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*Original Research Article*

# Isolation and Antibiotic Sensitivity Pattern of *Salmonella Enterica* Isolates from Livestock and Poultry of Haryana

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## ABSTRACT

*Salmonella enterica* is one of the main enteric pathogen of animals and humans, which leads to diarrhea and mortality. Livestock and poultry are not only the main economic activity of farmers of Haryana state but also contribute food security to the state and country as a whole. Study on disease caused by Salmonellosis in livestock and poultry of Haryana was performed along with detail of serotypes circulating and antibiotic resistance profile of isolates during a period of three years. In present study, a total of one-hundred and twenty-five samples of bovine and poultry were attempted for isolation on selective media, followed by serotyping and antibiotic sensitivity testing by disc diffusion method. Thirty-two isolates out of a total of eighty-one samples collected from commercial broiler farms, were found positive for *Salmonella* spp., out of which *S. typhimurium* was found predominant (26/32) followed by rarely reported *S. miami* (4/32) and *S. infantis* (2/32). Out of forty-four fecal samples collected from cattle and buffaloes, no sample was found positive for Salmonellosis in present study. Antimicrobials Norfloxacin, Kanamycin, Amikacin, Chloramphenicol and Streptomycin were found most effective against *Salmonella enterica* isolates. Resistance of *Salmonella enterica* isolates was found highest towards Sulfa drugs, Tetracycline, Nitrofurantoin, Nalidixic acid and Ciprofloxacin. Detection of *Salmonella enterica* serovars from Haryana state confirms its occurrence but with changing pattern of circulating serotypes, more of public health importance and observation of multiple drug resistance necessitates routine testing of *Salmonella* spp. in poultry, better food safety and judicious use of antimicrobials in animals.

***Key words:*** Salmonella, Broiler, Serotypes, Antibiotics, Haryana

## INTRODUCTION

Agriculture is one of the main contributors of economy of Haryana state and the country, which provides employment and food security to not only the state but also to other parts of the country. Poultry and livestock are major contributors to agricultural economy and help in reducing protein deficit. *Salmonella enterica*, a member of *Enterobacteriaceae*, is a major etiological agent of typhoid in human (*S. typhi* and *S. paratyphi*) and causative agent of diarrhea in animals, followed by mortality in severe cases, is reported by many investigators previously. Poultry and poultry products are major source of human infections caused by Salmonella, which has increased enormously over the years (EFSA,2007). [1] Serotype detection helps in better understanding of the disease as some Salmonella spp. are host adaptive (*Salmonella gallinarum* and *S.pullorum* in poultry, *S.anatum* in cattle) while others have diverse host range (*S. typhimurium, S. enteritidis*), which are of public health

importance. Multiple antibiotic resistance is currently biggest challenge for pharmaceutical and health sector, as a number of antibiotics are unresponsive against *Salmonella* infections. So, the present study was attempted to investigate the prevalent Salmonella serotypes from poultry, cattle and buffalo in the state and to know the antibiotic resistance pattern of these bacteria.

## MATERIALS AND METHODS

A total of eighty-one tissues (liver) and blood samples were collected from dead broilers of commercial poultry farms on postmortem examination. All these broilers were one to two months old and were suffering from diarrhea whereas 44 fecal swabs were collected from diarrheic cattle and buffalo from different parts of Hisar, Jind and Sonipat districts of Haryana state during three year period (2013 to 2016). Tissue and blood samples from poultry were transferred directly on MacConkey Lactose Agar (MLA) (Hi-Media) and incubated at 37$^{o}$C for 24 h. All fecal swabs were transferred on pre-enrichment Buffered Peptone Broth (Hi-Media) and incubated for 18-24 h at 37 $^{o}$C. Incubated broth (0.1ml) was then transferred for enrichment to 10 ml Tetrathionate broth (Hi-Media) and further incubated at 37$^{o}$C for 24 h, which was later on streaked on solid media for selective growth. Selective media used were Xylose Lysine Deoxycholate Agar (XLD) (Hi-Media), Brilliant Green Agar (BGA) (Hi-Media), Hekoen Enteric Agar (HE agar) (Hi-Media). After 24 h incubation at 37$^{o}$C, suspected colonies were selected for further confirmation by biochemical tests [2] and agglutination reaction against "O" antisera (IVRI, Izatnagar). Isolation and identification procedure was as suggested by Cowan *et al* (1993). [3] Thereafter *Salmonella* spp. positive isolates were reconfirmed and subjected to serotyping at were sent to Central research Institute, Kasauli (Himachal Pradesh). Isolates were stored in maintenance media (nutrient agar) at 4$^{o}$C till further use.

