Microbes and Health
ISSN: 2226-0153 (Print) 2305-3542 (Online)
http://journal.bsvmph.org/

Microbes and Health, June 2013, 2(1): 5-7
DOI: 10.3329/mh.v2i1.17254

Prevalence of Multidrug Resistant *Escherichia Coli* in Pigeon in Mymensingh, Bangladesh

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[**Received**: 28 March 2013, **Revised**: 12 May 2013, **Accepted**: 16 May 2013]

A B S T R A C T

To determine prevalence of antimicrobial resistant *Escherichia coli* in pigeon a total of 112 samples such as cloacal swabs (n=36), foot pads (n=36) and feces (n=40) were collected from pigeon aseptically. Samples were enriched in nutrient broth and then streaked onto eosine methylene blue agar, Salmonella-Shigella agar, MacConkey agar and blood agar. The cultural and biochemical characteristics of bacterial isolates of pigeon were indicative of *E. coli*. The prevalence of *E. coli* in cloacal swabs, foot pads and feces samples were 86.11%, 44.44% and 77.50%, respectively. The overall prevalence of *E. coli* in pigeon was 69.64% (78 of 122 samples were found positive for *E. coli*). The antibiotic sensitivity pattern showed that *E. coli* isolates were sensitive to erythromycin, ciprofloxacin, kanamycin, nalidixic acid and resistant to amoxicillin, tetracycline and sulphamethaxazole. It may be concluded that pigeons from Mymensingh locality in Bangladesh carry multidrug resistant *E. coli*. Data of this study will be helpful in creating public health awareness among people and help controlling transmission of drug resistant *E. coli* by undertaking effective sanitary and hygienic measures.

Keywords: Antibiotic sensitivity, *Escherichia coli,* Prevalence, Pigeon

Introduction

Pigeon (*Columba livia*) is a plump and rounded-bodied bird of the family *Columbidae,* order Columbiformes. The family *Columbidae* has 289 species worldwide among them 30 species were found in Indian sub-continent. In Bangladesh, there are 17 species of pigeons, of which 2 are migratory. It carries a number of zoonotic diseases that are transmissible to humans and other domestic animals. *Escherichia coli* 0157:H7 (*E. coli* 0157) is one of the recognized important food borne pathogen. Food animals such as cattle, pig and chicken appear to be reservoir of this organism (Beutin *et al.,* 1993).

E. coli is found naturally as intestinal microflora of many species including pigeons. Pigeons are extremely effective transmitters of *E. coli* to humans because their feces is a source of *E. coli* O157:H7 for birds, mammals and humans (Santaniello *et al.,* 2007). *E. coli* is a Gram-negative, rod-shaped, flagellated, motile, oxidase negative, facultative anaerobic bacteria under the family *Enterobacteriaceae* (Buxton and Fraser, 1977*).* It remains as commensal and is mainly encountered in the lower portion of the intestine and acts as opportunistic pathogens for human and animal (Levine, 1984). Most of the strains of *E. coli* are harmless; however some can cause food poisoning. *E. coli* food poisoning is usually caused by enteropathogenic *E. coli* (EPEC); enterotoxigenic *E. coli* (ETEC); enteroinvasive *E. coli* (EIEC) and enterohaemorrhagic *E. coli* (EHEC) strains.

E. coli causes septicaemia and diarrhoea in animals and birds. *E. coli* diarrheagenic strains, which are able to survive in pigeon feces has potential for human exposure and infection (Silva *et al*., 2009). It enters into the body through the consumption of food containing bacteria. Inadequate cooked meat is the most common source of *E. coli* food poisoning (Sonntag *et al.,* 2005).

The occurrence of antimicrobial resistance among pathogenic and commensal bacteria is a significant problem affecting medical treatment of infectious diseases. Humans (Ishikawa *et al.,* 2005), food animals (Asai *et al.,* 2005) and wildlife (Gilliver *et al.,* 1999) may act as reservoirs of drug-resistant bacteria through the food chain. The widespread occurrence of antimicrobial resistance in bacteria in wildlife populations may be caused by environmental pollution of antimicrobials or resistant bacteria through human activities such as antimicrobial use in medicine and agriculture.

In Bangladesh, isolation and characterization of *E. coli* was performed from normal flora of milk, water samples, poultry carcass and diarrhoeic feces of calf (Chowdhury *et al.,* 1967; Hasina, 2006). Apparently, there is no study so far conducted on multidrug resistant *E. coli* in pigeon in Bangladesh. Our goal for this study was to determine the prevalence of antimicrobial-resistant *E. coli* in apparently healthy pigeons at livebird market, farms and village of Mymensingh Sadar.

