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Short communication

Antimicrobial resistance profile of *Salmonella* present in poultry and poultry environment in north India

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ABSTRACT

The current study was carried out to detect *Salmonella* spp. contamination on poultry and poultry environmental samples of layer farms situated in Bareilly and to determine the antibiotic susceptibility profiles and serotype distribution of the isolates. A total of 720 samples of egg, feed, water, cloaca, and faeces were collected and screened for the presence of *Salmonella*. Twenty four (3.3%) of the samples tested were found to be positive for *Salmonella*. Out of 180 chicken eggs, 120 poultry feed samples, 120 poultry water samples, 120 fecal samples and 180 cloacal swabs, the isolation frequencies of *Salmonella* spp. were 3.3%, 2.5%, 3.3%, 2.5% and 4.4% respectively. Among the isolates, *Salmonella* Typhimurium was the predominant serovar. The antibiogram testing revealed differential multi-drug resistance among *Salmonella* isolates in poultry and poultry environment samples. All the isolates were resistant to clindamycin, oxacillin, penicillin and vancomycin whereas sensitivity was recorded for ampicillin, enrofloxacin and colistin. As a result, the relatively high resistance among the bacteria present in poultry could pose public health and therapeutic problems to consumers as potential vehicles of resistant *Salmonella* foodborne infections.

1. Introduction

Salmonella is widely distributed throughout the world and the most important bacterial agent implicated in outbreaks of the foodborne disease. It is a direct occupational anthropozoonosis disease of great economic and public health significance. Poultry egg and food products containing egg are the primary vehicle of infection caused by *Salmonella* Typhimurium and *Salmonella* Enteritidis and cause egg-borne human salmonellosis (Messens et al., 2007; Singh, Yadav, Singh, & Bharti, 2010). *Salmonella* also cause infection in poultry birds. Several factors contribute to the spread of *Salmonella* in poultry; of these feed and water contaminated with *Salmonella* are important sources of infection (Frederick & Huda, 2011). In addition, the drinkers, feeders, litter and the air inside poultry houses are also critical to horizontal transmission (Hoover, Kenney, Amick, & Hypes, 1997) of *Salmonella*. The contaminated poultry environment leads to *Salmonella* transmission either by vertical or horizontal transmission (Singh et al., 2010). However, adequate literature is not available about the presence of *Salmonella* in the poultry environment in Bareilly (north India) region. Therefore, studies are needed to verify the occurrence of *Salmonella* in the poultry environment in this region.

The antimicrobial resistance of *Salmonella* is an increasing problem and has become a public health issue worldwide (Kaye, Engemann, Fraimow, & Abrutyn, 2004). Eventually, most of the *Salmonella* isolates have developed resistance against multiple drugs due to their indiscriminate, repeated abusive applications. Overall frequencies and patterns of resistance can vary remarkably from one country to another. Variation in resistance is also associated with the time of the year, the serovar of *Salmonella*, broilers versus layer, one farm versus another and the particular antimicrobial agent (Yildirim, Gonulalan, Pamuk, & Ertas, 2011). Therefore, farm level screening of *Salmonella* for antimicrobial resistance could be of great importance in the control of *Salmonella* infection in poultry. The aim of the study was to detect *Salmonella* spp. contamination on poultry and poultry environmental samples of layer farms situated in Bareilly (north India) and to determine the antibiotic susceptibility profiles and serotype distribution of the isolates.

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2. Materials and methods

2.1. Sample collection

In the present study 180 chicken eggs, 120 samples each of layer feed, drinking water, faeces and 180 layer cloacal samples (total 720) were collected from 180 White Leghorn layers at five different farms near to the Bareilly of north India. The samples were transported to laboratory under the aseptic conditions and processed immediately for *Salmonella* isolation.

2.2. Isolation of Salmonella

2.2.1. Egg shell surface, egg yolk, cloaca, feed, water and faeces

For isolation of *Salmonella* from egg shell surface, a sterile cotton swab soaked in sterilized normal saline solution (NSS) was used for egg surface swabbing, and re-immersed into the same tube having 10 ml normal saline solution, then transferred to 90 ml of Buffered Peptone Water (BPW) and incubated at 37 °C for 18 h. For isolation of *Salmonella* from egg yolk, five ml of yolk was mixed with 5 ml of NSS and transferred to 90 ml of BPW and incubated at 37 °C for 18 h. Similarly, cloacal swabbings were re-immersed into the same tube with 10 ml of BPW and incubated at 37 °C for 18 h. For isolation of *Salmonella* from feed, 10 g of feed was inoculated in 90 ml of BPW and incubated at 37 °C for 18 h. For isolation of *Salmonella* from drinking water and faeces, 1 ml of drinking water and 1 g of faeces were separately inoculated in 9 ml of BPW and incubated at 37 °C for 18 h. All the samples were further processed for *Salmonella* identification as per protocol given in Section 2.2.2.