Confirmed Salmonella isolates were further subjected to antibiotic susceptibility testing by disc diffusion method (Baurer *et al*., 1996). [4] Briefly, a single colony was picked and inoculated on nutrient broth and incubated for 4-6 h at 37$^{o}$C. Around three ml of broth was spread on Mueller Hinton's Agar (MHA) (Hi-Media) by swab in duplicate plates. A total of 16 antibiotic disc (Hi-Media) namely Co-trimoxazole (25 µg), oxytetracycline (30 µg), kanamycin (30 µg), Cefotaxime (30 µg), Nitrofurantoin (300 µg), Ampicillin (10 µg), Amoxicillin (10 µg), Nalidixic Acid (30 µg), Norfloxacin (30 µg), enrofloxacin (05 µg), Chloramphenicol (30 µg), Ciprofloxacin(05 µg), Amikacin (30 µg), Streptomycin (10 µg), Cefalexin (30 µg) and Gentamicin (10 µg) were selected for antibiotic profiling of *Salmonella* spp. Zone of inhibition (mm) were recorded after incubation at 37$^{o}$C for 24 h.

## RESULTS

A total of 35 samples appeared small pin pointed transparent colorless colonies grown on MLA, red colonies with/without black center on XLD agar, pink colony on red discolored medium on BGA and transparent green colony on HE agar, gram negative rods on staining, were considered positive for *Salmonella enterica*. All test isolates showed biochemical test reactions (Table 1) typical of *Salmonella enterica*:-

All the samples showed positive agglutination reaction against "O" antisera of Salmonella. Out of these, thirty-two isolates were confirmed positive for *Salmonella enterica* by Central research Institute, Kasauli (Himachal Pradesh). Antigenic structure along with isolated *Salmonella enterica* serotypes (Table 2) revealed that three types of Salmonella serotypes were found, among these *S. typhimurium* was found to be predominant (26/32). However serotype *S.miami* confirmed from isolates under current study was rarely reported worldwide and detection of *S.infantis* in present study was rarely

reported from India and appears to be first report from poultry of Haryana state.

**Table 1: Biochemical characteristic of *Salmonella enterica* isolates**

| | | |
|---|---|---|
| 1 | Indole | Negative |
| 2 | Methyl Red (MR) | Positive |
| 3 | Vogus Proskaur (VP) | Negative |
| 4 | Citrate | Positive |
| 5 | Urease | Negative |
| 6 | H2S production on TSI | Positive |
| 7 | Lysine decarboxylation | Negative |
| 8 | ONPG | Negative |
| 9 | Lactose | Negative |
| 10 | Arabinose | Positive |
| 11 | Mannitol | Positive |
| 12 | Sucrose | Variable (60% positive) |
| 13 | Dulcitol | positive |

Sensitivity and resistance pattern against 16 antibiotics were recorded for all *S.enterica* isolates (Table 3). Most of isolates were found to be sensitive to Norfloxacin (32/32) and Streptomycin (32/32) followed by Kanamycin (30/32), Chloramphenicol (30/32), Amikacin (30/32), Cefotaxime (29/32), Ampicillin (27/32), Amoxicillin (26/32) and Gentamicin (23/32). Whereas maximum resistance was found against Enrofloxacin (100%) followed by Oxytetracycline (96.8%), Co-trimoxazole (90.6%), Nitrofurantoin (87.5%), Nalidixic Acid (53.12%), Cefalexin (46.8%) and Ciprofloxacin (43.75%).

