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Occurrence and distribution of *Salmonella* in a broiler chicken processing line in Thrissur district, Kerala#

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Abstract

Foodborne diseases are a major global concern and salmonellosis is one of the most frequently reported foodborne zoonoses. In this study, the occurrence of Salmonella in the broiler processing line was detected by conventional culture methods followed by confirmation by PCR against 16S rRNA gene. A total of 700 samples were analysed which included the samples from live broiler chicken at the unloading area, the carcass swabs from multiple stages of processing viz., scalding, defeathering, evisceration, final cutting and chilling and also the samples from the associated environments of processing unit. The study period was from January to August 2024. An overall occurrence of 4.63 per cent Salmonella spp. was detected from the processing line and all the isolates were confirmed by PCR against 16S rRNA gene. The major contamination points along the processing line were detected as defeathering, evisceration and final cutting and no Salmonella could be detected from broiler carcass after scalding and chilling. The data were analysed by chi-square test and there was no significant association (p> 0.05) between the occurrence of Salmonella spp. and the sources of samples.

Keywords: *Scalding, defeathering, evisceration, Salmonella, polymerase chain reaction*

Foodborne diseases are caused by consuming food or beverages contaminated with bacteria, viruses, parasites, toxins, heavy metals and prions. Globally, around 600 million people fall ill each year due to contaminated food, leading to 4.2 lakh deaths and the loss of 33 million healthy life years (WHO, 2022). The economic impact of foodborne illnesses extends far beyond healthcare expenses, affecting global trade and productivity. Among these illnesses, salmonellosis is one of the most frequently reported zoonotic diseases worldwide.

Salmonellosis is an infection caused by bacteria of the genus *Salmonella*, which belongs to the family Enterobacteriaceae. This genus includes two species: *Salmonella enterica* and *Salmonella bongori*. *Salmonella enterica* is divided into six subspecies, with several serovars, particularly those of *Salmonella enterica* subsp. *enterica*, being significant human pathogens. In humans, *Salmonella* infections manifest as typhoid fever, paratyphoid fever and nontyphoidal salmonellosis (NTS), caused by *Salmonella* Typhi, *Salmonella* Paratyphi and other serovars of *S. enterica*, respectively. The NTS is commonly associated with foodborne illnesses, ranging from mild gastrointestinal symptoms

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to severe invasive infections, especially in young children, the elderly, pregnant women and immunocompromised individuals. According to the CDC (2024), *Salmonella* causes about 1.35 million infections, 26,500 hospitalisations and 420 deaths annually in the United States. Kumar *et al*. (2022) studied samples from different places across India, such as hospitals, agricultural and veterinary research centres and laboratories from fisheries and food departments and found that 53.95 per cent of the samples were confirmed as NTS. Oommen *et al*. (2015) identified 51 NTS isolates from human samples collected from patients at a tertiary care centre in central Kerala, three of which resulted in bacteraemia and the serovars identified included *Salmonella* Typhimurium, *Salmonella* Enteritidis and *Salmonella* Weltevreden. According to the WHO (2018), *Salmonella* is one of the four primary global causes of diarrheal diseases.

Salmonella resides in the intestines of various animals, both domestic and wild. Humans often acquire salmonellosis by consuming contaminated animal products or by coming into contact with infected animals and the primary mode of infection is the faecal-oral route. The consumption of contaminated poultry meat is one of the main sources of infection for salmonellosis. In India, commercial broiler birds account for 80 to 85 per cent of total poultry meat production (BAHS, 2023). The growth of Indian poultry industry is fuelled by increasing disposable incomes and evolving dietary preferences. An example of these dietary preferences is the increasing popularity of chicken shawarma. The occurrence of *Salmonella* in chicken shawarma collected from various fast-food restaurants in Thrissur and Kollam districts of Kerala was eight per cent and 20 per cent, respectively (Anjali, 2023).

