

Original Research Article

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Risk of *Salmonella* in Various Poultry Farming and Processing Operations around Mumbai, India

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ABSTRACT

Shift in the process of traditional poultry supply chain to vertical integration has developed way for poultry meat availability in India, but there is food-borne risk for human health by contamination with *Salmonella* spp. Therefore, the present research work was planned to study risk of *Salmonella* spp. in chicken meat production. Samples (n=684) were collected from farms (non-integrated and integrated) and processing units (semi-automated and automated) located around Mumbai, India and subjected to *Salmonella* spp. detection. Isolation of *Salmonella* spp. was carried out following IS 5887. The obtained isolates were subjected to identification biochemical tests, PCR testing for specific *Salmonella* spp. gene (*invA*) and serological identification. The results indicate that, over all occurrences of *Salmonella* spp. in farming and processing system were 6.70% and 7.53%, respectively. Higher isolation rate was observed ($p < 0.05$) amongst various farms and processing units. Positive sample frequencies of *Salmonella* spp. found higher in litter (20.83%), faeces (10.41%), de-feathering (16.66%) and evisceration (25.00%). Amongst environmental samples of processing unit, knife (16.66%) and evisceration workers hands (12.50%) found to be major cross contaminating risk points. The results highlighted relevance of farm and processing level contamination for *Salmonella* spp. Therefore, it is essential to apply multiple interventions like, sourcing of *Salmonella* free chicks, litter management at farm, HACCP plan at the processing stages, use of chlorine wash and systematic cleaning or sanitation programs to avoid cross contamination.

Keywords

Salmonella
serotypes, Poultry
farms, Litter,
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Introduction

Microbiological risk factors are so prevailing that they can be found in almost all systems of poultry production (Yang *et al.*, 2014), if

products are improperly treated while handling, cooking or post cooking and storage. *Salmonella* has been pathogen of significance, and is a major cause of gastroenteritis in humans (Mead *et al.*, 1999).

Salmonella illness has linked with exposure to meat, a review of the Centers for Disease Control and Prevention (CDC) outbreak data from 2006 to 2011 indicated that 10 out of 25 outbreaks were related to live poultry, shell eggs, or further processed poultry products. Nine percent of United States (US) travellers with Salmonellosis were reported to acquire *Salmonella* infection while travelling from India (Jennifer and Watkins, 2017).

India is the world's second largest emerging economy with large and rapidly expanding poultry sector at annual growth rate of 10-12 percent for broiler production (Kotaiah, 2016). The consumer demand for processed poultry products like chilled or frozen chicken has also been increased. Indian broiler production for year 2016 estimated to be 4.2 million tons. with 3.1kg per capita consumption of poultry meat per year (USDA-GAIN, 2015). Shift in the process of traditional poultry supply chain to vertical integration has developed way for poultry meat availability and better value creation of poultry meat products. Still there is food-borne risk for human health which includes microbiological risks wherein, *Salmonella* spp. contamination is important risk (Kiilholma, 2007).

The problem of *Salmonella* in Indian poultry is exacerbated by modern conditions of intensive rearing, where large numbers of birds are kept together and high-rate processing; in which carcasses remain in close proximity throughout the operation which favours the spread of any pathogens that may gain access to the flock. It is often not possible to trace back *Salmonella* contamination to the original source because risk assessment on *Salmonella* in meat with information on food handling and processing practices is rarely available (EC, 2008). Risk assessment made by Food and Agriculture Organization (FAO) indicates that reduction

of *Salmonella* prevalence in poultry flocks helps to reduce the occurrence of Salmonellosis in humans. Microbial risk assessment of poultry for *Salmonella* has been considered as an essential basis for the management of foodborne hazards which results in reduction of human health risk (WHO/FAO, 2002 and FAO, 2016).

Research work carried out by various workers in India reported prevalence of *Salmonella* either in few variables of farms or retail shop final products (Bhuvaneswari *et al.*, 2015 and Waghmare *et al.*, 2017), but there is no complete strategic study on assessment of *Salmonella* in poultry farm and processing environment. The present study was planned for identifying risk of occurrence of *Salmonella* spp., in chicken meat production chain with different farming and processing systems.

