation is needed. Proper disposal of dustbin content, sewage water, dead carcass, waste food, and human stool should be needed to prevent transmission of *Salmonella* infection.

Authors' Contributions

AAF and MMH conducted the research and actively prepared the manuscript. AAF, MMH, MA, and AI designed the work and provided the information. MMU, MLR, AI, and TMR participated in the manuscript preparation and advice during the research work. All the authors read and approved the final manuscript.

Acknowledgments

The authors are grateful to the Chittagong Veterinary and Animal Sciences University, for providing financial (grant reference no. UGC/PUB/CVASU: P013) and technical support, and PRTC for laboratory facilities, to the project. This study was benefited from intellectual contributions from the PREDICT project of the United States Agency for International Development (USAID) Emerging Pandemic Threats Program.

Competing Interests

The authors declare that they have no competing interests.

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