Prevalence and antibiotic resistance profiles of *Salmonella* serotypes isolated from backyard poultry flocks in West Bengal, India

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Primary Audience: Researchers, Veterinarians

SUMMARY

The present study was conducted to determine prevalence, virulence gene profile, serotyping, and antibiotic resistance patterns of *Salmonella* in birds kept under the backyard system in West Bengal, India. The study also incorporated the detection of *Salmonella* prevalence in their environment, including feed, drinking water, utensils, litter, dried manure under the house, soil, and eggs, which helped to formulate a biosecurity strategy. The study was conducted in 4 agro-climatic zones, such as the terai, new alluvial, red laterite, and coastal. Out of 360 samples, 22 *Salmonella* isolates (6.1%) were identified. *Salmonella* were isolated from cloacal swabs of 6 birds (15%, n = 40), from 4 feed samples (10%, n = 40), 8 drinking water samples (20%, n = 40), and 4 eggs (10%, n = 40). Similar antigenic structure, nucleotide sequence (*invA*) of *Salmonella* Enteritidis and Typhimurium, and randomly amplified polymorphic DNA banding patterns of *Salmonella* Enteritidis were observed. It seems that the same *Salmonella* isolate was present in feed sample, cloacal swabs, and eggs in the terai zone, whereas, it was found in drinking water, birds, and eggs in the new alluvial and in drinking water and birds in the coastal zone. A zone-specific biosecurity strategy was formulated based on the findings. The isolates were found to be resistant to chloramphenicol, ciprofloxacin, gentamicin, levofloxacin, norfloxacin, and oxytetracycline. None of the isolates possessed genes for major extended spectrum β-lactamases. Thus, the present study identified the source of *Salmonella* contamination in the backyard chickens and their eggs in India with possible forms of biosecurity strategies. Our study was the first attempt in India to determine the prevalence, virulence gene profile, serotyping, and antibiotic resistance pattern of *Salmonella* in backyard birds, including the environment and product.

Key words: antibiotic resistance, backyard poultry, biosecurity, extended spectrum β-lactamase, India, *Salmonella*

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DESCRIPTION OF PROBLEM

Intensive poultry production has arisen in the past decades as part of the search for the most viable and economical protein sources. In developing countries such as India, adoption of this type of intensive system is limited due to the lack of resources or lack of utilization as a rational choice. In rural and semiurban West Bengal, a major egg-producing state in India, access to poultry meat and eggs largely depends on the backyard production system [1]. This backyard system provides 81% of the total eggs produced in West Bengal in a year [2]. However, such backyard flocks make a very minor contribution to rural livelihoods, as the net income per bird per month ranges from 4 to 13 Indian Rupees for an average flock size of 5 to 20 in a household [3]. Therefore, the backyard system does not appear to be a promising industry until the production level or flock size is increased [4].

Some constraints exist for increasing egg production, such as microbial infection, due to lack of biosecurity knowledge among farmers [5]. Furthermore, microbiological risk factors, specifically from *Salmonella*, cause major foodborne infections in humans. Poultry have mostly implicated in the spread of *Salmonella* [6, 7]; reports of *Salmonella* outbreaks, especially with *Salmonella enterica* serovar Enteritidis, have been noted due to consumption of homemade food containing raw eggs or undercooked chicken products, leading to hospitalization (15%) and death (0.03%) in the United States [8] and other countries, such as the Netherlands [9] and Portugal [10]. In India, *Salmonella* Typhimurium is one of the most common nontyphoidal serovars detected in humans [11]. Results from several studies indicated that poultry eggs are the major source of *Salmonella* Typhimurium and Enteritidis in India [12–14]. However, those studies are inadequate regarding the prevalence of *Salmonella* serovars in backyard birds, their environment, and eggs. A few reports related to the prevalence of *Salmonella* serovars among backyard birds were reported in other countries such as Argentina [15], Belgium [16], Iran [17], and Paraguay [18]. However, these studies did not incorporate the environmental samples of backyard birds and the prevalence of antimicrobial resistance genes in the isolated *Salmonella* spp.

Transmission of antimicrobial resistance genes into the nonpathogenic commensal flora may take place in the intestinal tract of animals, including birds [19]. Specifically, the extended spectrum β-lactamase (**ESBL**) enzymes are increasingly expressed by many strains of pathogenic bacteria with a potential for dissemination. These ESBL diminish the activity of wide-spectrum antibiotics, creating major therapeutic difficulties in treatment of the patients [20]. Among the ESBL, CTX-M (offering resistance against cephotaxime) production is most frequent in *Salmonella* and other *Enterobacteriaceae* isolates [21].

