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Prevalence and Antibiogram Profiles of *Salmonella* Isolated from Pigeons in Mymensingh, Bangladesh

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A B S T R A C T

This study was undertaken to determine the prevalence of *Salmonella* spp. in apparently healthy pigeons at the live bird markets, farms and villages in the Mymensingh district of Bangladesh. Cloacal swabs (n = 36), foot pads (n = 36) and feces (n = 40) of pigeons were collected aseptically and inoculated onto various culture media for isolation of *Salmonella*. Identification of *Salmonella* from culture positive samples was performed by cultural characteristics, Gram's staining and biochemical tests. The prevalence of *Salmonella* in cloacal swabs, foot pads and feces was 22.22%, 58.33% and 27.50%, respectively. The overall prevalence of *Salmonella* in pigeons was 35.71%. Pigeons at live bird markets, farms and villages showed a 40.48%, 20% and 30% prevalence of *Salmonella*, respectively. Antibiotic sensitivity tests of the *Salmonella* was performed by the disc diffusion method against 10 randomly used antibiotics. The highest rate of resistance was found with amoxicillin (90%) followed by ampicillin (80%), erythromycin (80%) and tetracycline (60%). The highest rate of sensitivity was recorded to ciprofloxacin (80%) followed by sulphamethoxazole (70%), chloramphenicol (60%), kanamycin (60%), gentamicin (60%) and nalidixic acid (60%). Data from this study suggest that pigeons carry multi-drug resistant *Salmonella* which can be transferred to humans through direct contact or the food chain and could cause a potential public health hazard.

Keywords: *Salmonella,* Pigeons, Prevalence, Antibiotic sensitivity

Introduction

Domestic and feral pigeons (*Columbia livia*) are found in the rural and urban areas of Bangladesh. The weather and vast areas of crop field along with housing premises of Bangladesh are suitable for pigeon farming (Asaduzzaman *et al.,* 2009). Farmers in rural areas rear pigeons for family nutrition and to sell in the markets for money. Some people rear pigeons in cages at their houses for recreation. Close contact of humans with pigeons at home, live bird markets and farms bear the risk of transmission of zoonotic infections since they are the potential reservoirs for *Salmonella, Campylobacter, Escherichia coli* and *Chlamydia* (Weber, 1979; Tanaka *et al*., 2005; Lillehaug *et al.,* 2005).

Pigeons play an important role in the fecal contamination of drinking water sources and agricultural crops, and may transfer infectious agents to outdoor domestic poultry through direct contact (Lillehaug *et al.,* 2005). The gut of the healthy pigeons is known to carry *Salmonella* (Casanovas *et al*., 1995; Fallacara *et al.*, 2001; Wahlstrom *et al.*, 2003). Pigeon's meat might be contaminated with *Salmonella* if proper hygienic measures are not maintained during scalding, defeathering, evisceration and giblet operations (Bryan and Doyle, 1995). Multidrug resistant *Salmonella* in food animals is an emerging issue all over the world. In developed countries majority of the zoonotic agents acquired drug resistance in an animal host before being transmitted to humans through the food chain (Molback *et al*., 2002; Threlfall, 2002).

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Pigeons usually live in close proximity to human residential areas. It is important to know their role in the transmission of different infectious organisms from the public health point of view. To the best of our knowledge one report is available on the characterization and antibiogram profile of *Salmonella* in pigeons of Dinajpur district of Bangladesh (Rahman *et al.,* 2011). The objectives of the present research work were to determine the prevalence of *Salmonella* with their antibiotic sensitivity profiles in the apparently healthy pigeons in the Mymensingh district of Bangladesh.

Materials and Methods

This study was conducted in the laboratory of the Department of Microbiology and Hygiene, Bangladesh Agricultural University (BAU), Mymensingh, during the period from January 2012 to May 2012.

Sources of samples

A total of 112 samples, comprised of cloacal swabs (n = 36), foot pads (n = 36) and feces (n = 40) were collected aseptically from apparently healthy pigeons at the live bird markets, farms and villages in and around BAU Campus, Mymensingh, Bangladesh. Samples were transported to the laboratory at 4°C.

Isolation of Salmonella

Samples were enriched in selenite broth by overnight incubation at 37°C. A loopful of enrichment culture was streaked onto selective media for *Salmonella* such as *Salmonella-Shigella* (SS) agar and brilliant green agar (BGA) media and incubated at 37°C for 24 hr.

Identification of Salmonella
Identification of *Salmonella* in pure culture was performed by observing colony morphology, Gram's staining and biochemical tests such as: sugar fermentation test, indole test, methyl red (MR) and Voges Proskauer (VP) tests (Cheesbrough, 1985).