2.2.2. Identification of Salmonella

After pre-enrichment in BPW, 0.1 ml of pre-enriched samples were added to 10 ml of Rappaport Vassiliadis broth for all the samples individually and incubated at 37 °C for 24 h. The enriched, samples were streaked on Hektoen Enteric Agar plates and incubated at 37 °C for 24 h. After incubation, typical black centred and smooth colonies showing greenish periphery giving bull eye appearance on plates were selected and subjected to biochemical characterization (motility, nitrate reduction, indole test, methyl red, Voges proskauer, citrate utilization, urease) as per the standard methods (Agarwal, Bhilegaonkar, Singh, Kumar, & Rathore, 2003). Serotyping of *Salmonella* isolates was done by standard method (Kauffmann, 1971) at National *Salmonella* Centre (Vet.), Indian Veterinary Research Institute, Izatnagar, Bareilly.

2.3. Antimicrobial sensitivity test

Antibiotic sensitivity of the isolates was performed according to agar disc diffusion method on Mueller–Hinton Agar (CLSI, 2008). The antibiotic discs (antibiotic concentration in mcg) used were consisted of ampicillin (10 μg), amoxicillin (20 μg), clindamycin (2 μg), ciprofloxacin (10 μg), tetracycline (30 μg), gentamicin (10 μg), nitrofurantoin (300 μg), oxacillin (1 μg), streptomycin (10 μg), colistin (10 μg), chloramphenicol (30 μg), penicillin (10 μg), co-trimoxazole (23.75 μg), enrofloxacin (10 μg), kanamycin (30 μg) and vancomycin (30 μg). Results were evaluated according to CLSI zone diameter interpretive standards and minimal inhibitory concentration (MIC) breakpoints or the manufacturer's recommendations. Strains were evaluated as susceptible, intermediate or resistant. Multiple antibiotic resistances (MARs) index for each resistance pattern was calculated by the formula given in Table 3 (Singh et al., 2010).

3. Results and discussion

3.1. Isolation of Salmonella from eggs, feed, water, faeces and cloaca

Of the total 720 samples screened, only 24 (3.3%) tested positive for the *Salmonella* spp. Out of 180 chicken eggs screened, 6 (3.3%) were found positive for *Salmonella* (Table 1). Of these, 4 (66.7%) had only surface contamination while the 2 (33.3%) revealed contaminated yolk and shell. A total 8 isolates were detected from 6 eggs (Table 1). The present results indicated the incidence of *Salmonella* in chicken egg samples at 3.3% with a higher incidence level on the egg shell surface than contents (yolk). The higher incidence of surface contamination may be through faeces, feed and insects, whereas the internal contamination may either be due to penetration of the organisms from the surface to the inside of egg or else the layer hens might have systemic infection in their reproductive tissue (Barnhart, Dressen, Bastien, & Pancorbs, 1991). Our results indicated that incidence of *Salmonella* in chicken eggs is low in the north India and almost similar to the previous studies conducted in the same region (Singh et al., 2010) with a higher incidence on shell surface than the yolk (Krishamoorthy, Paul, Premkumar, & Govindarajan, 2003). In contrast, a higher (10.8%) rate of *Salmonella* incidence has been reported in chicken egg in other part of India (Bajaj, Sharma, & Thakur, 2003). The low *Salmonella* incidence in poultry farms in this particular region may be due to the application of effective control measures.

Feed and water has been implicated as an important source of *Salmonella* infection to poultry birds (Frederick & Huda, 2011). We observed 2.5% *Salmonella* in poultry feed samples. Previous studies reported higher isolation rate (22.2%) of *Salmonella* from 36 bulk commercial poultry feed samples (Okoli, Ndujine, & Ogbuwa, 2006) which are high than our results. The incidence of *Salmonella* in drinking water was 3.3% which is less than 36% reported by Sasipreeyajan, Jerngklinchan, Koowtananukul, and Saitanu (1996) in Thailand. However, no information is available in literature on *Salmonella* occurrence in poultry feed and water in India. Therefore, we could not compare our results in an Indian context. Of the 180 samples of cloacal swabs collected from 180 birds in the present study, only 4.4% tested positive to *Salmonella*. Li, Payne, Santos, Levine, and AndersonSheldon (2007) reported a higher (30.8%) prevalence rate of *Salmonella* in faeces collected from layers. Sasipreeyajan et al. (1996) also reported higher isolation rate (13%)

Table 1
Occurrence and distribution of different serotypes of *Salmonella* in chicken eggs, cloaca, feed, drinking water and faeces.

