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ORIGINAL RESEARCH

Isolation, histopathology and antibiogram of *Escherichia coli* from pigeons (*Columba livia*)

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Abstract

Aim: To know the prevalence of antibiotic resistant *Escherichia coli* among dead and/or diarrhoic pigeons in and around greater Guwahati.

Materials and Methods: Samples were cultured from dead and/or diarrhoic pigeons and identification was done by standard methods. The sensitivity of the isolated *E.coli* strains to 15 antibiotics of human and veterinary use was also determined. Organs from those dead birds from which *E.coli* were recovered were processed according to the routine procedure for histopathological studies.

Results: Out of 150 pigeons subjected to microbiological investigation, 91(60.67 %) samples were found positive for *E. coli*. The most frequently occurring serotypes were O157 (9.89%), followed by O68, O121 (7.69%), O9, O75, O131 (5.49%), O2, O13, O22 (3.30%). Antibiogram investigation of the isolates revealed that 91isolates (100%) exhibited resistance against Ampicillin followed by Nitro-furantoin (73.62%), Tetracycline (65.93 %), Oxytetracycline (62.63 %) and Streptomycin (61.54). Gross changes of some birds showed fibrinous pericarditis and perihepatitis and coligranuloma in different organs like liver and serosal surface of intestine. Microscopically, severe congestion and haemorrhages in different organs such as liver, kidney, lung and intestine. In some cases thick layer of fibrinous exudates with large number of heterophills over the surface of liver and heart with early degenerative changes as well as focal necrosis.

Conclusion: The result of this study suggests that antimicrobial-resistant pathogenic *E.coli* is present in pigeons in and around greater Guwahati. Surveillance programs may be introduced to monitor antimicrobial resistance of pathogenic *E.coli* in pigeons in and around greater guwahati.

Keywords: drug sensitivity, *E.coli*, histopathology, pigeon

Introduction

Pigeons had been associated with human society both as a source of food and as cage birds from a long time. Out of 8600 nos. of known species of birds, 289 species are of pigeon. They are reared scientifically in Belgium and England, as the people used to exhibit the flock in racing competition.

Pigeons come into close contact with humans and animals, and may contribute to the spread of infectious agents. Workers from different countries have reported variable incidence of bacterial diseases in pigeons. Among the bacterial agents, *E. coli*, *Streptococci* and *Salmonella* infections are common in pigeons [1]. *E. coli* strains causing human diarrhea which may be able to survive in pigeon feces, may create potential human exposure. Use of antimicrobial therapy plays a major role in reducing incidence and mortality from avian colibacillosis [2]. Though many isolates of *E. coli* are sensitive to several antibiotics, numbers of strains are becoming resistant to common antibiotics especially those are widely used [2]. Frequent occurrence of respiratory, diarrhoea and joint problems caused by *E.coli* following treatment with antibiotics indicates drug resistant strains of the organism [3]. Resistance to two or more classes of antibiotics is become common in veterinary medicine [4].

Therefore, the present study was undertaken to identify the major serotypes of *E. coli* prevailing in pigeon, their antimicrobial susceptibility and histopathological studies in and around greater Guwahati.

Materials and Methods

One hundred and fifty samples were collected from organs of fresh carcasses and/or diarrheic pigeons in and around greater Guwahati and subjected to isolation of *E.coli* as per standard method [5].The isolates were identified on the basis of their cultural, morphological and biochemical characteristics [6]. The isolates were referred at National Salmonella and Escherichia Centre, Central Research Institute, Kasauli (Himachal Pradesh, India) for serotyping.