Antibiotic sensitivity assay
Antibiotic sensitivity was tested using 0.5 McFarland turbidity standard inoculum and freshly prepared, dried Mueller Hinton agar (Oxoid, UK) against 10 common antibiotics: erythromycin (15 µg/disc), ampicillin (10 µg/disc), amoxycillin (10 µg/disc), chloramphenicol (30 µg/disc), ciprofloxacin (5 µg/disc), tetracycline (30 µg/disc), kanamycin (30 µg/disc), gentamicin (10 µg/disc), sulphamethoxazole (25 µg/disc) and nalidixic acid (30 µg/disc) (Oxoid, UK). Three *Salmonella* isolates of cloacal swabs, 4 *Salmonella* isolates of foot pads and 3 *Salmonella* isolates of feces were selected randomly for the test. Disc diffusion or Kirby-Bauer method (Bauer *et al.,* 1966) was used to test the sensitivity patterns. The results were expressed as resistant, intermediate or sensitive according to the guidelines of Clinical and Laboratory Standard Institute (CLSI, 2007).

Results

Identification of *Salmonella*
Salmonella on SS agar produced smooth, small, round and black centered colonies. On BGA it produced translucent pink to red colonies. Gram's staining of *Salmonella s*howed Gram-negative (pink) rods arranged singly or pair. It fermented dextrose, maltose and manitol with the production of acid and gas but did not ferment lactose and sucrose. MR test was positive whereas VP and indole tests were negative.

Prevalence of *Salmonella*
The prevalence of *Salmonella* in the cloacal swab, foot pad and feces samples of pigeons is presented in Table 1. The highest prevalence of *Salmonella* was recorded in the foot pads (58.33%) and lowest prevalence of *Salmonella* was observed in cloacal swab samples of pigeons. However, the overall prevalence of *Salmonella* in all samples was recorded as 35.71%.

The prevalence of *Salmonella* in pigeons at live bird market, farm and villages is shown in Table 2. Pigeons at the live bird market showed the highest prevalence of *Salmonella* (40.28%). The lowest prevalence of *Salmonella* in pigeons was found at farms (20%).

Antibiotic sensitivity assay
In this study 80% of the *Salmonella* isolates were sensitive to ciprofloxacin followed by sulphamethoxazole (70%), chloramphenicol (60%), kanamycin (60%), gentamicin (60%) and nalidixic acid (60%). Antibiogram profiles of 10 *Salmonella* isolates are shown in Fig. 1. On the other hand 90% of the *Salmonella* isolates were found resistant to amoxicillin (90%), followed by ampicillin (80%), erythromycin (80%) and tetracycline (60%). The overall resistance patterns of pigeon's *Salmonella* isolates against antibiotics are given in Table 3.

Discussion

Pigeons are important birds in Bangladesh. Rearing of pigeons is profitable. There are high demands for pigeon meat since it is considered very delicious and contains high quality protein. Pigeons move from one place to another and have access to outside soil and water. This plays an important role in transferring infectious agents to humans, livestock and poultry. Very little is known about the food safety risk factors of pigeon meat. This study evaluated the role of pigeons as a carrier of *Salmonella* to assess public and animal health risks.

This study revealed the presence of *Salmonella* in cloacal swabs, foot pads and feces of pigeons ranging from 22.22 to 58.33%. Nassar and El-Ela (2000) reported 8-16% prevalence of *Salmonella* in the liver, hearts, gizzards and intestine of squabs

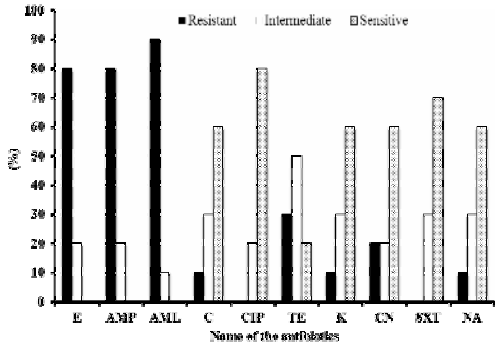

Fig. 1. Antibiogram profile of *Salmonella* isolates of pigeons against erythromycin (E), ampicillin (AMP), amoxicillin (AML), chloramphenicol (C), ciprofloxacin (CIP), tetracycline (TE), kanamycin (K), gentamicin (CN), sulphamethoxazole (SXT) and nalidixic acid (NA)

Table 1. Prevalence of *Salmonella* in cloacal swabs, foot pads and feces sample of pigeons

Name of samples	No. of samples	No. of *Salmonella* positive samples	Prevalence (%)
Cloacal swabs	36	8	22.22
Foot pads	36	21	58.33
Feces	40	11	27.50

Table 2. Prevalence of *Salmonella* in pigeons at live bird markets, farms and villages

Locations	No. of samples	No. of *Salmonella* positive samples	Prevalence (%)
Live bird markets	72	29	40.28
Farms	10	2	20
Villages	30	9	30

Table 3. Resistance profile of *Salmonella* isolates of pigeons against ten antibiotics

