Isolation of *Salmonella* Typhimurium from pigeons (*Columba livia*) in Greater Guwahati, its histopathological impact and antibiogram

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Introduction

World over pigeons are mainly consumed as food and also maintained as cage birds. In Belgium and England, pigeons are reared scientifically as done for poultry. A variable susceptibility of the birds to bacterial infections and other diseases, including Salmonellosis, has been periodically documented from different parts of the world. Salmonellosis has been documented as the most prevalent disease of aviaries as well as in racing pigeons (Granville 1973). Occurrence of *Salmonella* Typhimurium was also reported to be predominant among the isolated Salmonellae (Dominici et al. 1976).

The present study was conducted to isolate and identify the serotypes of *Salmonella* spp. in diarrhoeic faecal samples and/or from fresh carcasses of pigeon. The associated gross and histopathological lesions and antimicrobial susceptibility were also documented.

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Materials and methods

Fifty fresh diarrhoeic faecal samples and 100 fresh tissue samples from the lungs, liver, intestine, heart, spleen and kidney were collected from fresh pigeon carcasses in a sterile vial and brought to the laboratory. One gram of faecal or minced tissue sample was aseptically transferred into a sterile tube containing 10 ml of Tetrathionate Broth and/or Rappaport Vassiliadis medium. The tubes were aerobically incubated at 42 °C for 24 h, and then a loopful of broth culture was streaked onto Brilliant Green Agar plates and incubated aerobically at 37 °C for 24 h, and suspected pinkish colonies were further inoculated onto Mac Conkey's lactose agar plates. The plates were incubated aerobically at 37 °C for 24 h for isolation of the pathogen. Representative (lactose non-fermenting pale) colonies suspected to be *Salmonella* were obtained in pure culture on nutrient agar slants by conventional method, and the pathogen was identified as per the method described by Edward and Ewing (1972). The isolates were referred to the National *Salmonella* and *Escherichia* Centre, Central Research Institute, Kasauli (HP), India, for serotyping.

In vitro antibiotic sensitivity of the isolates was assessed using disc diffusion method (Bauer et al. 1966) using disc coated with 15 antibacterial drugs (Hi-Media), namely, ampicillin 10 µg, cephalixin 30 µg, cephalexime 30 µg, chloramphenicol 30 µg, ciprofloxacin 10 µg, colistin 10 µg, cotrimoxazole 25 µg, furazolidone 50 µg, gentamicin 10 µg, nalidixic acid 30 µg, nitrofurantoin 300 µg, norfloxacin 10 µg, oxytetracycline 30 µg, streptomycin 10 µg and tetracycline 30 µg.

Fig. 1 Section of small intestine showed severe congestions with heterophilic and few mononuclear infiltrations. H & E $\times 100$

Tissue samples with gross visible lesions were preserved in 10 % formalin and processed for histopathological investigations using standard protocol (Luna 1968). The paraffin-embedded tissues were cut into sections of 4- to 5- μ thickness and stained with haematoxylin and eosin (H & E) stain. Microscopic lesions in different organs were recorded.

Results and discussion

Out of 150 samples screened for *Salmonella* organisms from pigeons, 12 (8 %) *Salmonella* isolates could be recovered. All the strains were identified as *Salmonella* Typhimurium with antigenic structure 4, 5, I, 1, 2.

Gross lesions comprised of enteritis in 50 % cases followed by hepatic congestion and necrosis (25 %) and pericarditis along with congestion of the spleen and kidneys (12.5 %). Congestion of the lungs (12.5 %) was also recorded in 12.5 % cases. Histopathological investigation revealed intestinal congestion which extended into lamina propria and tunica muscularis (Fig. 1). There was infiltration of heterophils and few mononuclear cells with focal necrosis

Fig. 2 Section of small intestine showed severe congestions with heterophilic and few mononuclear infiltrations. H & E $\times 400$

Fig. 3 Section of intestine showed severe necrosis followed by sloughing mucosa from the surface. H & E $\times 100$

(Fig. 2). Sloughing of the intestinal mucosal layer was evident (Fig. 3). Further, the liver showed sinusoidal congestion and individualization of hepatocytes (Fig. 4) with various degrees of cellular degeneration and necrosis (Fig. 5). Congestion and haemorrhages in the lungs were noticed. Kidney revealed congestion and focal degeneration of tubular epithelial cells. Epicardium showed focal congestion. There was presence of severe congestion, haemorrhage and haemosiderosis on the spleen. Similar histopathological changes were earlier described and documented in poultry (Saikia 1984) and in pigeons (Phangcho 2001). Sukanuma (1960) observed endothelial cell proliferative foci on the liver, focal necrosis of the myocardium, catarrhal enteritis and interstitial infiltration of lymphocytes in salmonellosis of chicks, adult hens and roosters.

