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Prevalence of *Salmonella* in chicken eggs collected from poultry farms and marketing channels and their antimicrobial resistance

Sangeeta Singh [a,*], Ajit Singh Yadav [a], Satyendra Mohan Singh [b], Priyanka Bharti [a]

[a] Food Microbiology Lab, Post Harvest Technology Division, Central Avian Research Institute, Izatnagar, Bareilly-243 122, India
[b] Animal Science Department, MJP Rohilkhand University, Bareilly, India

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ABSTRACT

An investigation was carried out to study the dynamics of *Salmonella* occurrence in chicken eggs during production at farm level and subsequently in marketing channels (both whole sale and retail markets) in north India and to select an effective antimicrobial agent for the control of *Salmonella* in poultry birds. A total of 560 chicken eggs comprising 260 from poultry farms and 300 from marketing channels were collected and screened for the presence of *Salmonella* during the period of April 2006 to July 2007. Twenty seven (4.82%) of the samples tested were found to be positive for *Salmonella*. Among the chicken eggs from poultry farms and marketing channels, 10 (3.84%) and 17 (5.5%) eggs were positive for *Salmonella*, respectively. Among the isolates, *S.* Typhimurium was the predominant serovar. Antibiogram testing revealed multi-drug resistance among *Salmonella* isolates from chicken eggs collected from poultry farms and marketing channels in north India. All the isolates were resistant to bacitracin, polymyxin-B and colistin, whereas sensitivity was recorded for ciprofloxacin, streptomycin and enrofloxacin.

1. Introduction

Salmonellosis is one of the most important food-borne bacterial zoonotic diseases worldwide. It also causes great economic loss in poultry especially in young chickens in terms of heavy morbidity and mortality. Poultry eggs are frequently involved in the transmission of *Salmonella*. Among the serovars of *Salmonella*, Typhimurium and Enteritidis have been of great concern from egg-borne human salmonellosis (Henzler, Kradel, & Sischo, 1998; Krishnamoorthy, Paul, Premkumar, & Govindrajan, 2003; Messens et al., 2007 and Suresh, Hatha, Sreenivasan, Sangeetha, & Lashmanaperumalsamy, 2006). The intestinal tract is the primary reservoir of *Salmonella* in poultry birds leading to contamination to chicken eggs in cloacal region through horizontal route. Trans-ovarian transmission from infected chickens is another important route of contamination of chicken eggs leading to egg-borne salmonellosis.

Success of *Salmonella* control depends mainly on the choice of therapeutic agent used (Rimler & Glisson, 1997). Injudicious use of antibiotics without conducting antibiotic sensitivity profile may lead to development of resistance among the bacterial strains rendering control programme ineffective. This necessitates the periodic assessment of sensitivity/resistance profile of *Salmonella* serovars prevalent in a particular region to enable the *Salmonella* control programme effective. Moreover, antimicrobial-resistant bacteria in food animals threaten the efficacy of human drugs if antimicrobial-resistant genes become incorporated into human bacterial populations (Smith, Harris, Johnson, Silbergeld, & Morris, 2002). Information on prevalence of *Salmonella* serovars in India is fragmentary and no systematic literature is available on prevalence of *Salmonella* serovars from production to market level which is essential in understanding the dynamics of *Salmonella* contamination from poultry farm and in marketing channels.

Therefore, the present investigation was carried out to study the contamination of *Salmonella* serovars in chicken eggs at farm level and then subsequently in the marketing channels and to assess the antibiogram of *Salmonella* serovars to enable selection of effective chemotherapeutic agents.

2. Materials and methods

2.1. Sample collection

In the present study, a total of 560 chicken eggs were collected aseptically from various poultry farms (260 eggs) and marketing channels (300 eggs) with 150 eggs each from whole sale and retail outlets during the period of April 2006 to July 2007 in North India (Uttar Pradesh, Haryana, Punjab, Delhi and Uttrakhand). The samples were transported to lab under aseptic conditions and processed immediately for the isolation of *Salmonella*.

* Corresponding author. Food Microbiology Lab, Post Harvest Technology Division, Central Avian Research Institute, Bareilly – 243 122, India.
E-mail address: soniya_sony13@rediffmail.com (S. Singh).

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2.2. Isolation and identification of Salmonella

2.2.1. Egg shell surface

For the isolation of *Salmonella* from egg shell surface, a sterile cotton swab wetted in sterilized normal saline solution (NSS) was used for surface swabbing and it was 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.