S. No.	Source	No. of samples	No. of positive samples	Percentage of positive sample	Serotypes
1	Egg	180	6 (Yolk + shell = 2, shell = 4)	3.3%	*S.* Typhimurium (6), *S.* Senftenberg (1) and *S.* Kottbus (1)
2	Feed	120	3	2.5%	*S.* Kottbus (1), *S.* Typhimurium (1) and *S.* II (1)
3	Drinking water	120	4	3.3%	*S.* Kottbus (2) and *S.* Typhimurium (2)
4	Faeces	120	3	2.5%	*S.* Kottbus (2) and *S.* II (1)
5	Cloaca	180	8	4.4%	*S.* Typhimurium (5), *S.* Kottbus (2) and *S.* II (1)
	Total	720	24	3.3%	

from cloacal swab samples of 13 broiler flocks, 15 layer flocks and 7 parent breeder flocks in Thailand. The presence of *Salmonella* in faeces indicates its colonization inside the live birds and this may be due to poultry environment contamination. The low *Salmonella* incidence in poultry environment samples observed in the present study could be the reason of less *Salmonella* incidence in faeces. Apart from that environmental factors (water activity, temperature) and management practices (animal density, housing) may also influence the *Salmonella* status in a flock irrespective of their *Salmonella* positive or negative status (Frederick & Huda, 2011).

Out of 26 isolates, 14 serotyped as *S.* Typhimurium indicating a prevalence rate of 53.85%. Other isolates serotyped as *S.* Kottbus (8), *S.* II (3), and *S.* Senftenberg (1). Previously, Singh et al. (2010) reported 55.5% *S.* Typhimurium among all the *Salmonella* isolated in the similar region thereby corroborating the current findings. Murugkar, Rahman, Kumar, and Bhattacharyya (2005) reported a high pre-dominance of *S.* Typhimurium with some case of *S.* Enteritidis in cloacal swabs of poultry in north-eastern parts of India. Among all the serovars of *Salmonella*, *S.* Typhimurium and *S.* Enteritidis have been of great concern from the standpoint of egg-borne human salmonellosis. The detection of *S.* Typhimurium as a predominant serovar in this study therefore raises a public health issues in this particular region of India. The absence of *S.* Enteritidis, a main zoonotic serovar of poultry and poultry products, in the present study is of interest, and is similar to that reported earlier by Singh et al. (2010). The main reason for the prevalence of other *Salmonella* serovars instead of *S.* Enteritidis in poultry eggs and poultry environmental samples may be due to their adaptability to environmental conditions indicating the changing dynamics of *Salmonella* serovars occurrence in this region.

3.2. Antimicrobial sensitivity/resistance of pattern analysis

Results on the resistance profile of *Salmonella* strains against 16 antimicrobial agents evaluated in this study have been presented in Tables 2 and 3. The three antibiotics ampicillin, enrofloxacin, and colistin were found to be 100% effective, whereas varying degree of sensitivity are shown by other agents: ciprofloxacin (88.5), kanamycin (88.5), chloramphenicol (76.9), gentamicin (84.6), streptomycin (80.7), nitrofurantoin (46.1), co-trimoxazole (76.9), tetracycline (80.7) and amoxicillin (65.3). The isolates were resistant to the extent of 100% to clindamycin, oxacillin, penicillin and vancomycin. We observed variation in resistance of *Salmonella* spp.

Table 2
Antibiogram sensitivity/resistance pattern of *Salmonella* isolates.

Antimicrobial agent	Total no. of *Salmonella* isolates tested	Pattern of antibiogram of *Salmonella* isolates		
		Resistant (%)	Intermediate (%)	Sensitive (%)
Ampicillin (A)	26	–	–	26 (100)
Amoxicillin (Am)	26	–	9 (34.7)	17 (65.3)
Clindamycin (Cli)	26	26 (100)	–	–
Chloramphenicol (C)	26	6 (23.08)		20 (76.9)
Ciprofloxacin (Cf)	26	3 (11.5)	–	23 (88.5)
Oxacillin (O)	26	26 (100)	–	–
Colistin (Cl)	26	–	–	26 (100)
Enrofloxacin (Ex)	26	–	–	26 (100)
Gentamicin (G)	26	2 (7.69)	2 (7.69)	22 (84.6)
Kanamycin (K)	26	–	3 (11.5)	23 (88.5)
Penicillin (P)	26	26 (100)	–	–
Nitrofurantoin (Nf)	26	6 (23.08)	8 (30.77)	12 (46.1)
Co-trimoxazole (Co)	26	6 (23.08)	–	16 (76.9)
Streptomycin (S)	26	3 (11.5)	2 (7.69)	21 (80.7)
Tetracycline (T)	26	6 (23.08)	15 (57.69)	5 (19.2)
Vancomycin (V)	26	26 (100)	–	–