Materials and Methods

Sampling sites

The commercial broiler farms 24 each managed under non-integrated and integrated systems with capacity of 1500 -2000 birds per farm, birds aged between 35 to 42 days supplies birds to two different chicken processing units named semi-automated and automated processing plant, respectively situated around Mumbai city, Maharashtra, India were selected under study.

Sample collection

A total of 684 samples were collected in period of December 2015 to December 2017. Farm level samples (n=432) samples comprising of 216 each from non-integrated (n=12) and integrated (n=12) commercial broiler farms were collected. From these two farm types 48 samples of each source were collected which, comprises of water, feed,

faeces, litter and various swabs (cloaca, drinker, feeder, workers hand and walls). A total of 252 samples from semi-automated and automated processing unit comprising of a chicken processing stage wise swab samples (post bleeding, scalding, de-feathering, evisceration and chlorination) and environmental samples (water, ice, scalding water and swabs of utensil, defeaters, carcass contact surfaces, workers hand and packaging material) were collected. From both the processing unit a six replicates at every source from various processing stages and environment were collected except workers hands (twelve replicates) at evisceration and deboning stage. Neck sample of post eviscerated and chlorinated carcass were also collected. Swabbing was done using transportable sterile swab (Hi-Media) applied with firm rolling pressure. The swabs were placed immediately in a sterile container with Cairy Blair transport medium whereas, other samples were collected in sterile poly bags. All the samples after collection brought to the laboratory under chilled conditions.

Isolation and Confirmation of *Salmonella* spp.

Isolation of *Salmonella* spp. from various samples collected was carried out by pre enrichment with buffered peptone water, enrichment in Rapport Vassiliadis broth (Himedia) followed by selective plating on XLD and BGSA (Himedia), according to IS 5887 (Part 3): 1999 (Reaffirmed in 2005). Presumptive *Salmonella* colonies were streaked onto nutrient agar slants (Himedia.) and refrigerated (4°C) after incubation. Colonies showing typical Gram negative, non spore forming short rod shaped appearance were further subjected to biochemical characterization with biochemically negative for hydrolysis of urea, positive for TSI with alkaline slant (red), acid butt (yellow) with H₂S gas production and positive citrate

utilization considered as positive for *Salmonella* spp. Biochemically confirmed isolates were further characterized by PCR assay for *invA* gene as per protocol of Rahn *et al.*, (1992). Randomly selected PCR positive isolates were submitted to Poultry Diagnostics and Research Centre, Lonikalbhor, Pune for serotyping according to the Kauffmann-White scheme.

Statistical analysis

Results obtained were subjected for ANOVA and Paired t test analysis using WASP (ICAR, India) software.

Results and Discussion

The different rate of isolation of *Salmonella* from broiler poultry farms and processing units are shown in Table 01 and Table 02. The results indicate that, over all occurrences of *Salmonella* spp. in farming and processing system were 6.70 and 7.53 per cent, respectively. The occurrence of *Salmonella* in non-integrated and completely integrated farming system was 12.03 and 1.38, per cent respectively. Significant difference ($p < 0.05$) was observed within the farming systems. Amongst the various samples higher *Salmonella* occurrence rate was observed in litter (20.83%) and faeces (10.41%) followed by cloacal swabs (8.33%), water (8.33%) and drinker (6.28%).

As per the results depicted in Table No 02 occurrence *Salmonella* spp. for semi-automated and automated processing unit ($p < 0.05$) was 11.11 and 3.9, percent respectively. Considering the various stages of processing defeathering (16.66%) and evisceration (25%) were found to be critical stages of contamination. Environmental samples differ non significantly between the semi-automated and automated processing unit.