A range of control strategies are implemented to prevent *Salmonella* throughout the poultry production chain. However, the ability of *Salmonella* to survive in the environment and within the intestines of processed birds results in the contamination of carcasses during processing and complicate the control efforts. According to the CDC (2023) in United States, approximately one in every 25 packages of chicken sold in grocery stores is contaminated with *Salmonella*. This contamination of food products can negatively impact consumer demand and perception, beyond the health risks associated with *Salmonella*. This study aims to analyse the presence of *Salmonella* spp. in live broiler chicken, carcass swabs from multiple stages of processing (scalding, defeathering, evisceration, final cutting and chilling) and from the environment of the poultry processing plant.

Materials and methods

During the study period from January 2024 to August 2024, a total of 700 samples were collected from a broiler chicken processing plant in Thrissur district, Kerala. The samples included were from live broiler chicken, broiler carcasses and associated environmental sources. The swab samples were collected over 13 visits, with an average interval of 10 days, during the processing of 25 birds.

Swab samples from cloaca (n= 25) and breast swabs (n= 25) from live broiler chicken at the unloading area as well as breast swabs from carcasses at various stages of processing such as before scalding, after scalding, before defeathering, after defeathering, before evisceration, after evisceration, before final cutting and after final cutting (n= 25 swabs each) and breast swabs both before and after chilling (n= 12 swabs each) were collected. The other samples such as tap water used for cleaning purpose (n= 28), swabs from bird crates (n= 28), handwash of personnel, scalding water, swabs from evisceration tray, swabs from defeathering machine, swabs from carcass cutting machine, swabs from chopping board and knife swabs (n= 25 samples each before and after the processing) and samples of chilling water (n= 10) both before and after the processing were collected. All the swabs were collected using sterile cotton swabs (HiMedia, Mumbai) moistened with sterile buffered peptone water (BPW). Breast swabs were collected using 100 cm^2 templates. Handwash of the personnels involved in processing were collected by washing the hands of each worker in 100 mL of buffered peptone water in sterile polythene bags and tap water samples, scalding water and chilling water were collected (100 mL each) in sterile sample containers. All samples, including swabs and water samples, were stored in Thermocol boxes and immediately transported to the laboratory for further processing at 4 °C.

Isolation and identification of Salmonella spp.

All the samples were analysed for the presence of *Salmonella* spp. by conventional culture methods (Andrews *et al*., 2001) with certain modifications. The swabs were collected aseptically, placed in 10 mL of BPW and incubated at 37 °C for 18 h for pre-enrichment. The collected handwash samples in BPW were also incubated at 37 °C for 18 h. At the end of incubation, 1 mL of the sample was transferred to 9 mL of modified rappaport vassiliadis (MRV) *Salmonella* enrichment broth and incubated at 42 °C for 18 h. Other samples, including tap water used for cleaning the processing unit, scalding water and chilling water, were analysed (APHA, 2012) by enriching 50 mL of each sample in 50 mL of tetrathionate broth and incubating at 35 °C for 24 h. After incubation a loopful of inoculum from the enriched samples was plated to MacConkey agar and incubated at 37 °C for 18 h. The non-lactose fermenting transparent colonies were selected and plated on xylose lysine deoxycholate (XLD) agar and incubated at 37°C for 18 h. The presumptive colonies were subjected to cultural, morphological and biochemical tests (Barrow and Feltham, 1993).