**Table 2: Antigenic structure and serotypes of *Salmonella enterica* isolate**

| Sample Code | Serotypes | Total no. of Samples | Antigenic Structure |
|---|---|---|---|
| V-BT-1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 19, 21, 22, 23, 24, 25, 26, 27, 28, 30, 31, 32 | *S.Typhimurium* | 26 | 4,12:i:1,2 |
| V-BT-17,18,25,30 | *S.Miami* | 4 | 9,12:a:1,5 |
| V-BT-20,29 | *S.Infantis* | 2 | 6,7:r:1,5 |
| TOTAL | | 32 | |

In addition to presence of Salmonella, five out of 125 samples were found positive for *Escherichia coli* (*E.coli*), showing lactose fermenting pink colony on MLA after 24 hours incubation at 37$^{o}$C. This isolation was done from blood samples of broilers and no further study was performed on above.

**Table 3: Antibiogram of *Salmonella enterica* isolates**

| S.No | Serotype | Sample code | Antibiotic for which resistance was observed | No. of resistant antibiotics |
|---|---|---|---|---|
| 1. | *S.typhimurium* | V-BT-1 | O, Ex | 2 |
| 2 | *S.typhimurium* | V-BT-2 | Na,Ex | 2 |
| 3 | *S.typhimurium* | V-BT-3 | O,Nit,Ex | 3 |
| 4 | *S.typhimurium* | V-BT-22 | cot,O,Na,Ex | 4 |
| 5 | *S.miami* | V-BT-30 | cot,O,Na,Ex | 4 |
| 6 | *S.typhimurium* | V-BT-16,23,27,32 | Cot,O,Nit,Ex | 4 |
| 7 | *S.typhimurium* | V-BT-5,6 | Cot,O,Nit,Ex,Cn | 5 |
| 8 | *S.miami* | V-BT-25 | Cot,O,Nit,Ex,Cn | 5 |
| 9 | *S.typhimurium* | V-BT-15 | Cot,O,Nit,Ex,Cip | 5 |
| 10 | *S.typhimurium* | V-BT-7 | cot,O,Nit,Na,Ex,Cn | 6 |
| 11 | *S.typhimurium* | V-BT-8 | Cot,O,Nit, Ex,Cn,Gen | 6 |
| 12 | *S.typhimurium* | V-BT-9 | Cot,O,Nit, Amx,Na,Ex | 6 |
| 13 | *S.typhimurium* | V-BT-12,26 | Cot,O,Nit,Amp,Ex,Cip | 6 |
| 14 | *S.miami* | V-Bt-18 | Cot,O,Nit,Amp,Ex,Cip | 6 |
| 15 | *S.typhimurium* | V-BT-31 | cot,O,Nit,Na,Ex,Cip,,Gen | 7 |
| 16 | *S.typhimurium* | V-BT-13 | Cot,O,Ctx,Nit,Na,Ex,Cn | 7 |
| 17 | *S.typhimurium* | V-BT-14,19 | Cot,O,Nit,Na,Ex,Cip,Cn | 7 |
| 18 | *S.typhimurium* | V-BT-24 | Cot,O,Nit,Na,Ex,Cip,Gen | 7 |
| 19 | *S.infantis* | V-BT-29 | Cot,O,Nit,Na,Ex,Cip,Gen | 7 |
| 20 | *S.typhimurium* | V-BT-10 | cot,O,Nit,Amx,Ex,Cip,,Ak,Cn | 8 |
| 21 | *S.typhimurium* | V-BT-4,28 | Cot,O,Nit, Na,Ex,Cip,Cn,Gen | 8 |
| 22 | *S.miami* | V-BT-17 | Cot,O,Nit, Na,Ex,Cip,Cn,Gen | 8 |
| 23 | *S.typhimurium* | V-BT-11,21 | Cot,O,K,Ctx,Nit,Amp,Amx,Na,Ex,C,Cn,Gen | 12 |
| 24 | *S.infantis* | V-BT-20 | Cot,O,K,Ctx,Nit,Amp,Amx,Na,Ex,C,Cn,Gen | 12 |