2.2.2. Egg albumin and yolk

Five ml of Egg albumin and yolk were separately mixed with 5 ml of NSS and transferred to 90 ml of BPW and incubated at 37 °C for 18 h.

One ml and 0.1 ml of pre-enriched samples were added to 10 ml of Tetrathionate (TT) broth and Rappaport Vassiliadis (RV) broth, respectively, for all the samples individually and incubated at 37 °C for 24 h. After enrichment of the samples, they were streaked on Hektoen Enteric Agar (HEA) and incubated at 37 °C for 24 h. Typical black centered and smooth colonies showing greenish periphery, giving bull eye appearance on plates were selected and subjected for biochemical characterization (motility, nitrate reduction, indole test, methyl red, Voges proskauer, citrate utilization, urease, lysine decarboxylase, etc.) employing standard methods (Agarwal, Bhilegaonkar, Singh, Kumar, & Rathore, 2003). Serotyping of *Salmonella* isolates was done at National *Salmonella* Centre (Vet.), Indian Veterinary Research Institute, Izatnagar, Bareilly.

2.3. Antibiotic sensitivity of Salmonella serovars

All the *Salmonella* isolated in the present study were examined for their antimicrobial susceptibility/resistance pattern by disc diffusion technique (Bauer, Kirby, Sherris, & Turk, 1966) against a panel of 21 antibiotics. Antimicrobials used, and their concentrations are as follows: ampicillin (10 mcg), chloramphenicol (30 mcg), ciprofloxacin (10 mcg), gentamicin (10 mcg), kanamycin (30 mcg), neomycin (30 mcg), polymixin B (30 mcg), sulphamethoxazole (25 mcg), tetracycline (30 mcg), cephalexin (30 mcg), oxytetracycline (30 mcg), bacitracin (30 mcg), colistin (10 mcg), sulphaphenazole (30 mcg), erythromycin (15 mcg), amoxicillin (20 mcg), doxycycline (30 mcg), trimethoprim (5 mcg), sulphamethizole (25 mcg), streptomycin (10 mcg) and enrofloxacin (10 mcg). All the antimicrobial discs were purchased from Himedia, Mumbai. *Salmonella* isolates were grown in Brain Heart Infusion (BHI) broth, smeared on Muller-Hinton (MH) agar plates and antibiotic discs were placed on its surface and plates were kept for overnight incubation. The result was interpreted as sensitive, intermediate, or resistance by comparing with manufactures instruction.

Multiple antibiotic resistances (MARs) index for each resistance pattern was calculated by employing the formula given below.

$$MAR\,index = Number\,of\,resistance * antibiotics / total\,number\,of\,antibiotics\,tested$$

*Isolates classified as intermediate on the basis of inhibition zone were considered as sensitive for MAR index.

3. Results and discussion

3.1. Incidence of Salmonella in eggs

Since *Salmonella* is an important zoonotic bacterium with poultry as largest single reservoir of *Salmonella* (Gupta, Ray, & Sharma, 1999), the assessment of the contamination level and site of contamination is of utmost importance in deciding the control strategies against *Salmonella* in chickens. The contamination of *Salmonella* in the internal content of chicken eggs can be due to infection in the ovary of birds (Barnhart, Dressen, Bastien, & Pancorbs, 1991) while surface contamination of eggs can be through feces, feed, insects, or through handling, transport or storage material.

The present investigation indicated the incidence of *Salmonella* in chicken egg samples at 4.82% level with lower incidence level in fresh farm eggs as compared to eggs collected from markets. In an earlier study conducted in India, incidence level of 10.8% in 534 chicken eggs was observed by Bajaj, Sharma, and Thakur (2003) with higher incidence on egg shell surface than internal contents. Surveys conducted in England and Wales have also shown egg contamination level of *Salmonella* to be 0.2% to 1.6% (De Louvois, 1994) which is lower than the present study. Out of 260 chicken eggs collected from selected poultry farms, 10 (3.84%) were found positive for *Salmonella* (Table 1). Two eggs had only surface contamination, 7 eggs were contaminated only in their yolk contents and from one egg *Salmonella* was isolated from surface as well as from yolk. Out of the 300 eggs collected from marketing channels, 17 (5.6%) were found positive for *Salmonella*. Prevalence of *Salmonella* in eggs from whole sale and retail market was found to be 4% (6/150) and 7.4% (11/150), respectively. From the *Salmonella* isolates having origin from whole sale markets, 3 eggs each had surface as well as egg yolk contents contamination while isolates from retail markets, 7 eggs had only surface contamination, 2 eggs were contaminated only in their egg yolk contents and from 2 eggs *Salmonella* was isolated from surface as well as from yolk contents. Overall, the results indicated that incidence of *Salmonella* on egg shell surface and egg yolk was found to be similar. No *Salmonella* could be isolated from egg albumen of all the eggs screened.