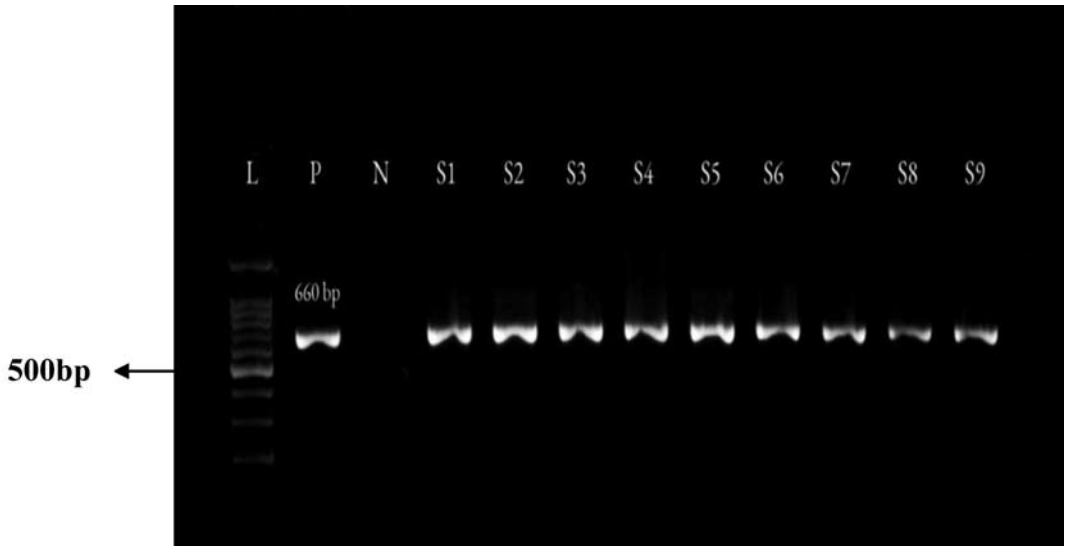

Fig. 1. PCR profile of *Salmonella* spp. using *16S* rRNA gene; L- Molecular ladder (100 bp), P- Positive control, N- Negative control, S1-S9- Samples [S1& S2- Water samples, S3- Breast swab after evisceration, S4- Breast swab after final cutting, S5- Breast swab after defeathering, S6-Breast swab before final cutting, S7 & S8- Cloacal swabs, S9- Knife swab after slaughter]

Polymerase chain reaction (PCR)

The isolates were further confirmed by polymerase chain reaction (PCR) targeting *16S* rRNA gene (Kaabi and Al Yassari, 2019). DNA extraction was done by boiling and snap chilling method (Ram *et al.,* 2019). The resultant cell lysate was used as template DNA and stored at -20 °C until further use. The PCR was performed in a total volume of 25 µL reaction mixture and the gene identification was standardised using the reference strain *S.* Enteritidis (ATCC-13076™) at an annealing temperature of 65 °C. The PCR products were detected by gel electrophoresis and the images were documented on gel documentation system (Syngene, USA).

The data were analysed using SPSS version 24.0. Fisher's exact chi-square test was used to assess significant association in the occurrence of *Salmonella* spp. across the samples.

Results and discussion

The pink colonies with or without black centre on XLD were subjected to primary and secondary biochemical tests of *Salmonella* spp. All the isolates were Gram negative, catalase positive, oxidase negative, motility test positive, indole negative, methyl red positive, Voges-Proskauer negative, citrate positive, triple sugar iron agar test with acid butt and alkaline slant with or without H_2S production and urease test negative. A total of 31 isolates were identified from a total of 700 samples and all the isolates further confirmed by PCR to detect the genus-specific *16S* rRNA gene (660 bp) (Fig. 1.). There was no significant association ($p > 0.05$) between the sources of samples and occurrence of *Salmonella* spp. along the processing line.

The results of the study are shown in the Table 1. The overall occurrence of *Salmonella* spp. in broiler chicken processing line was 4.63 per cent. This was in accordance with the findings of Beczkiewicz and Kowalcyk (2021) who reported 4.26 per cent prevalence of *Salmonella* spp. from 203 poultry processing establishments in United States. However, the result was not consistent with that of Lin *et al.* (2021), who detected *Salmonella* spp. in 32.6 per cent of carcass rinse samples from 45 chicken abattoirs in Taiwan and with Radhika *et al.* (2022) who reported a 14.22 per cent occurrence of *Salmonella* spp. in carcass rinse samples from chicken meat processing plants in Thrissur and Ernakulam.