Therefore, the present study was conducted to detect the prevalence, virulence gene profile, serotyping, and antibiotic resistance pattern of *Salmonella* serovars in birds kept under the backyard system, the major egg-producing system in the studied agro-climatic zones. The current study also comprised the samples from the environment, such as feed, drinking water, utensils, litter, swabs from the wall of the poultry house, dried manure under the house, soil, and eggs. Our study will help to formulate a suitable biosecurity strategy to prevent *Salmonella* contamination in backyard birds and their eggs.

MATERIALS AND METHODS

Collection of Samples

Three hundred sixty samples (n = 360) for bacteriological analysis from birds kept in the backyard system, their environment, and eggs were collected from 4 agro-climatic zones of West Bengal, India (the terai, red laterite, new alluvial, and coastal) during July to September. The birds belonged to the Rhode Island Red breed, represented both sexes, and were apparently healthy. The birds were divided into 3 age groups, 1 (1–4 wk), 2 (5–8 wk), and 3 (>8 wk), and 3 flock size groups, flock group 1 (1–10), 2 (11–18), and 3 (>19). The eggs were collected from the birds at 20 to 26 wk old (Table 1). Birds in each zone were procured from the same source and distributed among the farmers at the same time of the year under the Rastriya Krishi Vikas Yojana project of the University. The beneficiary farmers did not possess any existing flocks of birds. The collection area included

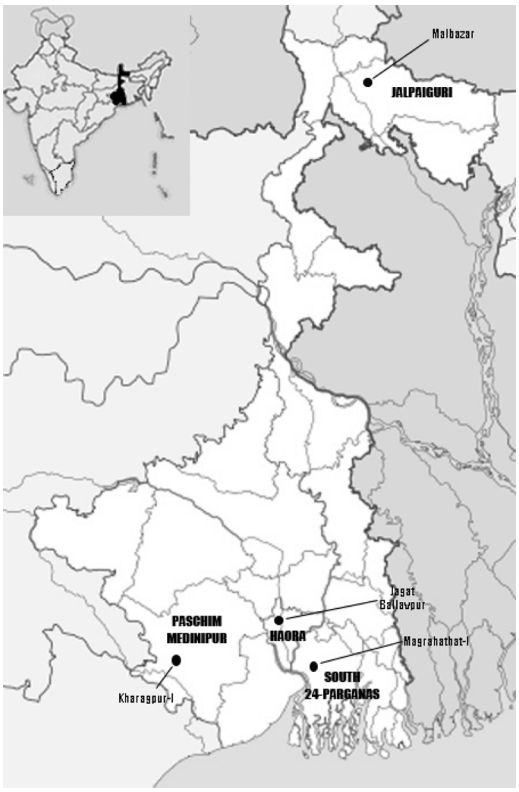

Figure 1. Geographic locations of the sample collection area in West Bengal, India.

the villages from Mal block, Jalpaiguri district (terai zone); Jagatballavpur block, Howrah district (new alluvial zone); Kharagpur-I block, West Midnapur district (red laterite zone); and Magrahat-I block, South 24 Parganas district (coastal zone; Figure 1). Each of the blocks in the collection area maintained 100 to 300 flocks composed of 5 to 25 birds in each flock. From 10 flocks in each agro-climatic zone, 9 different types of samples were collected, comprising (1) cloacal swabs of the birds (n = 10), (2) feed (4 g, n = 10), (3) drinking water (10 mL, n = 10), (4) utensil swabs (n = 10), (5) litter (4 g, n = 10), (6) swab from the wall of the poultry house (n = 10), (7) dried manure under the house (4 g, n = 10), (8) soil (4 g, n = 10), and (9) their eggs (n = 10); the total number of samples collected from each zone were 90, for a total of 360 from all 4 zones. The average minimum and maximum temperatures, respectively, and rainfall during the sample collection period were 22 and 31°C and 8 mm in the terai zone, 27 and 34°C and 5 mm in red latterite zone, and 26 and 33°C and 7 mm in both the new alluvial and costal zones.