The higher incidence of *Salmonella* in the market eggs may be due to surface contamination during handling, storage and transportation while contamination in the internal contents points to infection of hen. In the present investigation, Out of 27 *Salmonella* isolates, 15 (55.5%) were identified as *S.* Typhimurium, 6 (22.2%) as *S.* Lagos, 4 (14.8%) as rough *Salmonella* and 1 (3.7%) each as *S.* Africana and *Salmonella* II. Among the ten isolates from poultry farms, 9 (90%) were *S.* Typhimurium while 1 (10%) was *S.* Africana. Similarly, out of 17 *Salmonella* isolates from marketing eggs, 6 (35.29%) were *S.* Typhimurium, 6 (35.29%) *S.* Lagos, 4 rough *Salmonella* (23.53%) and 1 (5.89%) was *S.* II. *Salmonella* Typhimurium was the predominant serovar isolated in this region. Murugkar, Rahman, Kumar, and Bhattacharyya (2005) also reported isolation of *S.* Typhimurium in higher proportion as compared to other serovars from cloacal swabs of poultry in north-eastern part of India. Isolation of *S.* Typhimurium in eggs was also reported by other workers (Ohtsuka, Yanagawa, Takatoxi, & Hara-Kudo, 2005; Otomo et al., 2007). *S.* Enteritidis was not isolated in chicken eggs collected from poultry farms as well as in marketing channels in the present investigation indicating the changing dynamics of *Salmonella* serovars occurrence in this region (Table 2).

In the present study, two enrichment broths Tetrathionate (TT) and Rappaport Vassiliadis (RV) were compared for *Salmonella* isolation. RV broth was found to be comparatively better in recovering

Table 1
Isolation of *Salmonella* spp. from chicken eggs collected from poultry farm and marketing channels.

S. no.	Source		No. of chicken eggs	No. of egg samples positive for *Salmonella* spp. (%)	Serotypes identified
1	Poultry farms		260	10 (3.84%)	*S.* Typhimurium(9) *S.* Africana (1)
2	Marketing channels	Whole sale market	150	6 (4%)	*S.* Typhimurium(6) *S.* Lagos (6)
		Retail market	150	11 (7.4%)	Rough (4) *S.* II. (1)
	Total		*560*	*27 (4.8%)*	

Table 2
Distribution of *Salmonella* isolates in chicken eggs.

S. no.	Source		No. of samples positive for *Salmonella*	No. of samples positive for egg yolk and shell		
				Yolk	Shell	Yolk + shell
1	Poultry farms		10	7	2	1
2	Marketing channels	Whole sale market	6	3	3	–
		Retail market	11	2	7	2
	Total		*27*	*12*	*12*	*3*

Salmonella spp. as isolates grew better and luxuriantly in this broth as compared to TT broth where growth was very slow and sometimes not appreciable even after longer duration of incubation. Out of 27 *Salmonella* isolates 15 were isolated from RV broth. Valentine-Bon, Brackett, Sco, Mammock, and Andrews (2003) also observed higher isolation from RV broth in comparison to TT broth. The TT and RV broths contains brilliant green dye or magnesium chloride and malachite green oxalate, respectively, to inhibit the growth of Gram positive bacteria and other indigenous microflora. RV gives satisfactory results because it is very effective at suppressing growth of other bacteria. This may be the reason for comparatively more efficacy of RV broth than TT in the present study.

Moreover, the use of a three step protocol (pre-enrichment, selective enrichment and selective plating) as specified in the FDA's Bacteriological Analytical Manual (US Food Drug Administration, 2002) was found satisfactory for the recovery of *Salmonella* spp. from eggs. Sequential enrichment in non-selective and selective media allows enhanced detection and recovery of sub lethally injured *Salmonella* (Van Schothorst, & Van Leusden, 1975), because *Salmonella* can be present in eggs in such small number that they may not be detected by the direct method regardless of the size of eggs (Chen, Anantheswaran, & Knabel, 2001; Stephenson, Satchell, Allen, & Andraws, 1991).