Out of the 25 broiler chicken that arrived at the processing unit, two tested positive for *Salmonella* in their cloaca (eight per cent) and in the breast region (eight per cent). Additionally, *Salmonella* was detected in bird crates in the lairage (four per cent). This aligns with the findings of Afsal (2021), who reported an overall occurrence of 9.3 per cent from cloacal swabs in broiler chickens from both Kollam and Kottayam districts in Kerala. In contrast, Reiter *et al*. (2007) reported a higher occurrence of *Salmonella*, with 16.67 per cent detected in transport cages at a poultry slaughterhouse in Brazil. The presence of *Salmonella* in broiler chicken transported from the farm to the slaughterhouse may be attributed to inadequate hygiene, sanitation and improper biosecurity measures on the farms (Munuo *et al.*, 2022).

The water used for the cleaning purpose of the slaughter unit was found to be the major contamination source with a higher occurrence of *Salmonella* (10.7 per cent). Rather *et al*. (2013) reported a similar occurrence of *Salmonella* at 12 per cent in Kashmir, India, while Singh *et al*. (2013) found a lower occurrence of 3.3 per cent

in drinking water at a poultry layer farm in Bareilly. The occurrence of *Salmonella* in water could be attributed to infrequent chlorination of the water tank in the processing plant.

Along the processing line, *Salmonella* was detected in boiler carcasses after defeathering (eight per cent), after evisceration (four per cent) and both before (four per cent) and after final cutting (12 per cent).

The occurrence of *Salmonella* was detected at four per cent both before and after processing on the defeathering machine. Waghamare *et al*. (2020) in Mumbai reported a higher occurrence of *Salmonella* spp. on broiler carcasses post-defeathering (16.66 per cent), while Nidaullah *et al*. (2017) found a 91.67 per cent occurrence in defeathering machines at a small-scale processing plant in Malaysia, which was inconsistent with this study. The increase in *Salmonella* occurrence from undetectable to detectable levels after defeathering in this study is consistent with Nde *et al*. (2008), who identified similar *Salmonella* subtypes on the rubber fingers of the defeathering machine both before and after the process, highlighting the risk of cross-contamination from feathers to carcass skin.

Waghamare *et al*. (2020) reported a 25 per cent occurrence of *Salmonella* post-evisceration in chicken processing plants in and around Mumbai, India. In contrast, Ziba *et al*. (2020) found a lower occurrence of 2.5 per cent in broiler chicken carcass from six slaughterhouses in Zambia after evisceration. The occurrence of *Salmonella*

Table 1: Occurrence of *Salmonella* spp. in broiler processing line

Sl. No.	Sources	No. of samples analysed	No. of positive samples	Per cent occurrence
1	Cloacal swab	25	2	8.00
2	Breast swab of live bird	25	2	8.00
3	Breast swab before scalding	25	0	0.00
4	Breast swab after scalding	25	0	0.00
5	Breast swab before defeathering	25	0	0.00
6	Breast swab after defeathering	25	2	8.00
7	Breast swab before evisceration	25	0	0.00
8	Breast swab after evisceration	25	1	4.00
9	Breast swab before final cutting	25	1	4.00
10	Breast swab after final cutting	25	3	12.00
11	Breast swab before chilling	12	0	0.00
12	Breast swab after chilling	12	0	0.00
13	Water	28	3	10.7
14	Crate swab of birds	28	1	3.00
15	Handwash before slaughter	25	0	0.00
16	Handwash after slaughter	25	3	12.00
17	Evisceration tray swab before slaughter	25	1	4.00
18	Evisceration tray swab after slaughter	25	0	0.00
19	Scalding water before slaughter	25	1	4.00
20	Scalding water after slaughter	25	1	4.00
21	Defeathering machine swab before slaughter	25	2	8.00
22	Defeathering machine swab after slaughter	25	2	8.00
23	Carcass cutting machine before slaughter	25	3	12.00
24	Carcass cutting machine after slaughter	25	0	0.00
25	Chilling water before slaughter	10	0	0.00
26	Chilling water after slaughter	10	0	0.00
27	Chopping board surface before slaughter	25	0	0.00
28	Chopping board surface after slaughter	25	0	0.00
29	Knife swab before slaughter	25	1	4.00
30	Knife swab after slaughter	25	2	8.00
	Total	**700**	**31**	**4.63**