Table 3
Antibiotic sensitivity/resistance pattern of *Salmonella* isolates.

Salmonella isolate no.	Source	Antibiotic resistance profile	*Salmonella* serovar	MAR index
1	Egg	CliOPV	*S.* Typhimurium	0.25
2	Egg	CliOPV	*S.* Typhimurium	0.25
3	Egg	CliOPV	*S.* Typhimurium	0.25
4	Egg	CliOPV	*S.* Kottbus	0.25
5	Egg	CliOPV	*S.* Typhimurium	0.25
6	Egg	CliOPV	*S.* Typhimurium	0.25
7	Egg	CliOPV	*S.* Senftenberg	0.25
8	Egg	CliOPV	*S.* Typhimurium	0.25
9	Feed	CliOPVCCfNfCoSTG	*S.* Typhimurium	0.688
10	Feed	CliOPVCCfNfCoST	*S.* Kottbus	0.688
11	Feed	CliOPVCCfNfCoST	*S.* II	0.688
12	Drinking water	CliOPVCNfCoST	*S.* Typhimurium	0.25
13	Drinking water	CliOPV	*S.* Kottbus	0.25
14	Drinking water	CliOPV	*S.* Kottbus	0.25
15	Drinking water	CliOPV	*S.* Typhimurium	0.25
16	Faeces	CliOPV	*S.* Kottbus	0.25
17	Faeces	CliOPVCNfCoST	*S.* II	0.563
18	Faeces	CliOPV	*S.* Kottbus	0.25
19	Cloaca	CliOPV	*S.* Typhimurium	0.25
20	Cloaca	CliOPVG	*S.* Kottbus	0.313
21	Cloaca	CliOPVCNfCoST	*S.* Typhimurium	0.563
22	Cloaca	CliOPV	*S.* Typhimurium	0.25
23	Cloaca	CliOPV	*S.* Typhimurium	0.25
24	Cloaca	CliOPV	*S.* II	0.25
25	Cloaca	CliOPV	*S.* Typhimurium	0.25
26	Cloaca	CliOPV	*S.* Kottbus	0.25

Cli – clindamycin, Co – co-trimoxazole, Cf – ciprofloxacin, C – chloramphenicol, O – oxacillin, G – gentamicin, P – penicillin, Nf – nitrofurantoin, S – streptomycin, V – vancomycin, T – tetracycline.
MAR index = Number of resistance antibiotics/total number of antibiotics tested. Resistance antibiotics – intermediate isolates on the basis of inhibition zone were considered as sensitive for MAR index.

for a few antimicrobial agents viz. to penicillin (100%), oxacillin (97%), clindamycin (97%) and vancomycin (92.6%) in study performed earlier by Yildirim et al. (2011). The prevalence of resistant samples to penicillin, oxacillin, clindamycin and vancomycin can be explained by their frequent administration in veterinary medicine. Previous studies conducted in the north India demonstrated 100% resistance to colistin (Singh et al., 2010). However, we did not observe resistance to colistin which is a indication that resistance can vary remarkably from one farm to another (Yildirim et al., 2011). In the present study, the highest level of antimicrobial resistance profile was recorded for poultry feed isolates (0.688 MARs index) followed by faeces (0.563), cloaca (0.563), drinking water (0.25) and eggs (0.25). 23.08% of the isolates were resistant to co-trimoxazole, nitrofurantoin, and tetracycline. The results are in agreement with those of Okoli et al. (2006) and Chatlod (2007) where *Salmonella* isolates from commercial poultry feeds showed antimicrobial resistance against nitrofurantoin and tetracycline. Possible reasons for resistance against these antibiotics may have been their indiscriminate use in livestock production and animal husbandry (Cohen & Tauxe, 1986). Keeping in view the change in resistance showed by *Salmonella* serovars from one place to another, the assessment of antibiogram of *Salmonella* isolates at farm level is often advantageous for identification of effective antimicrobial agent.

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