3.2. Antimicrobial resistance patterns

The results of resistance analysis of the isolated *Salmonella* strains against 21 antimicrobial agents are presented in Table 3. The sensitivity pattern indicated that all strains were 100% sensitive to ciprofloxacin, tetracycline, streptomycin and enrofloxacin followed by pefloxacin (92.59%), kanamycin (81.48%), gentamicin (59.26%) and trimethoprim (59.26%). Complete susceptibility for ciprofloxacin was also reported by other workers (Gulsen, Eloin, & Arli, 2004; Lestari, Han, Wang, & Ge, 2009 and Zhao et al., 2006). Poultry farm isolates were sensitive to most of the antibiotics. All the strains exhibited absolute (100%) resistance against bacitracin, polymyxin-B and colistin. Kavitha, Chaturvedi, and Pandey (2008) and Suresh et al. (2006) also observed complete resistance for colistin and polymyxin-B, respectively. Resistance to sulphaphenazole and sulphamethazole was observed among the isolates from poultry farm and marketing channels (whole sale and retail outlet). Other workers have also reported resistance for sulphamethazole (Steven et al., 2006 and Zhao et al., 2006). While, most of the strains from poultry farm and retail market showed resistance against doxycycline but no resistance was observed for it among the isolates of whole sale market.

Among all the *Salmonella* isolates from different sources 24 antibiotic resistance patterns were observed indicating wide spread multi-drug resistance (Table 4). Two isolates from retail outlets were resistant to as many as 10 antibiotics whereas, 2 isolates were resistant to 9 antibiotics, 2 to 8 and 5 to 7 antibiotics. Multiple antibiotic resistances (MARs) index indicated that *Salmonella* isolated from eggs of retail market had highest MAR index (0.385) followed by eggs from whole sale (0.372) and poultry farms (0.328). The logical interpretation of the results of the multiple antibiotic resistances is

Table 3
Percentage of antimicrobial resistance among the *Salmonella* strains from poultry farm, whole sale and retail marketing channels.

S. no.	Antibiotic	Poultry farm (n = 10)	Whole sale market (n = 6)	Retail market (n = 11)
1	Cephalexin	0.0	33.3	18.2
2	Oxytetracycline	20	50	54.5
3	Ampicillin	0.0	33.3	45.4
4	Pefloxacin	10	0.0	0.0
5	Kanamycin	0.0	0.0	18.2
6	Bacitracin	0.0	100	100
7	Neomycin	0.0	16.7	0.0
8	Ciprofloxacin	0.0	0.0	0.0
9	Tetracycline	0.0	0.0	0.0
10	Polymyxin-B	100	100	100
11	Gentamicin	0.0	16.7	36.6
12	Sulphaphenazole	80	83.3	72.7
13	Streptomycin	0.0	0.0	0.0
14	Erythromycin	100	83.3	63.7
15	Enrofloxacin	0.0	0.0	0.0
16	Trimethoprim	0.0	66.7	9.9
17	Amoxycillin	50	33.3	27.3
18	Colistin	100	100	100
19	Chloramphenicol	0.0	50	9.99
20	Doxytcycline	70	0.0	90.9
21	Sulphamethizole	60	83.3	63.7

that all *Salmonella* isolated in the study might have originated from environments where antimicrobials are often used. Novick (1981) and Nowroozi, Mirzaii, and Norauzi (2004) also demonstrated that indiscriminate use of antibiotics in poultry production and eggs has increased the emergence and maintenance of MAR bacteria in the environment.

Antibiotic sensitivity pattern of *Salmonella* serovars is changing drastically and with the emergence of resistance against most commonly used antibiotics, the problem arises in treatment of salmonellosis in poultry birds. According to the investigation, ciprofloxacin, streptomycin and enrofloxacin can be drug of choice because no *Salmonella* serovar exhibited resistance against these antibiotics. Pefloxacin can also be useful in treatment of chickens because of low resistance among the *Salmonella* isolates from this region. Shivhare, Sharda, Sharma, and Reddy (2000) tested *in-vitro* susceptibility of *Salmonella* isolates of avian origin and found that majority of isolates were sensitive to ciprofloxacin, enrofloxacin, sparfloxacin, norfloxacin and pefloxacin. These drugs belonging to fluroquinolones group of antibiotics have rapid and prompt bactericidal action at a very low minimum inhibitory concentration against *Salmonella* (Hooper, 1995). Keeping in view the wide range of resistance showed by *Salmonella* serovars, the assessment of antibiogram of *Salmonella* isolates is often advantageous for identification of effective antimicrobial agent to be used by poultry practitioners for therapeutic purpose since without assessment of antibiogram there may be heavy morbidity and mortality in poultry. In addition to the need of pre-testing of antibiotic efficacy, the indiscriminate use of antibiotics in feed of poultry should be checked.