In vitro antibiotic sensitivity of the isolates (Table 1) revealed 100 % sensitivity towards ciprofloxacin followed by gentamicin, norfloxacin and chloramphenicol (91.67 % each), co-trimoxazole (75.00 %), cephalexin (66.67 %) and cephalexime (58.33 %). The strains showed lower sensitivity to tetracycline and nitrofurantoin (8.33 % each) followed by oxytetracycline, streptomycin, furazolidone (16.67 %

Fig. 4 Section of the liver showed sinusoidal congestion, individualization of hepatocytes. H & E $\times 400$

Fig. 5 Section of the liver showed various degrees of nuclear degeneration and hepatic necrosis. H & E×100

each) and colistin (33.33 %). None of the *Salmonella* isolates were sensitive to ampicillin. Jha et al. (1994) showed that ciprofloxacin, gentamicin, nalidixic acid, nitrofurantoin and ampicillin were highly effective against *Salmonella* isolated in chicken. However, in the present study, ampicillin and nitrofurantoin were found less effective against *Salmonella*. Asagi et al. (1976) recorded sensitivity of *Salmonella* isolates from pigeons to tetracycline, streptomycin and kanamycin, but in the present study, streptomycin and tetracycline were found to be least effective on *Salmonella*. Cicek and Kovarik (1994) recorded the resistance of *Salmonella* isolated from poultry against ampicillin, streptomycin and colistin. Besides, Pathan et al. (1998) observed the resistance of salmonellae recovered from poultry and pigs to tetracycline, oxytetracycline, streptomycin and trimethoprim. Similarly, Sharma and Joshi (1992) also recorded high sensitivity of salmonellae to gentamicin. Similar patterns of

sensitivity of *Salmonella* isolated from broilers to ciprofloxacin, gentamicin and amikacin was recorded earlier by Swaminathan (2001). He also observed high degree of resistance to ampicillin, oxytetracycline, co-trimoxazole, chloramphenicol, norfloxacin and enrofloxacin. However, in the present study, resistance to these drugs was not seen except to ampicillin and oxytetracycline.

Salmonella Typhimurium strains in the present study have shown higher resistance towards ampicillin, tetracycline and nitrofurantoin. This could be attributed to many reasons like indiscriminate use of these antibiotics without prescription, lack of proper veterinary consultation, not performing an antibiogram when the situation warrants, improper treatment regimens, etc. The routine practice of giving antimicrobial agents to domestic livestock as a means of preventing and treating diseases, as well as promoting growth, is an important factor in the emergence of antibiotic-resistant bacteria that are subsequently transferred to humans through the food chain. Most infections with antimicrobial-resistant *Salmonella* in human are acquired by eating contaminated foods of animal origin (Andoni 1982; Avril et al. 1988).

Knowing the resistance of *Salmonella* Typhimurium strains towards different antimicrobial agents helps the veterinarians to offer a better and more effective treatment to livestock diagnosed with salmonellosis by using the indispensable antibiotic and replacing the resistant antibiotics with new ones. Thus, the present investigation indicated the prevalence of important multidrug-resistant pathogens like *Salmonella* Typhimurium in pigeons, which is of zoonotic importance.

Table 1 Antibiogram of *Salmonella* Typhimurium isolates

Sl no.	Antibiotic	Not tested	Sensitive	Percent	Not tested	Resistant	Percent
1	Ampicillin	12	0	0	12	12	100
2	Chloramphenicol	12	11	91.67	12	1	8.33
3	Cephalexin	12	8	66.67	12	4	33.33
4	Cephotaxime	12	7	58.33	12	5	41.67
5	Ciprofloxacin	12	12	100	12	0	0
6	Colistin	12	4	33.33	12	8	66.67
7	Co-trimoxazole	12	9	75	12	3	25
8	Furazolidone	12	2	16.67	12	10	83.33
9	Gentamicin	12	11	91.67	12	1	8.33
10	Nalidixic acid	12	6	50	12	6	50
11	Nitrofurantoin	12	1	8.33	12	11	91.67
12	Norfloxacin	12	11	91.67	12	1	8.33
13	Oxytetracycline	12	2	16.67	12	10	83.33
14	Streptomycin	12	2	16.67	12	10	83.33
15	Tetracycline	12	1	8.33	12	11	91.67

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