Note: Cot: Co-trimoxazole, O:Oxytetracycline, K: Kanamycin, Ctx: Cefotaxime, Nit: Nitrofurantoin, Amp: Ampicillin, Amx: Amoxicillin, Na:Nalidixic Acid, Nx: Norfloxacin, Ex: Enrofloxacin, C: Chloramphenicol, Cip: Ciprofloxacin, Ak: Amikacin, S: Streptomycin, Cn: Cefalexin, Gen: Gentamicin

## DISCUSSION

Detection of *Salmonella* spp. from poultry of Haryana state is being reported by many workers during recent past and this study further adds to the knowledge that poultry especially broilers of Haryana have

some incidence of the disease which needs to be decreased. In addition to isolation of *Salmonella enterica* from poultry, serotyping and antibiotic sensitivity assays results of present study were analyzed with the information already available. Detection of thirty-two *Salmonella enterica* isolates form a total eighty-one suspected cases suggested that isolation from all suspected cases were not possible and this high test positivity does not reflect prevalence of the disease, but surely emphasize the need for development of control strategies. It is water borne bacteria and rapidly spread through oral-fecal route by horizontal transfer as well as vertical transmission to chicks from parent at time of hatching. Serotyping results shows that *S. gallinarum* and *S. pullorum*, could not be confirmed from cases of poultry. Detection of *S. typhimurium* predominantly from poultry of Haryana is in contrast to previous studies of Kumar *et al.* (2012), [5] and Arora *et al.* (2015) [6] where it was reported that *S. gallinarum* and *S. pullorum* were predominant in this area, whereas *S. typhimurium* was fourth predominant serotype reported from poultry of Haryana preceded by *S. enteritidis*. The possible explanation for this changing pattern may be that poultry farmers of Haryana have some-how reduced vertical transmission of *S. gallinarum* and *S. pullorum* from parents to chicks at the time of birth. This may also be result of management at hatcheries or companies supplying chicks, by way of better sanitary-phyto sanitary practices or some possible genetic improvement. Second possibility might be choice of sample, as samples were taken from adult broilers (more than one month old), not from chicks or young (less than 2-3 weeks) which are mostly affected by host adaptive *S. gallinarum* and *S.pullorum* serotypes. So, this high detection of *S. typhimurium* may be just because of relatively less detection of other serotypes rather than actual increase in incidence. This observation was found similar to report of Upadayay *et al.* (2016) [7] where S. typhimurium was found predominant from chicken meat but S. gallinarum and S.pullorum were not detected. Similar predominant detection of S. typhimurium was reported by Sudhanthirakodi *et al.* (2016) [8] from Kolkata, India. This emphasize the need for continuous monitoring of local serotypes of Salmonella spp. circulating in the state and country, as present study suggest that pattern of circulating serotypes may be changing with time in Haryana state over period of time. *S. typhimurium* was reported as predominant serotype by Singh et al. (2013) [9] from Bareilly area form poultry and poultry environment, which was also in agreement with the present study.

Although detection of *S.typhimurium* may be real increase or relative increase in this serotype, this finding suggests that there are higher chances of human cases of Salmonellosis in farmers handling poultry and increased risk in human through consumption of improperly cooked poultry products.

Documentation of *S.miami* is not available to investigator and need to be explored in detail. Detection and confirmation of four isolates of *S.miami* form broilers of Haryana state with antigenic structure 9,12:a:1,5 in present study necessitates more research in this area. This may be new introduction or isolated cases as no report from India was available. Any further report of *S.miami* from Haryana state or country from human, livestock or poultry can suggest the link and possible importance of finding.

