Isolation and Antibiogram of *Salmonella* from Poultry

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**ABSTRACT**

Salmonellosis is one of the most common and widely distributed disease affecting both man and animals. Presence of drug resistant *Salmonella* in poultry and poultry products further pose a risk to public health. This study was undertaken to determine the prevalence of *Salmonella* in poultry. Of 350 poultry faecal as well as cloacal swab samples analyzed, 6 (1.7%) yielded *Salmonella* which comprised of *Salmonella Paratyphi* B (3), *Salmonella Typhimurium* (2) and *Salmonella Stanley* (1). The culture protocol used included pre-enrichment in buffered peptone water, enrichment in Rappaport-Vassiliadis broth and selective plating on brilliant green agar. In order to study the antibiogram pattern of isolated *Salmonella* organisms, seven antimicrobial drugs comprising chloramphenicol, ciprofloxacin, oxytetracycline, nalidixic acid, gentamicin, erythromycin and cotrimoxazole were subjected to drug sensitivity test. Interestingly, all the isolates were found sensitive to chloramphenicol, ciprofloxacin, cotrimoxazole, oxytetracycline and gentamicin while 83.3% of the isolated *Salmonella* serovars were resistant to nalidixic acid and erythromycin.

**Keywords:** Antibiogram, poultry, prevalence, salmonellosis

**Introduction**

Salmonellosis is an important public health problem in affecting both humans and animals and remains one of the leading causes of foodborne illness throughout the world. Salmonellosis accounts for 30% of deaths resulting from foodborne illness in the United States (Mead *et al.*, 1999). About 95% of human salmonellosis cases have been reported to be associated with the consumption of contaminated food products (Foley *et al.*, 2008). Poultry and poultry products have frequently been implicated as a source of *Salmonella* contamination and consequently thought to be the major source of the pathogen in humans.

Salmonellosis is bringing new challenges to the protection of the public health in the form of increasing anti-microbial resistance, the growing problem of salmonellosis in the immunocompromised population, the growing importance of new food vehicles and the potential for enormous and widely dispersed outbreaks related to the increasing centralized production of foods. There are economic constrains in eradication of *Salmonella* infections on the poultry farms by test and slaughter policy. Facultative intracellular nature, endemicity of infection, presence of carrier state in the host further complicates the control of this disease. Bacterial isolation remains the “gold standard” for *Salmonella* detection, since it permits investigators to obtain antibiotic sensitivity patterns and serologic typing. This study was undertaken to assess the prevalence of *Salmonella* among poultry in the region along with their antibiogram.
Materials and Methods

As many as 325 faecal/cloacal swab samples collected from apparently healthy (317) and diarrhoeic (8) chicken and faecal samples (25) from apparently healthy guinea fowl were processed for the isolation of *Salmonella* organisms following the methods described in standard ISO 6579:2002 and by Edwards and Ewing (1972) with slight modifications.

Approximately one gram of faecal sample and cloacal swabs were transferred into a sterile test tube containing 10 ml buffered peptone water (BPW). The sample was mixed thoroughly and was incubated at 37°C ± 1°C for 18-24 h. One milliliter of buffered peptone water was transferred into a tube containing 10 ml of Rappaport-Vassiliadis medium (RV broth), and incubated at 42°C for 18-24 h. After incubation, a loop-full of material from top of the RV broth was taken and streaked separately onto brilliant green agar (BGA) plates, which were incubated at 37°C ± 1°C for 18-24 h. Simultaneously, a positive control of *Salmonella* culture was also streaked on BGA plate for comparative evaluation. After incubation, the plates were checked for growth of typical *Salmonella* colonies.

Appearance of moderately large, moist, smooth and colourless colonies with pink background on BGA were suspected to be of salmonellae and five such representative colonies characteristic of *Salmonella* were picked up and transferred onto MacConkey lactose agar (MLA) plates in order to get pure isolated colonies. After incubating plates at 37°C ± 1°C for 18-24 h, colourless colonies were picked up from MLA and streaked on nutrient agar slant.

