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Short Communication

Isolation, Characterization and Antibiogram Pattern of Salmonella from Poultry in Parts of Haryana, India

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ABSTRACT

In the present study, a total of 150 samples were collected from broiler birds suspected of fowl typhoid from different parts of Haryana state, India. Isolation of the causative pathogen was done and subsequently the obtained isolates were characterized biochemically and for culture characteristics. Serotyping and antibiotic sensitivity pattern was also drawn. Results revealed that 126 (84%) isolates were *Salmonella* Gallinarum, 15 (10%) were *Salmonella* Enteritidis, and 9 (6%) were *Salmonella* Typhimurium. Antibiograms of isolates revealed that most of the isolates were sensitive to gentamicin (76%) followed by amikacin (72%) and kanamycin (71%). Maximum resistance was obtained against Nalidixic acid (68.0%) followed by Carbenicillin (56%). All the isolates of *S.* Typhimurium and *S.* Enteritidis were 100% resistant against Nalidixic acid. The detection of *S.* Enteritidis and *S.* Typhimurium from fowl typhoid cases assumes significance from public health point of view and their emerging antibiotic resistance is also of major concern, for which effective prevention and control measures need to be carried out timely.

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Salmonellosis is an endemic disease in India and its prevalence in the animals acts as a continuous threat to man. About more than 2,300 serotypes of *Salmonella* have been identified and only about 10% of these were isolated from poultry (Kabir, 2010). Vertical transmission of infection from breeding hens to progeny is an important aspect of the epidemiology of *Salmonella* spp. infection within the poultry industry (Keller et al. 1997). Salmonellosis in poultry is primarily caused by *Salmonella* enterica serovar Gallinarum and serovar Pullorum causing the diseases Fowl Typhoid and Pullorum Disease, respectively. Prakash et al. (2005) revealed that the most predominant serotypes from poultry were *S.* Gallinarum accounting for 69.6% followed by *S.* Enteritidis for 21.7%. The detection of *S.* Enteritidis and *S.* Typhimurium from fowl typhoid cases assumes significance from public health point of view.

Continuous monitoring on antibiotic resistance properties of pathogens are of significance, so as for *Salmonella*. Since the consumption of poultry products is often associated with salmonellosis, therefore, it becomes interesting to be updated about *Salmonella* resistance scenario to antibiotics used in poultry production (Carraminana et al. 2004; Kabir, 2010; Tiwari et al. 2013). Therefore, the present study was planned to study the isolates of Salmonella from broiler population, followed by their characterization, serotyping and to draw the antibiogram pattern of the pathogen

A total of 150 samples (one pooled sample/flock) of heart blood, liver and bile were collected aseptically from birds showing typical lesions of fowl typhoid *viz.* necrotic foci on liver, enlarged liver, dark and friable with a distinctive coppery bronze sheen (Shivaprasad, 2000; OIE, 2008) during post–mortem examination of poultry birds at Disease Investigation Laboratory in the Department of Veterinary Public Health and Epidemiology, LLRUVAS, Hisar.

Identification of bacterial isolates and biochemical tests were performed as per O.I.E (2008). Briefly, inoculums prepared from tissues were streaked on McConkeys' Lactose Agar (MLA) and brilliant green agar (BGA) plates and kept at 37^0C for 24 hours. Impression smear of liver from affected birds was also made on a clean glass slide and Gram's staining as well as methylene blue staining was done for presumptive diagnosis. Bacterial colony from each culture was further stained by Gram's staining. Organisms giving smooth pin point, pale transparent colonies (non lactose fermentor) on MLA were also streaked on Brilliant green agar (BGA) medium plates and after 24 hours of incubation showed typical small, smooth, dew drop like colonies with pink background. Culture characteristics on MLA and BGA were used for initial identification of *Salmonella* spp. Different biochemical tests such as Indole, Methyl Red, Citrate,Voges–Proskauer, Urease, Catalase, Oxidase, Sugar fermentation, and growth patterns tests were carried out for the characterization of the organism. Based on biochemical characterization, the isolates were maintained in the Maintainance Medium in duplicate at 4 C^o for further study. Serotyping was performed as per the methodology adopted by National *Escherichia* and *Salmonella* Centre, Kasauli, Solan (Himachal Pradesh), India.

In vitro susceptibility of the organisms to various antimicrobial agents was determined by the disc diffusion technique (Bauer et al. 1996). Five ml of 16–18 hours growth of positive isolates in brain heart infusion (BHI) broth was streaked on Mueller–Hinton agar (Himedia) plates by pour plate method. Sixteen antibiotic discs (Himedia) namely

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amikacin (30 mcg), ampicillin (10 mcg), ampicillin–salbactum (10 mcg), Co–Trimazole (25 mcg), ciprofloxacin (5mcg), chloramphenicol (30 mcg), cephotaxime (30 mcg), ceftriaxone (10 mcg), enrofloxacin (5 mcg), carbenicillin (100 mcg), nalidixic acid (30 mcg), norfloxacin (10 mcg), spectinomycin (100 mcg), tetracycline (30 mcg), sulphafurazole (300 mcg), kanomycin (30 mcg), and gentamicin (10 mcg). Plates were incubated at 37^0C for 24 hours. Results were recorded using antibiotic zone scale and interpreted as sensitive, intermediate and resistant based on values given in zone size interpretative chart (Himedia, India).

