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# Prevalence of *Salmonella enterica* in poultry processing lines of central Kerala#

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**Abstract**

*Salmonella enterica is the most pathogenic species frequently responsible for foodborne Salmonella infections in humans. Occurrence of Salmonella enterica in poultry processing lines is a potential risk for the contamination of poultry meat and processing environment. Cross-contamination of carcasses by Salmonella spp. during processing could pose food safety risks. Therefore, the present study involved the detection of Salmonella enterica in the poultry processing lines. The cloacal swabs, caecal contents and carcass rinsates of the birds were collected from processing lines of two processing plants one each from Thrissur and Ernakulam districts of Kerala. The Salmonella spp. were isolated by conventional culture technique on Xylose–lysine deoxycholate agar. The molecular confirmation of Salmonella spp. and S. enterica was carried out by targeting inv*A *and iro*B *gene, respectively. Out of 450 samples analysed from both the processing lines, 14.22 per cent of the isolates were found to be positive for Salmonella spp. by PCR. The inv*A *gene was detected in 67.19 per cent of the isolates and iro*B *genes was detected in 81.4 per cent of the Salmonella spp. isolates. Implementation of standard sanitation protocol and hygiene strategies is required to reduce cross-contamination of poultry carcasses.*

**Keywords:** *Salmonella spp., poultry processing line, Salmonella enterica, cross-contamination*

*Salmonella* spp. is one of the leading cause of foodborne illness across the world. *Salmonella enterica* represents the most pathogenic species which includes more than 2600 serovars. *Salmonella* spp. cause variety of infections in humans including gastroenteritis, bacterimia, enteric fever and rarely extraintestinal complications (Swetha *et al*., 2022). Non-

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typhoidal Salmonella cause approximately 94 million cases of gastroenteritis resulting in 1.55 lakh deaths each year across the world. Out of these 94 million cases, 80.3 million cases are due to contaminated food sources (Antunes *et al*., 2016). Human salmonellosis is frequently reported to be caused by consumption of contaminated poultry products like chicken and eggs. Foods of animal origin, particularly poultry and poultry products, are regarded a key reservoir for several serotypes of *S. enterica*. Contamination of chicken or chicken meat can occur at any point in the production process. *Salmonella enterica* species are usually harboured in the alimentary tract and reproductive system of carrier chicken and they can be transported from farm to abattoir through poultry production chain (Ren *et al*., 2016). Several researchers have reported variable prevalence rates of Salmonella infection in different parts of India (Yadav *et al*., 2011; Ramya *et al*., 2012). Diverse number of serovars of Salmonella have been reported from poultry worldwide and more than 53 serovars have been reported from India. Various serovars like *Salmonella* Enteritidis, *Salmonella* Typhimurium, *Salmonella* Virchow and *Salmonella* Newport are important non-typhoidal Salmonella causing human salmonellosis and are usually caused by consumption of contaminated poultry products (Singh *et al*., 2004). Thus, the present study involved the detection of *Salmonella enterica* from poultry processing lines.

## Materials and methods

### *Sample collection*

The samples were collected from two poultry processing plants located at Thrissur (Plant A) and Ernakulam (Plant B) districts, respectively. Seventy-five samples each of cloacal swabs, caecal contents and carcass rinsates were collected from both the processing plants. Cloacal swabs of birds at the lairages were collected randomly using sterile cotton swabs dipped in sterile buffered peptone water (BPW) (Kadry *et al*., 2019). The caeca of selected birds were cut using sterile scalpel and five grams of caecal contents were collected (Ali *et al*., 2020). The carcass rinsate of the birds were collected after final wash by dipping the carcasses in 400ml of BPW (Rivera-Perez *et al*., 2014).