The stock cultures prepared on nutrient agar slant were subjected to various biochemical tests *viz.*, triple sugar iron test, urease test, citrate test, indole test, VP test, methyl red test and sugar fermentation tests. The isolated cultures showing positive results in these tests for the presence of *Salmonella* were sent for serotyping to National *Salmonella* Centre, Indian Veterinary Research Institute, Izatnagar, Bareilly.

All the serologically confirmed *Salmonella* isolates were subjected to antibiotic sensitivity test. An isolated colony from the nutrient agar slant was transferred into 5 ml nutrient broth and incubated at 37°C for 24 h. A sterile cotton swab was dipped into the adjusted suspension and rotated several times pressed firmly on the inside wall of the tube above the fluid level. The dried surface of a nutrient agar plate (150 mm) was inoculated by streaking the swab over the entire sterile agar surface. The predetermined antimicrobial discs were dispensed onto the surface of the inoculated agar plate. Each disc was pressed down individually to ensure complete contact with the agar surface. A total of 7 discs consisting of chloramphenicol (30 µg/disc), ciprofloxacin (5 µg/disc), erythromycin (15 µg/disc), nalidixic acid (30 µg/disc), cotrimoxazole (25 µg/disc), oxytetracycline (30 µg/disc) and gentamicin (30 µg/disc) were placed and incubated at 37°C for 24 h. The diameters of the zone of complete inhibition were measured, and compared with the zone size interpretation chart provided by supplier so as to determine the resistant, intermediate and susceptible pattern of the isolates.

Results and Discussion

Initially, five faecal samples from apparently healthy chicken and one of 8 diarrhoeic chicken yielded the putative colonies of salmonellae. On serological typing, the isolates were identified as *Salmonella* Paratyphi B (3), *S. Typhimurium* (2) and *S. Stanley* (1). The details of isolation are depicted in Table 1. One isolate serotyped as *S. Typhimurium* was recovered from diarrhoeal faeces, while rest of the isolates were from non diarrhoeal faecal sample. None of the faecal sample from guinea fowl yielded *Salmonella*. Previous study, however, revealed the prevalence rate of 6.49% in poultry (Sharma and Thapliyal, 1995), which was higher than the present one (1.7%). Murugkar *et al.* (2005) reported a higher prevalence of *Salmonella* (14.7%) in poultry faecal samples from north eastern part of India. The occurrence of salmonellosis in poultry with prevalence rates ranging from 11.42% to 69% has been reported by several workers (Rall *et al.*, 2005; Islam *et al.*, 2006). In the present study, the recovery of salmonellae was found to be lower (1.7%). This
Table 1. Isolation of salmonellae from faecal/cloacal swabs of poultry

<table>
<thead>
<tr>
<th>Sl.</th>
<th>Sample</th>
<th>Total No. of samples examined</th>
<th>Total samples yielded salmonellae</th>
<th>Serovars identified</th>
<th>Number of isolates</th>
<th>Antigenic structure</th>
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<tbody>
<tr>
<td>1.</td>
<td>Chicken faeces/ cloacal swab</td>
<td>325</td>
<td>6</td>
<td>S. Paratyphi B</td>
<td>3</td>
<td>1, 4, [5], 12 : b : 1, 2</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>S. Typhimurium</td>
<td>2</td>
<td>1, 4, [5], 12 : i : 1, 2</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>S. Stanley</td>
<td>1</td>
<td>1, 4, [5], 12, 27 : d : 1,2</td>
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<td>2.</td>
<td>Guinea fowl faeces</td>
<td>25</td>
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Table 2. Antibiotic sensitivity pattern of Salmonella serovars

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<tr>
<td>1.</td>
<td>S. Paratyphi</td>
<td>3</td>
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<tr>
<td>2.</td>
<td>S. Typhimurium</td>
<td>2</td>
<td>0</td>
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<tr>
<td>3.</td>
<td>S. Stanley</td>
<td>1</td>
<td>0</td>
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C= Chloramphenicol (30 µg), Cf= Ciprofloxacin (5 µg), N.A= Nalidixic acid (30 µg), E= Erythromycin (15 µg), Co= Cotrimoxazole (25 µg), G= Gentamicin (30 µg), O= Oxytetracycline (30 µg); R= Resistant, I= Intermediate, S= Sensitive

could be probably attributed to the fact that the most of the samples collected were from non diarrhoeic birds. Interestingly, of eight faecal samples of diarrhoeic chicken processed, one (12.5%) yielded S. Typhimurium indicating that only diarrhoeic faecal samples if analyzed, could have yielded higher number of salmonellae.