Table 1: Relative occurrence of different serotypes of *Salmonella* from poultry

Serotypes	No. isolated	Relative Occurrence (%)	Antigenic structure
S. Gallinarum	126	84	9,12:–:–
S. Enteritidis	15	10	9,12:g,m:–
S. Typhimurium	9	6	4,12:1:1,2
Total	150		

Impression smears of affected liver when stained with methylene blue revealed small bacterial rods under oil emersion which was confirmed by isolation. Isolation was done mainly from heart blood, liver and bile. On the basis of cultural and biochemical characteristics *Salmonella* was isolated from all the suspected tissues for fowl typhoid. From bile, 100% isolation was recorded followed by heart blood. *Salmonella* was also isolated from the ova of a dead parent bird.

Serotyping of 150 positive cultures of *Salmonella* isolates was carried out in Central Research Institute, Kasauli which revealed that 126 isolates were S. Gallinarum, 15 were S. Enteritidis and the remaining 9 were S. Typhimurium.

Table 2: *In–vitro* chemotherapeutic drug sensitivity (%) of *Salmonella* spp. isolated from different tissues.

Drug	Sensitive (%)	Intermediate Sensitivity (%)	Resistant (%)
Amikacin	72	10	18
Ampicillin	67	15	18
Cefoperazone	60	21	19
Co–Trimoxazole	69	14	17
Ciprofloxacin	20	63	17
Chloramphenicol	71	19	10
Enrofloxacin	66	20	14
Carbenicillin	20	24	56
Cephotaxime	15	65	20
Ceftriaxone	17	62	21
Nalidixic Acid	12	20	68
Norfloxacin	28	20	52
Tetracycline	21	64	15
Sulphafurazole	68	18	14
Kanomycin	71	15	14
Gentamycin	76	10	14
Amoxy–Clav	69	13	18
Streptomycin	70	18	12
Amp–Salb	66	21	13

The antibiogram pattern (Table 2) of the 150 isolates revealed that most of the isolates were sensitive to gentamicin (76%) followed by amikacin (72%), kanamycin (71%) and chloramphenicol (71%) which was more or less in accordance with the findings of Park et al. (1995), Oh et al. (2000) and Selvaraj et al. (2010). Intermediate sensitivity was found towards cefotaxime (65%), tetracycline (64%), ciprofloxacin (63%) and ceftriaxone (62%). All the isolates of S. Typhimurium and S. Enteritidis were 100% resistant against nalidixic acid that was also observed in the studies of Kumar et al. (2011).

Above all, maximum resistance was obtained against nalidixic acid (68.0%) followed by Carbenicillin (56%) which was also observed in studies of Lee et al. (2007). A study by Taddele et al. (2012) also showed the high prevalence of nalidixic acid resistance among *Salmonella* isolates. In the present study, all the isolates were resistant to more than one antimicrobials used, indicating the prevalence of multiple drug resistance which substantiates the findings of earlier workers (Shivhare et al. 2000; Shah et al. 2001; Bhattacharya et al. 2001; Carraminana et al. 2004; Siemon et al. 2007). The high rates of resistance found in this study can be explained by the widespread of use of antibiotics agents given to poultry as prophylaxis, growth promoters or treatment assumed that isolates which are resistant to two or more antibiotics have originated from high–risk sources of contamination like commercial poultry farms, where antibiotics are commonly used (Hatha and Lakshmanaperumalsamy, 1995).

The isolation and biochemical results were in accordance with the Bergey's Manual of Determinative Bacteriology (Holt et al. 1994) as well as with the findings of Lee et al. (2003). Some variability in the biochemical characteristics as reported by Shah et al. (2001) was observed in these isolates too. The present study also revealed that the prevalence of *Salmonella* Gallinarum was maximum followed by S. Enteritidis and S. Typhimurium. S. Gallinarum, the causative agent of fowl typhoid, is the most prevalent host–adapted *Salmonella* strain of poultry in India (Gupta et al. 1999). Prakash et al. (2005) also revealed that serovars of S. Gallinarum were maximum followed by S. Enteritidis. The detection of S. Enteritidis and S. Typhimurium from fowl typhoid cases assumes significance from public health point of view. Similar findings were reported by Rahman et al. (2002) and Prakash et al. (2005).

In recent years, antibiotic resistance in *Salmonella* has assumed alarming proportions worldwide (Kabir, 2010; Tiwari et al. 2013). The more prudent use of antibiotics by farmers, veterinarians, and physicians suggests the appearance of substantial multi resistance in foodborne *Salmonella* isolates (Carraminana et al. 2004). The antibiogram pattern of the isolates revealed that most of the isolates were sensitive to gentamicin, followed by amikacin and chloramphenicol which was more or less in accordance with the findings of Park et al. (1995) and Oh et al. (2000) and maximum resistance was obtained against nalidixic acid, followed by Carbenicillin which was also observed in studies of Lee et al. (2007). It is generally assumed that isolates which are resistant to two or more antibiotics have originated from high–risk sources of contamination like commercial poultry farms, where antibiotics are commonly used (Hatha and Lakshmanaperumalsamy, 1995).

Therefore, the present study concluded that S. Gallinarum was most prevalent serovar of salmonella in the region. Majority of isolates were resistant to nalidixic acid and carbencillin, while most sensitive antibiotics were gentamicin, amikacin and kanamycin. Necessary corrective measures need to be adapted for prevention and control of Salmonella and its emerging antibiotic resistance in poultry

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