The present investigation indicated occurrence of *Salmonella* in chicken eggs at low level, however, detection of *Salmonella* from chicken egg yolk needs greater concern for effective control of salmonellosis in poultry birds. Moreover, measures to reduce surface contamination of chicken eggs with *Salmonella* during transport and handling needs to be improved. Most serovars of *Salmonella* isolated in the study also showed resistance to other commonly used antibiotics and this problem is increasing because of injudicious use of antibiotics both as prophylactic as well as therapeutic agents. Therefore, it is suggested that the most effective control programme, following assessment of antibiogram profile, be used in a particular region where outbreaks of *Salmonella* are encountered.

Table 4
Antibiotic resistance profile and multiple antibiotic resistance index of individual *Salmonella* isolates from different sources.

S. no.	*Salmonella* serovar	Source	Antibiotic resistance profile	MAR index
1	*S.* Typhimurium	Poultry farm	B, Cl, E, Pb, Sm	0.238
2	*S.* Typhimurium		Am, B, Cl, Do, E, Pb, Pf, Sp, Sm	0.429
3	*S.* Typhimurium		Am, B, Cl, Do, E, Pb, Sp, Sm	0.381
4	*S.* Typhimurium		Am, B, Cl, Do, E, Pb, Sp, O	0.381
5	*S.* Typhimurium		Am, B, Cl, E, Pb, Sp, Sm	0.333
6	*S.* Typhimurium		B, Cl, Do, E, Pb, Sp	0.284
7	*S.* Typhimurium		B, Cl, Do, E, Pb, Sp	0.284
8	*S.* Typhimurium		B, Cl, Do, E, Pb, O, Sm	0.333
9	*S.* Typhimurium		Am, B, Cl, E, Pb, Sp, Sm	0.333
10	*S.* Africana		B, Cl, Do, E, Pb, Sp	0.284
Average				*0.328*
11	*S.* Lagos	Whole sale	B, Cl, Cp, E, O, Pb, Sp, Sm, Tr	0.429
12	*S.* Lagos		B, Cl, G, N, Pb, Sm, Tr	0.333
13	*S.* Typhimurium		A, Am, B, C, Cl, O, Pb, Sp	0.381
14	*S.* Typhimurium		B, C, Cp, Cl, Pb, Sm, Sp, Tr	0.381
15	*S.* Typhimurium		B, Cl, Pb, Sm, Sp, Tr	0.284
16	*S.* Typhimurium		A, Am, B, C, Cl, O, Pb, Sm, Sp	0.429
Average				*0.372*
17	*S.* Rough strain	Retail	A, Am, B, Cl, Do, O, Pb, Sp, Sm	0.429
18	*S* Rough strain		A, Am, B, Cl, Do, G, Pb, Sp, Sm	0.429
19	*S* Rough strain		A, B, Cl, Do, E, G, O, Pb, Sp, Sm	0.476
20	*S* Rough strain		A, Am, B, Cl, Do, E, O, Pb, Sm, Sp	0.476
21	*S.* Lagos		B, Cl, Cp Do, E, O, Pb, Sp	0.381
22	*S.* Lagos		A, B, Cl, Cp, Do, E, Pb, Sp	0.381
23	*S.* Lagos		B, Cl, Do, K, Pb, Sp, Sm	0.333
24	*S.* Lagos		B, Cl, Do, K, O, Pb, Sp	0.333
25	*S.* Typhimurium		B, Cl, Do, E, G, Pb, Sm,	0.333
26	*S.* Typhimurium		B, Cl, Do, E, G, Pb, Sm,	0.333
27	*S.* II		B, C, Cl, E, O, Pb, Tr	0.333
Average				*0.385*

Cp—cephalexin, O—oxytetracycline, A—ampicllin, K—kanamycin, B—bacitracin, N—neomycin T—tetracycline, Cl—colistin, G—gentamicin, Pb—polymyxin, Sp—sulphaphenazole, E—erythromycin, Sm—sulphamethizole, Am—amoxycillin, Do—doxycycline, Tr—trimethoprim, C—chloramphenicol, Sm—sulphamethizole.

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