In-vitro antibiotic sensitivity of the isolates were assessed using disc diffusion method [7] using disc coated with 15 antibacterial drugs (Hi-Media) viz. ampicillin (A)-10µg, cephalexin (Cp) -30µg, cephotaxime (Ce) -30µg, chloramphenicol (C) -30µg, ciprofloxacin (Cf) -10µg, colistin (Cl) -10µg, co-trimoxazole (Co) -25 µg, furazolidone(Fr) -50µg,

Table-1. Antimicrobial resistance of *E.coli* isolated from pigeon

Sr. No.	Antibiotic	No. tested	Sensitive	%	Resistant	%
1	Ampicillin(A)	91	0	0.00	91	100.00
2	Chloramphenicol(C)	91	70	76.92	21	23.08
3	Cephalexin (Cp)	91	55	60.44	36	39.56
4	Cephotaxim (Ce)	91	68	74.73	23	25.27
5	Ciprofloxacin (Cf)	91	88	96.70	3	3.30
6	Colistin (Cl)	91	47	51.65	44	48.35
7	Cotrimoxazole (Co)	91	58	63.74	33	36.26
8	Furazolidone(Fr)	91	38	41.76	53	58.24
9	Gentamicin(G)	91	83	91.21	8	8.79
10	Nalidixic acid(Na)	91	59	64.84	32	35.16
11	Nitrofurantoin (Nf)	91	24	26.37	67	73.63
12	Norfloxacin (Nx)	91	85	93.41	6	6.59
13	Oxytetracycline (O)	91	34	37.36	57	62.64
14	Streptomycin (S)	91	35	38.46	56	61.54
15	Tetracycline (T)	91	31	34.07	60	65.93

gentamicin (G) -10µg, nalidixic acid (Na) -30µg, nitrofurantoin (Nf) -300µg, norfloxacin(Nx) -10µg, oxytetracycline (O) -30µg, streptomycin(S) -10µg and tetracycline (T) -30µg. Sensitivity was determined on the basis of diameter of the zone of inhibition around the disc as per the chart supplied by the firm (Hi-Media).

After inoculation, the remaining parts of the organ samples were preserved in 10 per cent formalin for histopathological examination. Organs from those dead birds from which *E.coli* were recovered were processed according to the routine procedure for histopathological studies [8]. The paraffin embedded tissues were cut into sections of 4 to 5 µ thickness and stained with Haematoxyline and Eosin (H & E) stain. Histopathological changes in the tissues were recorded.

Results

Out of 150 samples, 91 samples yielded *E.coli* based on cultural, morphological and biochemical characteristics. All the isolates were serotyped as *E.coli* by National *Salmonella* and *Escherichia* Centre, Central Research Institute, Kasauli (H.P.). Of these 91 isolates, 75 were typable, 6 rough and 10 untypable. Serogroup O157 was found to be most predominant (9 strains) followed by O86, O121 (7 each), O9, O75, O131 (5 each), O2, O13, O22 (3 each), O68, O74, O78, O88, O127, O138, O148, O162 (2 each) O4, O20, O44, O55, O69, O87, O91, O97, O100, O102, O132, O154 (1 each).

In-vitro antibiotic sensitivity of the isolates (Table-1), revealed high sensitivity towards Ciprofloxacin (96.70%) followed by Norfloxacin (93.41%), Gentamicin (91.21%), Chloramphenicol (76.92%) and Cephotaxime (74.73%). Comparatively lower susceptibility was recorded against Nitrofurantoin and Tetracycline. Ampicillin was not at all effective.

Grossly, the pigeon carcasses were highly congested. There were severe congestion and haemorrhage in the intestinal mucosa and in some cases showed catarrhal enteritis. Fibrinous perihepatitis, pericarditis, and peritonitis were present in some cases.

Histopathologically, the mucosal epithelium of intestine had congestion, haemorrhage, necrosis and desquamation. Focal areas of diphtheritic enteritis characterized by the presence of necrotic tissue with heterophilic debrises were present over the intestinal mucosa. In certain cases the mucosa showed vacuolation and flattening with occasional villus hypertrophy projecting towards intestinal lumen. Liver showed a thick layer of fibrinous exudates with large number of heterophills over the hepatic capsule along with congestion and haemorrhage. The hepatocytes showed early degenerative changes as well as focal necrosis (Fig. 2.i). Lungs showed congestion with fibrinous exudate and hemorrhages filling the bronchiolar lumina. Presence of fibrinous sheets over the epicardial surface intermingling with heterophills, few mono nuclear cells and erythrocytes were also noticed. Kidneys revealed congestion and haemorrhages with focal areas of interstitial nephritis associated with degeneration and areas of necrosis.