*S.infantis* (2/32) detection in poultry was also a new to the state and only some reports may be available from the country. This serotype is also of public health importance and is not host adaptive. Kaushik et al (2014) [10] reported detection of *S.infantis* from chicken meat with positivity rate of 3.7% compared to predominant serotype *S. typhimurium* (51.8%). Report of human cases of S. infantis by Taneja *et al.* (2014) [11] from Chandigarh along with detection of *S. typhimurium* confirms public health

importance of both of these serotypes. This observation suggests that a new serotype might have introduced in poultry recently which needs to be studied by further investigations.

Detection of *E.coli* (5/125) from cases of poultry seems normal as this is common enteric pathogen available in the environment. Further study of *E.coli* was not done as this was beyond scope of present investigation. Similar study in Haryana by Mahajan *et al*. (1994) [12] reported that *E.coli* and *Salmonella* spp. caused major infections of broilers, which was in agreement with detection of *E.coli* from poultry in present study and this agent may be found very commonly.

The absence of *Salmonella* spp. from random diarrhea cases of cattle and buffalo in present study, suggest that Salmonella may not be causes of diarrhea in large animals in the state. Other cause of infection needs to be studied for better management. Further investigation is required to confirm reduced prevalence of *S.gallinarum* and *S.pullorum* from poultry and other *Salmonella enterica* spp. agent investigation from livestock.

The result of antibiotic sensitivity profiling shows more sensitivity was found towards Kanamycin and Amikacin which was in agreement with the study of Arora *et al*. (2015). [6] This indicates that both of these antibiotics are still selectively being used and may remain useful in control of disease presently and in near future. Less resistance to Norfloxacin, Kanamycin and nitrofurantoin may be because of negligible use in poultry now a days. More sensitivity to Chloramphenicol and streptomycin indicates that these drugs are being relatively less used at present and recent past. This re-emergence of sensitivity to chloramphenicol and streptomycin, was in agreement with the results documented by Kumari *et al*. (2013) [13] where chloramphenicol sensitivity re-emergence due to possibly less use in poultry industry, was observed. Similar observation of Kanamycin, Amikacin and Chloramphenicol sensitivity of *Salmonella* isolates reported by Selvaraj *et al.* (2010) [14] suggest that these antibiotic sensitivity patterns can be found in different parts of the country.

Resistance to less used antibiotics Co-trimoxazole, Oxytetracycline, Nitrofurantoin and Nalidixic acid might be due to inherent resistance in bacteria and may not be an event of conjugational transfer of antibiotic resistance. This observation was found in agreement with the report of Sudhanthirakodi *et al*. (2016). [8] Multiple antimicrobial resistance was very high in present study which may be result of frequent use of antibiotics in poultry rather than scientific use. This multiple resistance against *Salmonella* spp. is a serious issue as these serovars are of public health importance. Multiple drug resistance was also reported by Singh *et al*.(2010), [15] Taddele *et al.* (2012), [16] Singh *et al.* (2013), [9] Kaushik *et al.* (2014) [10] and Sudhanthirakodi *et al.* (2016). [8] Judicious use of antibiotics along with advances in antimicrobials can help in reducing this resistance to many drugs and hence in control of disease within time with less loss of production.

## CONCLUSION

This study found that Salmonella spp. causes loss to poultry farmers by affecting broilers causing diarrhea and mortality. Detection of *S. typhimurium* predominantly along with no detection of host specific *S. gallinarum* and *S. pullorum*, indicates that somehow due to changes in poultry farming loss due to host specific Salmonella are getting reduced and emergence of *S. typhimurium*, a public health pathogen still remains a serious concern. Detection of *S. infantis* from broilers was found new in this area but reports of human cases confirm that this serotype was already circulating in area and need of more study for effective control is required. Multidrug resistance observed in this study suggests that bacterial pathogen might be evolving for their survival against

antibiotics by possible resistance transfer mechanism and again emphasize that judicious use of antibiotics is needed for timely effective control of the disease.