The cloacal samples, swabs from the walls of the poultry houses, and utensils were collected aseptically with sterile cotton swabs [22] as per the Office International des Epizooties recommended method [23]. All the collected swabs were kept in sterile peptone water [22] for transport. Feed, drinking water, litter, dried manure under the house, and soil samples were collected aseptically with a sterile spatula and were put into sterile collection vials for transport [22].

Table 1. Agro-climatic zone, flock size, flock age, and number of eggs collected

Agro-climatic zone	Flock size	Flock age (wk)	Number of eggs collected
Terai	17	25–26	4
	23	3–4	0
	10	20–22	4
	12	1–3	0
	15	4–6	0
	17	2–3	0
	19	20–22	1
	08	2–3	0
	05	1–2	0
	21	20–22	1
New alluvial	20	24–25	5
	15	3–5	0
	22	9–11	0
	05	5–6	0
	08	4–5	0
	22	10–12	0
	11	4–6	0
	05	1–3	0
	09	8–9	0
	23	23–25	5
Coastal	10	2–3	0
	11	3–5	0
	20	24–25	5
	06	3–5	0
	07	7–9	0
	21	20–22	5
	15	3–5	0
	08	2–5	0
	07	8–9	0
	23	8–9	0
Red laterite	11	20–22	5
	19	7–8	0
	08	4–7	0
	09	2–5	0
	05	7–11	0
	22	5–7	0
	13	2–4	0
	06	20–22	5
	11	6–7	0
	21	8–9	0

Procured eggs were kept in sterile plastic resealable bags. All the samples, including the swabs in peptone water, were transported to the laboratory as reported previously [24].

Processing of Samples

All the swabs (cloacal, utensil, and wall of the poultry house) were taken out from peptone water and inoculated into the selenite broth [22] aseptically and were incubated at 37°C for 24 h for enrichment. Feed samples, litters, dried manures, and soils were also directly inoculated into the selenite broth (4 g of sample into 36 mL of broth) and incubated for enrichment. Similarly, the drinking water samples were inoculated into the selenite broth (5 mL of sample in 45 mL of broth). Yolk and white portions of collected eggs were aseptically taken, homogenized with 200 mL of buffered peptone water, incubated at 37°C, and subcultured in enrichment broth at 37°C for 24 h [23].

When the enrichment with selenite broth failed to detect *Salmonella* in utensil swabs, litter, swab from the wall of the poultry house, dried manure under the house, and soil, a primary enrichment in universal preenrichment broth [22], followed by a secondary enrichment in Rappaport-Vassiliadis broth [22] supplemented with 100 µg of rifampin/mL, was performed for the detection of *Salmonella* spp.

Isolation of Salmonella

Following enrichment, one loop-full of culture was streaked on brilliant green agar [22] and incubated at 37°C for 24 h. Convex, pale red, translucent colonies were identified on the basis of standard biochemical tests, such as catalase, oxidase, indole, methyl-red, Voges-Proskauer, citrate, and growth pattern, in triple sugar iron agar [25].

PCR-Based Detection of Salmonella Virulence Genes

Bacterial DNA for PCR-based detection of *Salmonella* virulence genes was extracted as previously indicated [26]. Virulence genes for *Salmonella* (invasin, *Salmonella* Enteritidis fimbrial protein) isolates were confirmed by PCR using the primer sequences and cycle condition of earlier studies [27, 28] with the standard reagents in a thermocycler [29, 30]. One each of *Salmonella* (ATCC 13076) and *Escherichia coli* (ATCC 35218) isolates were used as positive and negative controls, respectively. The amplified product was visualized by gel documentation system [31] after electrophoresis in 2% (wt/vol) agarose gel containing ethidium bromide (0.5 µg/mL) [32, 33].

Nucleotide Sequencing of the Selected PCR Products of the Virulence Genes

Both strands of selected PCR products were sequenced in an ABI 3730 XL automated sequencer [34]. Sequence homology searches were conducted using the BLAST algorithm (www.ncbi.nlm.nih.gov/BLAST). Sequence analysis and similarities or dissimilarities between the sequences were detected in DNASTAR MegAlign software [35].

Serotyping of the Pathogenic Isolates

All virulence gene-possessing *Salmonella* isolates were sent to the National *Salmonella* and *Escherichia* Centre, Central Research Institute (Kasuli, Himachal Pradesh, India), for detection of serovar and antigenic structure. The serovar was detected by the standard technique using antisera against all of the important serovars.