Table.1 Occurrence of *Salmonella* spp. in samples analyzed from various poultry farming systems

Sr. no.	Farming system	Total number of samples from various sources	Various sources of contamination (n=48 each source/systems)									Total number of positive samples with per cent occurrence
			Cloacal Swab	Feeder	Drinker	Wall dust	Worker Hand	Feed	Litter	Feces	Drinking water	
			Number of positive samples									
1	Non integrated	216	4	0	3	1	1	0	9	4	4	26 ^a (12.03)
2	Integrated	216	0	0	0	1	0	0	1	1	0	3 ^b (1.38)
Total		432	4 [8.33]	0	3 [6.25]	2 [4.16]	1 [2.08]	0	10 [20.83]	5 [10.41]	4 [8.33]	29 [6.7]

Values in the same column with the different superscripts letters are significantly different at 1 and 5% level of significance

Table.2 Occurrence of *Salmonella* spp. in samples analyzed from poultry processing units

Sr. No	Sample source	Poultry processing unit		No of isolates (% occurrence)
		Semi-automated	Automated	
A) Processing stages (n=6 per stage /unit)		n=42	n=42	n=84
1	Post bleeding	00	00	0
2	Post scalding	00	00	0
3	Post defeathering	02	00	2 (16.66)
4	Post evisceration	02	01	3 (25.00)
5	Neck sample of post eviscerated carcass	02	01	3 (25.00)
6	Post chlorination	01	00	1 (8.33)
7	Neck sample of post chlorinated carcass	01	00	1 (8.33)
Number of Positive isolates (percent occurrence)		08 (19.04)	02 (4.76)	10 (11.90)
t test : Significant difference at 5% level for processing stages				
B) Environmental samples(n=6 per source /unit except # where n =12)		n=84	n=84	n= 168
8	Washing water	00	00	0
9	Scalding water	00	00	0
10	Defeathering machine	01	00	1(8.33)
11	Worker hands# (Evisceration)	01	02	3 (12.50)
12	Carcass contact platform	01	00	1(8.33)
13	Chopping board	01	00	1(8.33)
14	Knife swab	02	00	2 (16.66)
15	Deboning table	00	00	0
16	Worker hand (Deboning)#	00	00	0
17	Deboning cone	00	01	1 (8.33)
18	Ice	00	00	0
19	Cloacal swab	00	00	0
Number of Positive isolates (percent occurrence)		06 (7.14)	03 (3.57)	9 (5.35)
Total Number of Positive isolates (percent occurrence)		14 (11.11)	5 (3.9)	19 (7.53)
t test : Non significant at 5% level for environmental samples				

Table.3 Serotypes of *Salmonella* spp. isolated from poultry farms and processing units

Sr. No.	Sample Code	Source	Serotype
A) Poultry Farms			
I) Non-integrated			
1	NICS 9	Cloacal swab	<i>S. Virchow</i>
2	NICS8	Cloacal swab	Non-Typable
3	NIDW 7	Drinking water	Non Typable
4	NIFL1	Litter	<i>S. Newport</i>
5	NIFL9	Litter	<i>S. Virchow</i>
6	NIFL 7	Litter	Non Typable
7	NIF8	Faeces	<i>S. Virchow</i>
8	NIDR 7	Drinker	<i>S. Virchow</i>
9	NIDR6	Drinker	<i>S. Typhimurium</i>
10	NIWH6	Worker hand	<i>S. Typhimurium</i>
11	PIL1	Litter	<i>S. Virchow</i>
12	PIL3	Litter	Non Typable
13	PIF7	Faeces	<i>S. Virchow</i>
14	PIF3	Faeces	Non Typable
15	PIDW5	Drinking water	<i>S. Typhimurium</i>
III) Integration			
16	VVAL	Litter	Non Typable
B) Poultry Processing units			
I) Semi-automated			
17	SCDM4	Defeathering machine	<i>S. Newport</i>
18	SCWH4	Worker hand (evisceration)	<i>S. Virchow</i>
19	SCCP4	Carcass contact platform	Non Typable
20	SCCB5	Chopping board	<i>S. Typhimurium</i>
21	SCKS4	Knife swap	<i>S. Virchow</i>
22	SCPD2	Post defeathering	<i>S. Virchow</i>
23	SCPE 4	Post evisceration	<i>S. Virchow</i>
24	SCCLWash4	Post washing / chlorination	Non Typable
25	SCNC4	Neck skin of eviscerated bird carcass	<i>S. Virchow</i>
II) Automated			
26	ACWH6	Worker hand (evisceration)	<i>S. Typhimurium</i>
27	ACDC6	Deboning cone	<i>S. Newport</i>
28	ACPE3	Post evisceration	Non Typable