Name of samples	No. of isolates tested	No of *Salmonella* isolates resistant to antibiotics									
		E	AMP	AML	C	CIP	TE	K	CN	SXT	NA
Cloacal swabs	3	3	3	3	0	0	1	0	0	0	0
Foot pads	4	2	3	4	1	0	3	1	1	0	0
Feces	3	3	2	2	0	0	2	0	1	0	0

E = Erythromycin; AMP = Ampicillin; AML= Amoxicillin; C = Chloramphenicol; CIP = Ciprofloxacin; TE = Tetracycline; K = Kanamycin, CN= Gentamicin; SXT = Sulphamethoxazole; NA= Nalidixic Acid

and 8% prevalence of *Salmonella* in the liver of wood pigeons. A study of Copenhagen reported 3.3% prevalence of *Salmonella* in feral pigeons and 22.8% prevalence of *Salmonella* in the pooled fecal samples of pigeons (Pasmans *et al.,* 2004). Kobayashi *et al.* (2007) reported 9.2% prevalence of *Salmonella* in foot pads and 5.8% prevalence of *Salmonella* in cloacal swab samples in the wild birds of Japan. Another study conducted in Iran recorded 15.55% prevalence rate of *Salmonella* in pigeons (Akbarmehr, 2010). Higher prevalence rate of *Salmonella* recorded in this study might be the effects of bird density, increased success of fecal-oral transmission, strain differences of the birds, immune status of the flocks and geographical variation (Carraminana *et al.,* 1997; McCrea *et al.,* 2006). *Salmonella* is known to cause food-borne infection in humans and estimated to cause 1.3 billion cases of gastroenteritis and 3 million deaths worldwide (Bhunia, 2008). Poultry and eggs are the most common vehicles of salmonellosis (Pui *et al.,* 2011). Meat of pigeons may be contaminated with *Salmonella* if proper hygienic measures are not followed during slaughtering and processing operations. Handling of pigeons may also transfer *Salmonella* to humans if contaminated hands are used for eating and drinking of food and beverages. Therefore, proper hygienic and sanitary methods must be undertaken during meat processing and handling of pigeons to prevent transfer of *Salmonella* to humans. Effective measures must be also undertaken to prevent the entry of pigeons in the livestock and poultry farms to prevent transmission of *Salmonella.*

Pigeons are sold in the small and large live bird markets in Bangladesh. Chickens and ducks are also sold in the same live markets. Huge public gatherings at live bird markets with unhygienic environment may favor the transmission of infectious agents from bird to bird and bird to human. This study recorded the highest prevalence of *Salmonella* at the live bird markets (40.28%) as compared to farms (20%) and villages (30%). McCrea *et al.* (2006) reported the lowest prevalence rate of *Salmonella* in squabs on farm (1.3%) compared to post transport squabs (2.1%) and carcass swabs of squabs (23.8%) in processing plants. Lack of proper hygiene and sanitary practices, cross contamination with other species of birds and overcrowding might be responsible for the highest prevalence of *Salmonella* in pigeons at the live bird markets. Data of this study underscore the need for strict hygienic and sanitary practices at the live bird markets.

The frequency of resistance among food-borne pathogens has increased dramatically, presumably due to the extensive use of antimicrobial agents in human and veterinary medicine **(**Bronzwaer *et al.*, 2002**).** Many species of *Salmonella* are known to carry multi drug resistant genes **(**Gebreyes and Altier, 2002) which has been a matter of concern. Drug resistance profiles of *Salmonella* have been investigated by others (Nazer and Safari, 1994; Van Duijkeren *et al.,* 2003; Lee *et al.,* 2005; Adesiym *et al.,* 2007). This study recorded the presence of multidrug resistance *Salmonella* in pigeons. In this study amoxycillin showed the highest resistance (90%) to *Salmonella* isolates of pigeons while the highest rate of sensitivity (80%) of *Salmonella* isolates of pigeons to ciprofloxacin. Jahantigh and Nili (2010) found the highest resistance of *Salmonella* isolates of pigeons was to Tetracycline (50%) and 100% sensitivity to Ciprofloxacin. *Salmonella typhimurium* strains isolated from human and animal sources in Italy were examined for their antimicrobial susceptibility. Resistance to tetracycline (73.6%), ampicillin (67.6%), chloramphenicol (32.3%), gentamycin (2.5%), nalidixic acid (13.5%), and ciprofloxacin (0.1%) were observed (Graziani *et al.,* 2008).

Conclusion

This study suggests that pigeons are a carrier of S*almonella* spp which might constitute a public health hazard if proper hygienic and sanitary measures are not undertaken during rearing on farms, selling at live bird markets and during meat processing. Data of this study also indicated that pigeons harbor multidrug resistant *Salmonella* which might be alarming as this resistance may gain access to man and animals resulting in difficulties in treatment of bacterial diseases, particularly salmonellosis.

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