Randomly Amplified Polymorphic DNA-PCR

The molecular characterization of all the virulence gene-possessing *Salmonella* Enteritidis isolates was done by randomly amplified polymorphic DNA-PCR (**RAPD-PCR**) using a single primer, P1254 (CCGCAGCCAA) [36], in a thermalcycler [30] as per the earlier protocol [37]. The PCR products were then electrophoresed in 2% (wt/vol) agarose gel containing ethidium bromide (0.5 µg/mL) [32, 33].

Phenotypical Detection of Antibiotic Resistance

All PCR-confirmed virulent *Salmonella* isolates were tested for sensitivity and resistance to

different antibiotics by the disc diffusion method [38]. The antibiotic discs used in our study included ceftriaxone (30 μg), ceftizoxime (30 μg), cephalexin (30 μg), cephotaxime (30 μg), cephaloridine (10 μg), chloramphenicol (30 μg), ciprofloxacin (10 μg), colistin (10 μg), co-trimoxazole (25 μg), erythromycin (15 μg), furazolidone (50 μg), gentamicin (10 μg), levofloxacin (5 μg), nalidixic acid (30 μg), nitrofurantoin (300 μg), norfloxacin (10 μg), oxytetracycline (30 μg), and tetracycline (30 μg) [22].

PCR-Based Detection of ESBL Producers

For the detection of ESBL-producing organisms, PCR for the major ESBL genes (bla_{TEM}, bla_{SHV}, $bla_{CTX\text{-}M}$) was performed on all of the virulent *Salmonella* isolates, as described previously [39, 40].

Statistical Analysis

Univariate ANOVA for detection of significant difference between isolate numbers of *Salmonella* with age group of the birds, flock size, and agro-climatic zone was performed in SPSS version 21 [41]. The correlation between temperature and rainfall of the 4 agro-climatic zones with the occurrence of *Salmonella* in backyard birds and other samples were analyzed by Chi-squared test at 95% CI in SPSS version 21 [41].

RESULTS AND DISCUSSION

In the studied flocks, farmers offered well water in the terai and red laterite zones and locally available pond water in the new alluvial and coastal zones to the birds. Rice, raw vegetable waste, and seeds were used as feed of the birds in all 4 zones. In most cases, drinking water was not regularly changed in the troughs. The birds generally roamed around the farmer's house in the daytime and took shelter at night in poultry houses constructed of bamboo. The birds were called for feed and drinking water at a specific time by women on the farms.

Out of the 360 samples, 22 isolates (6.1%) of *Salmonella* were identified on the basis of biochemical tests from cloacal swabs, drinking water, feed, and eggs (Table 2). Lower isolation rates were reported by previous workers, who found 6, 5.8, and 3.5% *Salmonella* prevalence in backyard chickens in Iran, Belgium, and Paraguay, respectively [16–18]. Among the 4 studied agro-climatic zones, the isolates were obtained from 3 zones [i.e., the terai (Jalpaiguri), new alluvial (Howrah), and coastal (South 24 Parganas)]. This pattern of isolation may be due to the variation in temperature and average rainfall. The prevalence of *Salmonella* was observed significantly higher ($P < 0.05$) in areas of high rainfall and comparatively low temperature zones, which is in corroboration with earlier work [42].

The 22 *Salmonella* isolates were distributed among cloacal swabs of 6 birds (15%, n = 40), 4 feed samples (10%, n = 40), 8 drinking water samples (20%, n = 40), and 4 eggs (10%, n = 40) (Table 2). A similar seroprevalence rate of *Salmonella* (16%) in backyard chickens was found in Argentina [15]. An equivalent isolation rate of *Salmonella* was detected from broiler poultry feed (15%, n = 20) in Nigeria [43], and from the drinking water of broilers (13%, n = 40) in Nashville, Tennessee [44]. However, the incidence of *Salmonella* in broiler feed and feed ingredients was seen to vary widely, between 0 to 78% depending upon husbandry practices [45]. Similarly, *Salmonella* was isolated from 13% of the studied table eggs (n = 184) in Trinidad [46].