Hjarre's disease or coligranuloma: The gross and microscopic lesions observed in three cases were suggestive of Hjarre's disease or coligranuloma.

Grossly, liver in two cases showed round and hard caseated nodular structures on its surface, deeply embedded into hepatic parenchyma. In one case focal to diffuse areas of necrosis and haemorrhage were seen on the liver surface with multiple nodular lesions of 2-4 mm diameter in the mesentery and serosal surface of intestine (Fig-1).

Histopathologically, liver showed large granulomatous inflammatory reaction characterized by central areas of caseonecrosis occasionally mixed with cellular debrices and at the periphery surrounded by epitheloid cells, lymphocytes and giant cells. The whole structure was surrounded by a connective tissue capsule leading to atrophy of the surrounding hepatocytes (Fig. 2.ii).

The duplicate section from these granulomas stained by modified Gram's stain revealed gram-negative large rods morphologically indistinguishable from *E. coli*.

Discussion

Majority of *E.coli* serotypes identified in this

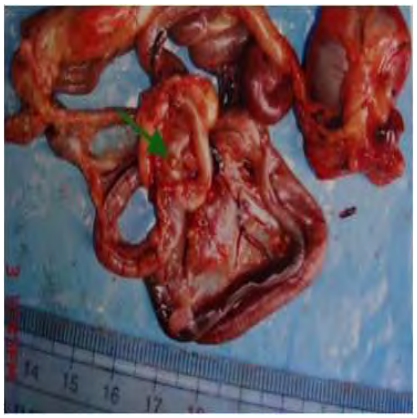

Figure-1. Minute caseated nodular (arrow) lesions over the serosal surface of intestine and mesentery.

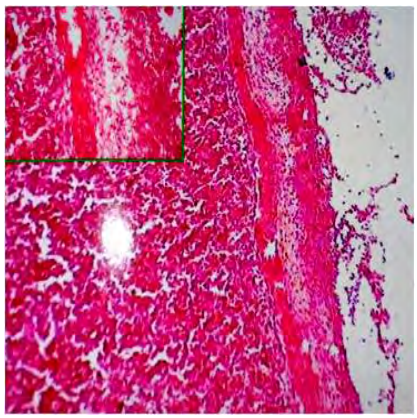

Figure-2.i. Liver showing perihepatitis with fibrinous exudates over the hepatic capsule in colibacillosis. H & E X 10 (inset higher magnification)

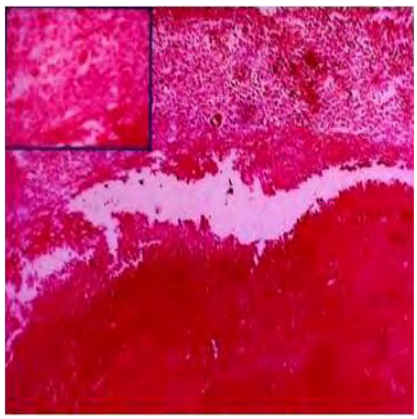

Figure-2.ii. Hjarre's granuloma in liver. H & E X 10 (Inset higher magnification showing gient cells)

study, were reported to be potential pathogens for both young and old poultry, although the pathogenicity varies among different serotypes [9]. In the present study, the most frequent serotype found was O157 (9.89%), followed by O68, O121 (7.69%), O9, O75, O131 (5.49%), O2, O13, O22 (3.30%). Strains belonging to sero-group O157, isolated in the present study were earlier shown to be verocytotoxigenic [10]. There is evidence of *E. coli* serotypes O133, O50, O79, O21, O55, O2 and O125 in pigeon prevalent in Assam [11], [12]. Multiple-antimicrobial-resistant APEC (Avian Pathogenic Escherichia coli) strains i.e. O78, O86, O141, O1 and O2 among rural chickens in the arid region of north-eastern Nigeria [13] is also reported. Variation in feed and water supply along with geographical variation might be the cause of variation in prevalence of serotypes.