Materials and Methods

Collection 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 in different areas of Mymensingh such as Sanki para bazar, Machowa bazar, Kewatkhali bazar, Kamal-Ranjit live bird market, Bangladesh Agricultural

To cite this article: Dey RK, MM Khatun, MA Islam and MS Hosain, 2013. Prevalence of multidrug resistant *Escherichia Coli* in pigeon in Mymensingh, Bangladesh. Microbes Health, 2(1): 5-7.

University poultry farm, Sheshmor, Sutiakhali and Boira villages. Cloacal swabs and fecal samples were collected by using sterilized cotton buds and foot pads were collected after slaughtering the pigeons. Samples were transported to the laboratory maintaining 4°C.

Isolation of *E. coli*
Samples were enriched in nutrient broth (NB) by overnight incubation at 37°C. After enrichment in NB, a loopful of cultured broth was streaked on Eosin Methylene Blue (EMB) agar, Salmonella-Shigella (SS) agar, MacConkey (MC) agar and Blood Agar (BA) media and incubated at 37° C for 24 hrs.

Identification of *E. coli*
Identification of *E. coli* in pure culture was performed by observing colony morphology, Gram's staining and motility test. Colonial morphology of *E. coli* such as size, margin, elevation and color were recorded on EMB agar, SS agar, MC agar and BA to study cultural characteristics (Cheesebourgh, 1985). Biochemical characterization of *E. coli* was performed by sugar fermentation reactions (dextrose, sucrose, lactose, maltose and mannitol). The methyl red-Voges Proskauer (MR-VP) and indole tests were performed according to the method described by Cowan (1985).

Antibiotic sensitivity assay
Disc diffusion or Kirby-Bauer method (Bauer *et al.,* 1966) was used to test the sensitivity patterns of *E. coli* isolates of pigeon. Briefly, 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 *E. coli* isolates of cloacal swabs, four *E. coli* isolates of foot pads and three *E. coli* isolates of feces were randomly selected for the test. The results were expressed as resistant, intermediate or sensitive according to the guidelines of Clinical and Laboratory Standard Institute (CLSI, 2007).

Results

Identification of *E. coli*
E. coli on EMB agar produced greenish black colony with metallic sheen On MC agar it produced bright, pink colored, transparent smooth and raised colonies Pink colored, rod shaped, short chain, single or paired Gram negative bacilli were observed after Gram's staining. It fermented five basic sugars such as dextrose, sucrose, lactose, maltose and mannitol with the production of both acid and gas. Acid production was indicated by the color change from reddish to yellow and gas production was noted by the presence of gas bubbles in the inverted Durham's tubes. All *E. coli* isolates were catalase, indole, M-R positive and V-P negative.

Prevalence of *E. coli*
The overall prevalence of *E. coli* in all samples were recorded as 69.64%. The highest prevalence of *E. coli* was recorded in the cloacal swabs (86.11%) and the lowest prevalence in foot pads (44.44%) samples of pigeons (Table 1).

The highest prevalence (80%) of *E. coli* was found in pigeon reared at farms. The lowest prevalence (58.33%) was recorded in pigeons at live bird markets (Table 2).

Antibiotic sensitivity assay
In this study the representative *E. coli* isolates were found sensitive to erythromycin (100%), ciprofloxacin (80%), nalidixic acid (80%), chloramphenicol (70%) and kanamycin (70%). On the other hand, *E. coli* isolates were found resistant to two or more antibiotics. The higher rate of resistance was found with sulphamethoxazole (90%), tetracycline (90%), amoxicillin (70%). Antibiogram profiles of *E. coli* isolates are shown in Fig. 1. The overall resistance patterns of *E. coli* isolates are given in Table 3.

Table 1. Prevalence of *Escherichia coli* in cloacal swabs, foot pads and fecal samples of pigeons

Name of samples	No. of samples	No. of *Escherichia coli* positive samples	Prevalence (%)	Overall prevalence (%)
Cloacal swabs	36	31	86.11	69.64
Foot pads	36	16	44.44	
Feces	40	31	77.50	

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

Location	No. of samples	No. of *Escherichia coli* positive samples	Prevalence (%)
Live bird markets	72	47	58.33
Farms	10	8	80
Villages	30	23	76.66