Salmonella Paratyphi B is primarily an organism that causes human infection known as paratyphoid fever. The role of infected attendants in transmission of S. Paratyphi B either directly or through the agency of clothes, boots, dust, rodents, flies etc. cannot be ruled out. Alternatively, infected feed also might have played a role in transmission of the organism to poultry. In a previous study at Pantnagar, two isolates of S. Paratyphi B were recovered from poultry (Sharma and Thapliyal, 1995). Several other workers have also reported the isolation of Salmonella Paratyphi B in poultry faecal samples (Basu et al., 1975; Murugkar et al., 2005).

Nagappa et al. (2006) reported meat and egg samples of Tarai region of Uttarakhand to be contaminated with S. Typhimurium. Since, S. Typhimurium has earlier been reported in lizard, goat, toad, pig, shrew, mice, birds and rat from the study area (Sharma and Thapliyal, 1995), the recovery of S. Typhimurium isolates from both diarrhoeic as well as apparently healthy chicken confirms that this particular serovar is still existing in the study area. Presence of S. Typhimurium in chicken may constitute a potential risk to human health.

Only one isolate of S. Stanley was recovered during our investigation. In past, there have been reports of isolation of S. Stanley from poultry meat (Sharma et al., 1995). Basu et al. (1975) also reported 35 isolates of S. Stanley from poultry during the period of 1958-1973. It is worth mentioning that the association of this serotype with outbreaks of poultry disease in India underlines the importance of this organism. Contaminated feed, water, litter, rodents, insects, free flying birds and dogs might have aided in the transmission of this serovar to the poultry.

Two isolates of S. Typhimurium from poultry faeces were also recovered during present study.
All the six isolates of *Salmonella* were tested against seven commonly used antibiotics and chemotherapeutic agents. The sensitivity patterns determined on the basis of zone of inhibition (as per the interpretative chart) has been depicted in Table 2.

All the six *Salmonella* isolates were found to be sensitive to chloramphenicol, ciprofloxacin, cotrimoxazole, oxytetracycline and gentamicin. One of the isolates of *S. Paratyphi B*, *S. Typhimurium* (2) and *S. Stanley* (1) were found to be resistant to erythromycin and nalidixic acid. In most of the study conducted world over on establishing the sensitivity pattern of nalidixic acid, many of the isolated *Salmonella* organisms were found resistant to this antimicrobial agent (Laxmi *et al.*, 2006). A high frequency of antibiotic resistance (100%) to erythromycin from veterinary sources in India is supported by the finding of Willinga *et al.* (2002) and Aksakal *et al.* (2009).

All the *Salmonella* recovered during the course of present study were found sensitive to cotrimoxazole. Aksakal *et al.* (2009) also had similar observation. However, contrary to the present finding, resistances to cotrimoxazole among poultry isolates from USA (Zhao *et al.*, 2006) have been reported.

*Salmonella* isolated in the present study were found to be sensitive to chloramphenicol, which is similar to the study of Mandal *et al.* (2004) who reported that the *Salmonella* isolates in India from 1996-99 and 2001 were 100% chloramphenicol sensitive. This chloramphenicol sensitivity could be attributed to the limited use of this antimicrobial agent during the last decade in India.

All the *Salmonella* serovars isolated during the present study were found sensitive to gentamicin, oxytetracycline and ciprofloxacin. Similar observations were recorded by several workers (Willinga *et al.*, 2002; Aksakal *et al.*, 2009).

### References