All the *Salmonella* isolates were obtained from birds of the higher age group (≥8 wk) and larger flock size (≥10 birds). A difference ($P < 0.01$) was observed in the number of *Salmonella* isolates between age group 2 (5–8 wk) and age group 3 (>8 wk) and between age group 1 (1–4 wk) and age group 3. Similarly, a difference ($P < 0.05$) was observed in the number of *Salmonella* isolates between flock group 2 (11–18) and 3 (>19) and between flock group 1 (1–10) and 3. These findings are in corroboration with other researchers who observed that the prevalence of *Salmonella* infection increased with the age of the birds [47]. Likewise, in Bangladesh, the highest prevalence of *Salmonella* was detected at higher age group (64 wk and above) of the broilers than the lower age group (16–23 wk) [48]. Furthermore, similar reports were observed where the *Salmonella* infection rate increases with the flock size of broiler birds [48, 49].

The highest isolation rate of *Salmonella* from all kinds of samples was seen in the terai (9/22;

Table 2. Distribution of *Salmonella* isolated from backyard birds, the environment, and eggs in different agro-climatic zones in West Bengal, India

Agro-climatic zone	Flock size	Age (wk)	Number of birds infected with *Salmonella* (cloacal swab)	Number of environmental samples infected with *Salmonella*	Number of eggs infected with *Salmonella*
Terai	17	25–26	0	1 (Feed)	1
	10	20–22	1	1 (Feed)	1
	19	20–22	1	1 (Feed)	0
	21	20–22	1	1 (Feed)	0
New alluvial	20	24–25	1	1 (drinking water)	1
	22	9–11	0	1 (drinking water)	0
	23	23–25	1	1 (drinking water)	1
Coastal	20	24–25	0	2 (drinking water)	0
	21	20–22	1	2 (drinking water)	0
	23	8–9	0	1 (drinking water)	0
Total (22)			6 (n = 40)	12 (n = 280)	4 (n = 40)

40.9%) followed by the new alluvial (7/22; 31.8%) and coastal zone (6/22; 27.2%). This result could be due to high rainfall and comparatively low temperatures in the terai zone ($P < 0.05$) rather than other zones during the sample collection period, which is positively correlated with higher *Salmonella* isolation [42]. However, no *Salmonella* was detected from any of the collected cloacal swabs, eggs, or other environmental samples in the red laterite (West Midnapur) zone. Furthermore, no *Salmonella* was detected from utensil swabs, litter, swab from the wall of the poultry house, dried manure under the house, or soil collected from all the studied agro-climatic zones even after secondary enrichment with Rappaport-Vassiliadis broth. Similarly, *Salmonella* was not detected by culture or PCR in the broiler litter samples in an earlier study [50]. In contrast, a few previous reports showed that the environmental samples from the broiler or layer farm samples, such as soil, dust, manure, or utensils, may harbor *Salmonella* [51, 52]. A report of *Salmonella* isolation from backyard bird feed, drinking water, or other environmental samples was apparently not available to compare with the present finding. Probably the presence of *Salmonella* in the collected samples of the present study was below detectable limits, even after secondary enrichment, as observed previously [53].

Among the 22 *Salmonella* isolates identified biochemically, 8 isolates (8/22; 36.3%) were found to possess the *invA* gene following PCR. The *invA*-positive isolates were obtained from feed, cloacal swabs, and eggs in the terai zone, as well as drinking water, cloacal swabs, and eggs in the new alluvial zone and drinking water and cloacal swabs in the coastal zone (Table 3). The nucleotide sequence of the purified *invA* PCR products (3) of *Salmonella* Enteritidis isolated from feed, cloacal swabs, and eggs in the terai zone and *invA* PCR products (2) of *Salmonella* Typhimurium isolated from cloacal swabs and drinking water in the coastal zone revealed the similarities of the isolates (100% cognate) with the *invA* of other *Salmonella* strains isolated throughout the world (accession no: DQ644618.1, AM933172.1) in a BLAST search (http://blast.ncbi.nlm.nih.gov/Blast.cgi). All 6 *Salmonella* Enteritidis isolates were found to possess the *sefA* gene following PCR. The isolates were obtained from feed, cloacal swabs, and eggs in the terai zone and drinking water, cloacal swabs, and eggs in the new alluvial zone (Table 3). The isolates belonged to *Salmonella* Enteritidis (6) and *Salmonella* Typhimurium (2) serovars (Table 3). In southern India, *Salmonella* Enteritidis was solely isolated from 164 cloacal swabs of backyard chickens [54]. Similarly, in other countries, such as Iran, the number of *Salmonella* Enteritidis isolates (15/27) was more than the number of *Salmonella* Typhimurium (6/27) in backyard chicken, and all of the *Salmonella* Enteritidis and Typhimurium isolates possessed *sefA* and *invA* genes in PCR [55].