Higher occurrence rate of *Salmonella* spp. was observed in knife swabs (16.66%) and evisceration workers hand (12.50%), followed by defeathering machine, carcass contact platform chopping board and deboning cone (8.33%).

Table 03 shows the source wise serotypes of *Salmonella*, out of total 48 isolates randomly selected 28 isolates were subjected for serotyping these isolates showed three distinct serotypes namely *S. Virchow*, *S. Typhimurium* and *S. Newport*. The non identified serotypes were categorised as Non

Typable.

The integrated and non integrated broiler poultry farms and processing plants selected under study were located in vicinity of Mumbai and supplying the live and processed birds to Mumbai city. In the chicken distribution chain, a farming and processing management have a significant effect on the level of *Salmonella* contamination (Kim *et al.*, 2007). However in Indian processing systems though retail processing may important role but study of newly established modern poultry processing units which cater need of urban population is essential. Therefore, it is essential to understand the epidemiology of *Salmonella* in chicken meat production to enhance the food safety of poultry products.

The overall isolation rate was found to be 6.7% for non integrated (12.03%) and integrated broiler farms (1.38%), respectively. For the comparison of these findings with results from other studies in Indian and abroad, differences in study design, number of samples and types of microbiological samples must be considered. The higher isolation rate in non integrated farming system pose serious hazard to public health as majority of the farms supply birds to Indian retail shops. Over all *Salmonella* occurrence in this study was lower or equal than that in Japan (14.3%) (Limawongpranee, 1999); USA (5.2 -13.4%) (Bailey *et. al.* 2001); Senegal (35.1%) (Dione *et al.*, 2009) and Iraq (9.2%) (Abadi and Mayah, 2012).

In this study Litter, faeces and cloaca swabs found to be major source of cross contamination at farm. The frequency of *Salmonella* recovery from poultry litter, faeces and cloacal swabs was similar to that of previous studies (Ayachi *et al.*, 2010; Kumar *et al.*, 2014; Abunna *et al.*, 2016).

Salmonella colonize in intestinal tract, shed in the faeces and become a source of contamination for other animals, humans and the environment (Poppe, 2000). Water, drinker and workers hand samples were positive at the non-integrated farms could be due to cross contamination with the litter and faecal material. Feed samples from both the farming system found to be negative which might be result of proper procurement of feed. Abadi and Mayah (2012) also reported negative occurrence of *Salmonella* spp. in feed and feeder.

A large number of environmental factors including the poultry house environment, water, old litter, farm handlers, equipment and transport vehicles have been suggested as source of *Salmonella* infections in broiler and layer flocks, but the relative importance of these potential sources is not clearly understood (Meerburg, 2006). Low prevalence in integrated farming system may be attributed management practices carried out by integrator where, they can support farmers with procurement of chicks and feed along with technical support on biosecurity measures; while lack of operational control in non integrated farming systems might be resulted in high prevalence. The control of dissemination of *Salmonella* in poultry production chain is dependent on the control of transmission sources, especially litter treatment is essential to reduce *Salmonella* population in litter (Frederick and Huda, 2011).