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**Conflict Of Interest:** It is declared that there is no conflict of interest in the present paper.

## REFERENCES

1. EFSA. European Food Safety Authority. EU-wide survey on Salmonella levels in broilers. Available at: http://www.efsa.europa.en/en/press_room/press-release/pr-zoon_Salmonella broilers.html. 2007; Accessed on 28/05/2015.
2. Mac-Faddin JF. Biochemical tests for identification of Medical Bacteria. Williams and Wilkins. Baltimore. 1976.
3. Cowan ST, and Steel KJ. Cowan and Steels manual for the identification of medical bacteria. 3rd edn. Barrow.G.I. and Feltham.R,K,A,eds. Cambridge University Press. Cambridge.U.K. 1993; 97-164.
4. Baurer AW, Kirly WMM, Sherris JC, and Turck M. Antibiotic susceptibility testing by a standardized single disc method. *Am J Clin Pathol.* 1996; 45:493-49.
5. Kumar T, Mahajan NK, and Rakha NK. Isolation and prevalence of Salmonella serovars form poultry in different parts of Haryana, India. *Indian J Anim Sci*. 2012; 82(6).
6. Arora D, Kumar S, Jindal N, Narang G, Kapoor P,. and Mahajan NK. Prevalence and epidemiology of *Salmonella enterica* serovar *gallinarum* from poultry in some parts of Haryana, India. *Vet World*. 2015; 1300-1304.
7. Upadayay AK, Kiran, Mansi, Singh PK, Ipshita, and Kumar A. Epidemiology and antimicrobial resistance of *Salmonella* isolated from meat. *IRJNAS*. 2016; 3(2):40-45.
8. Sudhanthirakodi S, Jai P, Chattopadhyay UK, Dutt S. Non-typhidal *Salmonella* isolates from livestock and food samples, Kolkata. India. *J Microbiol Infect Dis.* 2016; 6(3):113-120.
9. Singh R, Yadav AS, Tripathi V, and Singh R.. Antimicrobial resistance profile of Salmonella prevalent in poultry and poultry environment in north India. *Food Control*. 2013; 3(2):545-548.
10. Kaushik P, Anjay, Kumari S, Bharti SK, and Dayal S. Isolation and prevalence of Salmonella from chicken meat and cattle milk collected from local market of Patna, India. *Vet World*. 2007; 7(6):62-65.
11. Taneja N, Appannanavar SB, Kumar A, Verma G, Kumar Y, Mohan B, and Sharma M. Serotype profile and molecular characterization of antimicrobial resistance of non-typhoidal *Salmonella* isolated from gastroenteritis cases over nine years. *J Med Microbiol*. 2014; 63: 63-73.
12. Mahajan NK, Jindal N, and Kulshrestha RC. Major broiler diseases in some parts of Haryana. *Indian J Anim Sci*. 1994; 64:1118-1112.
13. Kumari D, Mishra SK, and Lather D. Pathomicrobial studies on *Salmonella gallinarum* infection in broiler chickens. *Vet World*. 2013; 6: 725-729.
14. Selvaraj R, Das R, Ganguli S, Ganguli M, Dhanalakkshmi S, Mukhopadhayay SK. Characterization and antibiogram of *Salmonella* spp. from poultry specimens. *J Microbiol Antimicrob.* 2010; 2(9): 123-126.
15. Singh BR, Agarwal M, Chandra M, Verma M, Sharma G, Verma JC, and Singh VP. Plasmid profile and drug resistance pattern of zoonotic *Salmonella* isolation of Indian Buffalo. *J Infect Dev Ctries*. 2010; 4(8):477-483.
16. Taddele MH, Rathore R, and Dhama K. Antibiogram assay of *Salmonella gallinarum* and other *Salmonella enterica* serovars of poultry origin in India. *Asian J Anim Vet Adv*. 2012; 7(4): 309-317.

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