The MegAlign analysis of the 3 *invA* gene sequences of *Salmonella* Enteritidis and 2 *invA* sequences of *Salmonella* Typhimurium revealed

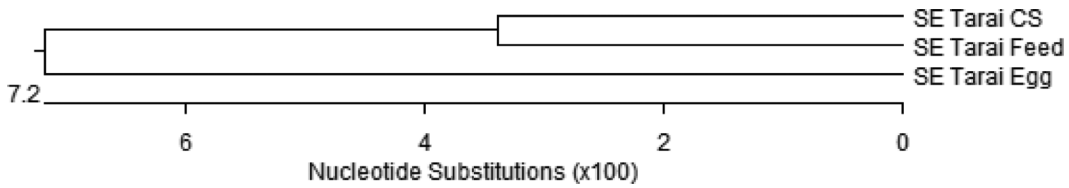

Figure 2. Dendogram showing 2 invasin sequences of *Salmonella* Enteritidis isolated from cloacal swab and feed in the same cluster.

similarities. The percent identity analysis detected 94.4% similarity between the *invA* sequences of *Salmonella* Enteritidis isolated from cloacal swab and feed, 90.2% similarity between cloacal swabs and eggs, and 85.6% similarity between eggs and feed. Similarly, the percent identity analysis detected 96.5% similarity between the *invA* sequences of *Salmonella* Typhimurium. The dendogram also revealed 2 *invA* sequences of *Salmonella* Enteritidis from cloacal swabs and feed in the terai zone and 2 *invA* sequences of *Salmonella* Typhimurium from cloacal swabs and drinking water in the coastal zone in the same cluster (Figure 2).

All the *Salmonella* Enteritidis isolates (6) were typeable with the primer P1254 in RAPD-PCR. The RAPD-PCR produced the same banding pattern for 3 *Salmonella* Enteritidis strains isolated from feed, cloacal swabs, and eggs in the terai zone and for 3 *Salmonella* Enteritidis strains isolated from drinking water, cloacal swabs, and eggs in the new alluvial zone. It seems that the same *Salmonella* Enteritidis strain was present in feed samples and birds in the terai zone as well as drinking water and birds in the new alluvial and the coastal zones due to their similarity in antigenic structure, nucleotide sequences (*invA*), and RAPD-PCR banding patterns (*Salmonella* Enteritidis). Therefore, the feed in the terai zone and drinking water in the new alluvial and coastal zones were identified as major sources of *Salmonella* infection in the studied birds. Similarly, in the literature, drinking water is considered a major source of salmonellosis in poultry [56]. In the new alluvial and coastal zones, water collected from the local ponds in the vicinity was used for drinking by birds.

The ponds were otherwise used for bathing and washing of the anus and hands after defecation by villagers in a remote area unrelated to the ponds. The use of the ponds for bathing and washing after defecation may make the villagers a source of *Salmonella* contamination, specifically *Salmonella* Typhimurium, a major nontyphoidal serovar detected in the Indian population [11]. Furthermore, infrequent change of the water in the drinking trough might aggravate the

Table 3. *Salmonella* serovars and virulence gene profiles of isolates from cloacal swabs, drinking water, and eggs of birds in West Bengal, India

Salmonella serovar	Antigenic structure	Invasin gene	*Salmonella* Enteritidis fimbrial protein gene	Sample	Agro-climatic zone
Enteritidis[1]	9,12:g, m:-	+	+	Cloacal swab	Terai
Enteritidis[1]	9,12:g, m:-	+	+	Feed	Terai
Enteritidis[1]	9,12:g, m:-	+	+	Egg	Terai
Enteritidis[1]	9,12:g, m:-	+	+	Cloacal swab	New alluvial
Enteritidis[1]	9,12:g, m:-	+	+	Drinking water	New alluvial
Enteritidis[1]	9,12:g, m:-	+	+	Egg	New alluvial
Typhimurium[2]	4,12:i:1,2	+	-	Cloacal swab	Coastal
Typhimurium[2]	4,12:i:1,2	+	-	Drinking water	Coastal

[1]*Salmonella* Enteritidis: 9, 12 = O (somatic antigen) group D1; g, m = phase-1 H-antigen; - = absence of phase-2 H-antigen (monophasic).