Indian poultry processing industry undergoing the changes but majority of Indian consume chicken from retail shop but processed chicken market gaining importance. *Salmonella* isolation rate with respect to stages of processing and environment was compared amongst the two processing units analysed under study. *Salmonella* Processing stage wise sampling

showed higher isolation rate ($p < 0.05$) for semi-automated unit (19.04%) than automated unit (4.46%). *Salmonellae* isolated at various stages of processing during study revealed that *Salmonellae* were more frequently isolated from post defeathering (27.77%) and post evisceration stages (33.33%). Nde *et al.*, (2007) also reported that defeathering and evisceration has been a significant source of carcass contamination, which further leads to direct contact between contaminated and uncontaminated carcasses. The rubber fingers in defeathering machine harbour pathogens, which in turn contaminates the carcass while careless manual evisceration could contaminate the carcass during evisceration as a result of spillage of intestinal content (FAO /WHO 2001 and Silva, 2009). Environmental samples showed Overall per cent occurrence of 5.35 for *Salmonella* spp. Samples of chopping board (22.22%), knife (16.66%) and evisceration worker's hand (13.33%) revealed as major source of *Salmonella* spp. contamination. Surprisingly scalding water, deboning table, worker's hand (Deboning) and ice were found negative for *Salmonella* spp. Morris and Wells (1970) reported that processing stages results in recontamination of the carcass but noticed higher isolation rate from environmental samples to which carcasses frequently come in contact. Ishola and Taiwo (2014) reported higher *Salmonella* isolation rate from environmental samples due to non systematic cleaning or sanitation programs. According to our knowledge this is first time in India sampling of automated chicken processing plant was done to analyse risk of *Salmonella* spp. in processing environment. Chlorination was found to be effective control tool as only one sample from post-chlorination stages of semi-automated process was found positive, which might be attributable to proper chlorine concentration

used while washing the carcasses. Bailey *et al.*, (2000) reported that the use of 40 to 50 ppm chlorine in the chill tank reduces the rates of *Salmonella* in processing plant.

The higher incidence of *Salmonella* spp. in samples obtained from semi-automated processing unit at the processing stages could be due to cross contamination during processing of poultry (Kottawatta *et al.*, 2017). The issue of *Salmonella* should be addressed by implementing multifaceted plans which govern all phases of production from breeder farms through transport of the chicks to farms and then live birds to slaughter (Bailey *et al.*, 2001 and Fluckey *et al.*, 2003).

Serotypes confirmed as *Salmonella* Virchow, *Salmonella* Newport and *Salmonella* Typhimurium in farms and processing environments indicates that these *Salmonella* serotypes circulating in all farming systems and poultry processing units in and around Mumbai. Results are depicted in Table No: 03 Isolates which were positive by PCR assay but negative by serotyping has been termed as untypable. In this study, *Salmonella* Virchow serovar was the most frequently found in poultry farms and processing units. In general, this is consistent with previous research carried out by Khanna, 2009. *Salmonella* serovar Enteritidis and Typhimurium were the main serotypes isolated from poultry (Kaushik *et al.*, 2014). Report of *S. Newport* in poultry farm and processing environment is a serious issue, as CDC (2005) has raised concern over the rapid rise of *Salmonella* serovar Newport isolates over the last decade as important causes of human Salmonellosis. Several serotypes are consistently found at a higher incidence and the distribution of *Salmonella* serotypes from poultry sources varies geographically and changes over a period of time (Myint,

2004). The high rates of serogroup S. Virchow in our studies, may suggest that these serogroup may be more adapted to poultry farm and processing environments under study.

It is concluded that higher risk of *Salmonella* spp. was observed in non-integrated farms and semi-automated processing unit. This study provides important cross sectional epidemiological information on the *Salmonella* spp. contamination status in the poultry farms and slaughter units in and around Mumbai. To reduce *Salmonella* spp. in chicken meat production system, use of single intervention strategy for *Salmonella* control will not eliminate it as different farming and processing systems are operated together. Therefore, it is essential to apply multiple interventions like, sourcing of *Salmonella* free chicks, litter management at farm, HACCP plan at the processing stages and environmental sanitation programme to avoid cross contamination.

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Authors' Contributions

Author RNW is the main author performed field work an analysis of samples under the supervision of AMP. VMV, RJZ and SDM helped in literature survey, drafting and finalized the manuscript.

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