was found to be four per cent on knife surface before processing and eight per cent after processing. Additionally, an eight per cent occurrence was noted on the evisceration trays used for holding visceral organs, both before and after processing. In contrast, a higher percentage of *Salmonella* occurrence was detected on knife swabs, with Waghamare *et al*. (2020) reporting 16.66 per cent in Mumbai, India and Siddiky *et al*. (2022) finding 17.58 per cent in Bangladesh. In this study, the knives used for evisceration were not sterilised and manual evisceration was performed. This may cause damage to the intestines, resulting in contamination of the carcass and its surroundings.

An occurrence of 12 per cent *Salmonella* was found in handwash samples from personnel after processing, while no *Salmonella* was detected in handwash collected at the start of operations following routine handwashing before entering the processing plant. In contrast, Shamsul *et al.* (2016) reported a *S.* Enteritidis occurrence rate of 20 per cent to 100 per cent in hand swabs of workers after processing at a cattle abattoir in Malaysia. Continuous contact with carcasses by the workers may contribute to further contamination of both the carcasses and equipment, posing risks that extend beyond those related to personnel hygiene.

In this study, *Salmonella* (12 per cent) was detected in the carcass cutting machine before cutting the broiler chicken. Zhu *et al*. (2017) reported a higher occurrence of *Salmonella* in chicken meat after the cutting operation in a slaughterhouse in China when compared to the current findings. The presence of *Salmonella* in the broiler carcass may be attributed to inadequate cleaning of the cutting machine that harboured *Salmonella* or the use of water contaminated with *Salmonella* for cleaning the cutting machine.

No *Salmonella* could be detected from the broiler carcasses both before and after the scalding and chilling process, scalding water after the processing and chilling water both before and after the processing. However, the scalding water taken at the start of operations was positive for *Salmonella* (four per cent). Waghamare *et al*. (2020) in Mumbai, India and Nidaullah *et al*. (2017) in Malaysia reported undetectable levels of *Salmonella* in broiler chicken carcasses and scalding water after scalding which was consistent with the findings of the present study. According to Perez-Arnedo *et al*. (2021) in Spain and Thames *et al*. (2022) in Alabama, *Salmonella* was not detected on broiler chicken carcasses after chilling which align with the findings of the present study. In this study, the temperature of the scalding water was maintained between 57 °C and 60 °C for an average of two minutes. Immersion chilling was performed using chlorinated water at a concentration of 25 ppm and a pH range of 5.5 to 6 for approximately 20 minutes.

In the present study, the chopping board surfaces did not reveal the presence of *Salmonella* either before or after the processing. In contrast, Nidaullah *et al*. (2017) reported 100 per cent occurrence of *Salmonella* on chopping boards from small-scale poultry processing plants in Malaysia, while Siddiky *et al*. (2022) found 19.31 per cent occurrence in samples from chicken processing environments in Bangladesh, which could be attributed to the use of chlorinated water sprays on the chopping boards.

Conclusion

In the present study, the occurrence of *Salmonella* from the broiler chicken processing line was studied. The overall occurrence of *Salmonella* was 4.63 per cent and the major contamination points on the line were detected as defeathering, evisceration and final cutting. Implementing proper hygiene practices, effective management, sanitary measures, personal hygiene, implementation of hazard analysis and critical control points (HACCP) principles and a multiple-hurdle approach from production to consumption can significantly mitigate food safety risks.

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Conflict of interest

The authors declare that they have no conflict of interest.

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