[2]*Salmonella* Typhimurium: 4, 12 = O group B; i = phase-1 H-antigen; 1, 2 = phase-2 H-antigen.

contamination level, as isolation of *Salmonella* from 7-d-old poultry drinking water infrequently changed in Nashville, Tennessee, was reported earlier [44]. In the other studied agro-climatic zones, well water was used for drinking by the birds, which was free of contamination.

Furthermore, as evident from the antigenic characteristics, *invA* nucleotide sequence, and RAPD-PCR pattern, the same strains of *Salmonella* Enteritidis were also isolated from the eggs in the terai and alluvial zones. It seems that the feed and drinking water in the both zones acted as major sources of *Salmonella* Enteritidis, which was further transmitted into eggs. The transmission might be due to the possession of the fimbriae, as confirmed by the detection of fimbrial protein gene (*sefA*) by PCR in the isolates, which helps in effective colonization of the bacteria in chicken reproductive tract and subsequent transmission into the eggs [57]. However, due to lack of fimbriae as evidenced from PCR, *Salmonella* Typhimurium isolates were not detected in the studied eggs in the coastal zones where the bacteria were present in drinking water and the studied birds.

Thus, based on the identification of the major source of *Salmonella* in different zones in the present study, a need was felt to formulate a biosecurity strategy to prevent *Salmonella* contamination in backyard birds and their subsequent transmission into eggs, considered a major source of *Salmonella* in humans, specifically *Salmonella* Enteritidis and Typhimurium in India. The following suggestions based on the current study were conveyed to farmers. (1) The drinking water for the birds in the new alluvial and coastal zones should be potable, preferably boiled, and changed frequently in troughs. (2) The feed mixture in the terai zone offered to the birds should be prepared (rice) or washed (seeds, vegetable wastes) with potable drinking water. (3) The eggs should be washed with sterile water and preserved at cooling temperature. The formulation was based on the source of infection in an agro-climatic zone. An earlier study regarding the survival of *Salmonella* Enteritidis in eggs showed that the numbers of surviving organisms was inversely related to the storage temperature [58], and storage of eggs without prior washing helped to disseminate the pathogens within the contents of the eggs [59]. Implementation of the biosecurity strategies by the farmers in the studied zones might reduce the *Salmonella* contamination in eggs. Furthermore, implementation will also reduce the bacterial load in the birds, which is beneficial for backyard farmers as well as for the local commercial poultry farms [15].

All the PCR-confirmed virulent *Salmonella* isolates were found resistant to chloramphenicol (100%), ciprofloxacin (100%), gentamicin (100%), levofloxacin (100%), norfloxacin (100%), oxytetracycline (100%), tetracycline (100%), co-trimoxazole (75%), and erythromycin (75%). The isolates were sensitive to ceftriaxone (100%), ceftizoxime (100%), cephalexin (100%), cephotaxime (100%), cephaloridine (100%), furazolidone (75%), nitrofurantoin (62.5%), nalidixic acid (37.5%), and colistin (25%). However, an intermediate reaction was observed against colistin (75%), nalidixic acid (62.5%), and nitrofurantoin (37.5%). Similarly, the majority of the *Salmonella* isolated from backyard birds in Iran was resistant to tetracycline [17]. In another study in northeastern India, resistance of poultry *Salmonella* to tetracycline and chloramphenicol was detected [60]. In contrast, in a study in Iran, *Salmonella* isolates from the backyard birds were mostly sensitive to norfloxacin [55]. The differences in antibiotic resistance patterns might be due to the differences in use of antibiotic types in birds in India compared with those used in other countries.

None of the isolates possessed major ESBL-producing genes (bla_{TEM}, bla_{SHV}, $bla_{CTX\text{-}M}$), as revealed by PCR. In the studied zones, third-generation cephalosporins and ampicillin were apparently never used by the farmers in the backyard birds due to either higher cost or lack of poultry preparation.

CONCLUSIONS AND APPLICATIONS

1. The present study identified drinking water and feed as the source of *Salmonella* contamination in the chickens and their eggs produced in backyard farming systems in 4 agro-climatic zones of India.
2. A zone-specific biosecurity strategy was developed to mitigate the *Salmonella* contamination.

3. Absence of major ESBL genes in *Salmonella* isolated from the backyard birds makes these birds a safe food in terms of antimicrobial resistance gene transfer.
4. Adaptation of the biosecurity strategy by the farmers may make backyard birds and their products more hygienic.

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