In-vitro antibiotic sensitivity of the isolates (Table-1), revealed high sensitivity towards Ciprofloxacin (96.70%) followed by Norfloxacin (93.41%), Gentamicin (91.21%), Chloramphenicol (76.92%) and Cephotaxime (74.73%). Similar results have been reported by earlier worker [13]. Comparatively lower susceptibility was recorded against Nitrofurantoin and Tetracycline. Ampicillin was not at all effective [13], [14]. Lower sensitivity of the isolates towards these drugs might be due to frequent and inadequate use in the pigeon.

Grossly, the pigeon carcasses were highly congested. Fibrinous perihepatitis, pericarditis, and peritonitis were present in some cases. Haemorrhage and congestion were observed in kidneys with congestion in various other organs indicating septicemic nature of the disease. These findings agree with the earlier reports [11].

Histopathologically, the mucosal epithelium of intestine had congestion, haemorrhage, necrosis and desquamation. Changes like desquamation, necrosis and heterophilic infiltration in the intestinal mucosa were also observed earlier workers [11]. In certain cases the mucosa showed vacuolation and flattening with occasional villus hypertrophy projecting towards intestinal lumen indicating a concurrent viral infection. Liver showed a thick layer of fibrinous exudates with large number of heterophills over the hepatic capsule along with congestion and haemorrhage. The hepatocytes showed early degenerative changes as well as focal necrosis. The changes in the liver and intestine are possibly due to the response and reaction in detoxification and excretion of endotoxins, enterotoxin release by *E.coli*.

Lungs showed congestion with fibrinous exudate and haemorrhage filling the bronchiolar lumina. The similar changes like congestion, haemorrhage were described by Phangcho [11].

Presence of fibrinous sheets over the epicardial surface intermingling with heterophills, few mono nuclear cells and erythrocytes were also noticed. Fibrinous exudates are due to acute inflammatory reaction caused by toxin beta haemolycin released by *E.coli* resulting marked increased in vascular permeability. This violent type of injury leads to escape of fibrinogen into the surrounding tissue [15].

Kidneys revealed congestion and haemorrhages with focal areas of interstitial nephritis associated with degeneration and areas of necrosis. Degeneration and necrosis are due to *E.coli* toxins followed by inflammatory reaction leading to interstitial type of nephritis. The histopathological changes as observed in the present study were also recorded by Ficken [16].

In coligranuloma, liver showed large granulomatous inflammatory reaction characterized by central areas of caseo-necrosis occasionally mixed with cellular debrices and at the periphery surrounded by epitheloid cells, lymphocytes and giant cells. The whole structure was surrounded by a connective tissue capsule leading to atrophy of the surrounding hepatocytes (Fig. 2.i & 2.ii). Similar granulomatous lesions were also observed by earlier workers [17,18] in mesentery and serosal surface of intestine, liver and caecum.

The pigeons may acquire infection from contaminated environment, feed and water or from other carrier birds. But the commonest source of *E. coli* in captive birds may be the bird's own commensals intestinal flora which may cause the diseases under stressful conditions.

Conclusion

This study indicates that antimicrobial-resistant *E.coli* isolates are present in pigeons in and around greater Guwahati. Animal health problems created by such resistant strains might being potential danger to human health because through food chain, these strains could easily infect humans. Consequently, introduction of surveillance programmes to monitor emergence of antimicrobial resistant strains requires attention.

Author's contribution

PD and RS participated in the preparation of experimental design and the facilities of the research. PD collected samples and involved in identification, antibiogram and serotyping of the pathogen. PD and MKB involved in gross as well as histopathological studies. PD, RG and RS analyzed the data, drafted and revised the manuscript. All authors read and approved the final manuscript.

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Competing interests

Authors declare that they have no competing interests.

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