Table 3. Antibiotic resistance profile of *Escherichia coli* isolates of pigeons

Name of samples	No. of isolates tested	No. of *E. coli* isolates resistant to antibiotics										Summary			
		E	AMP	AML	C	CIP	TE	K	CN	SXT	NA	0	1	2-5	5+
Cloacal swabs	4	0	0	3	0	0	4	0	0	4	0	0	0	4	0
Foot pads	3	0	0	2	0	0	3	0	0	3	0	0	0	3	0
Feces	3	0	0	2	0	0	2	0	0	2	0	0	0	3	0
Total	10	0	0	7	0	0	9	0	0	9	0	0	0	10	0

0= Susceptible to all antibiotics; 1= Resistant to one antibiotic; 2-5= Resistant to 2-5 antibiotics;
5+= Resistant to more than 5 antibiotics; E= Erythromycin; AMP =Ampicillin; AML= Amoxicillin;
C= Chloramphenicol; CIP = Ciprofloxacin; TE = Tetracycline; K= Kanamycin, CN= Gentamicin;
SXT = Sulphamethoxazole; NA= Nalidaxic Acid

Discussion

Pigeons are sold in the small and large live bird markets in Bangladesh. Chicken and ducks are also sold in the same live bird markets. Huge public gatherings at live bird markets with unhygienic environments may favor the transmission of infectious agent from bird to bird and bird to human. This study recorded 69.64% prevalence of *E. coli* in pigeons. Other studies recorded 78.86% prevalence of *E. coli* in poultry (Jakaria, 2011), 66.67% prevalence in duck (Avishek, 2010), 32% prevalence in pig (Arefin, 2009), 46% prevalence in diarrhoeic buffaloes (Paul, 2009), 41.67% prevalence in diarrhoeic sheep (Purkayastha, 2007) and 72.22% prevalence in diarrhoeic goats (Abdullah, 2007).

Colony characteristics of *E. coli* recorded onto EMB agar, MC agar, SS agar and blood agar were similar to the findings of other authors (Buxton and Fraser, 1977; Nazir, 2005; Hasina, 2006; Purkayastha, 2007; Arefin, 2009; Avishek, 2010 and Jakaria, 2011). Gram's staining reaction and biochemical test results of

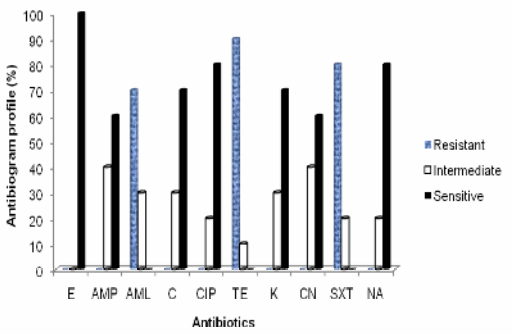

Fig. 1. Antibiogram profile of *Escherichia coli* isolates of pigeons against erythromycin (E), ampicillin (AMP), amoxicillin (AML), chloramphenicol (C), ciprofloxacin (CIP), tetracycline (TE), kanamycin (K), gentamicin (CN), sulphamethoxazole (SXT) and nalidixic acid (NA)

the *E. coli* isolates were in agreement with the findings reported by other investigators (Buxton and Fraser 1977; Freeman, 1985; Jones *et al.,* 1987; Beutin *et al.,* I991; Mckec *et al.,* 1995; Sandhu and Clarke, 1996; Purkayastha, 2007; Arefin, 2009 and Avishek, 2010).

The development and use of antibiotics has been one of the most important steps towards controlling of infectious bacterial diseases in the 20th century. However, the subsequent appearance and spread of antibiotic resistance in pathogenic organisms have made many currently available antibiotics ineffective (Neu *et al.,* 1992). To successfully fight the increasing numbers of drug resistant and multi-drug resistant bacteria, extensive knowledge of the molecular mechanisms of acquiring antibiotic resistance and updated information regarding current distribution of resistance pattern are required. The antibiogram profiles suggested that *E. coli* isolates were multidrug resistant. Antibiotic resistance of *E. coli* isolates of pigeon recorded in this experiment might have resulted from indiscriminate use of antibiotics as suggested by Jawetz *et al.,* (1984). Blanco *et al.* (2009) reported that antimicrobial resistance in wild birds was associated with agricultural manure. These drug resistant bacteria can be spread in the environment where humans and animals acquire infections resulting to difficulties in treating infection caused by *E. coli* (Joseph *et al.,* 1979).

Conclusion

Data of this study suggests that pigeon at household, farms and live bird markets are reservoir of multidrug resistant *E. coli* that might contaminate food chain if proper hygienic measures are not undertaken during rearing, handling and processing of pigeon.

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