### *Isolation and identification*

The isolation and identification of *Salmonella* spp. was done by conventional culture techniques (Andrews *et al*., 2001). Initially, the samples were pre-enriched in BPW. The cloacal swabs were pre- enriched by dipping the swabs in 10ml of BPW and caecal contents by adding five grams of caecal contents to 45 ml of BPW. The carcass rinsates were pre-enriched by adding 30ml of carcass rinsates to equal volume of BPW and incubated for 18-20 h at 37°C. For enrichment, 0.1 ml of pre-enriched samples were transferred to 10 ml each of modified Rappaport-Vassiliadis broth and incubated at 42.5°C for 24 h. The enriched samples were streaked on Xylose-lysine deoxycholate agar. The characteristic pink colonies with /without black centre of *Salmonella* spp. were selected as presumptive colonies and subjected to biochemical tests.

### *DNA extraction and polymerase chain reaction*

The DNA of the *Salmonella* spp. positive isolates was extracted by boiling and snap chilling technique (Ram *et al*., 2019). The molecular confirmation of *Salmonella* spp. was done by targeting *inv*A gene using primers as shown in Table 1. The *iro*B gene was targeted by PCR to confirm *Salmonella enterica* isolates and the primers used for identification are listed in table 1. The *inv*A gene and *iro*B genes were standardised at 244 and 606 base pairs respectively (Fig 1 and 2). The PCR amplification of *inv*A gene was carried out with initial denaturation 94°C for 5 min, followed by 35 cycles at 94°C for 30 sec, annealing at 60°C for 30 sec and extension at 72°C for 35 sec, followed by final extension at 72°C for 5 min. The *iro*B gene was amplified with 35 cycles of denaturation at 94°C for 40 sec, annealing at 65°C for 40 sec and extension at 72°C for one min.

### *Statistical analysis*

The results obtained were statistically analysed using SPSS version 24.0 software.

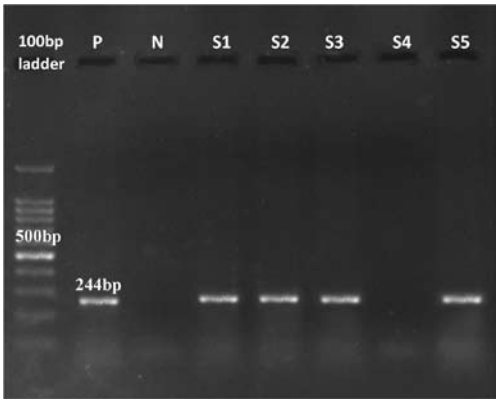


**Fig.1** Agarose gel electrophoresis of PCR product – *inv*A gene

P- Positive Control
N- Negative Control
S1, S2, S3, S4, S5, - Samples

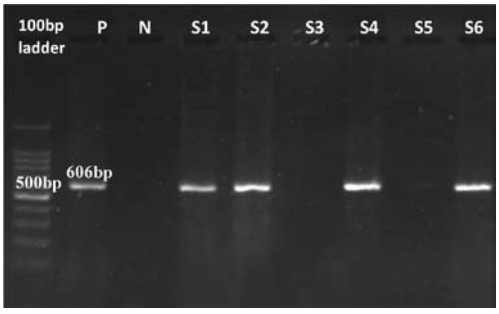


**Fig.2** Agarose gel electrophoresis of PCR product – iroB gene

P- Positive Control
N- Negative Control
S1, S2, S3, S4, S5, S6 - Samples

Chi-square test was used to study the statistical difference in the occurrence of *Salmonella* spp. between Plant A and Plant B.

## Results and discussion

### *Occurrence of Salmonella spp. in poultry processing lines*

The samples showing presumptive colonies were selected and confirmed by biochemical tests. The occurrence of *Salmonella* spp. in the processing lines of both Plant A and Plant B is shown in table 2. In total 45 and 19 samples were positive for *Salmonella* spp. from Plant A and Plant B, respectively. There was significant difference in the occurrence of *Salmonella* spp. in cloacal swabs and caecal contents of Plant A and Plant B. The overall occurrence of *Salmonella* spp. in poultry processing lines of both the processing plants by conventional culture technique was 14.22 per cent. The occurrence of *Salmonella* spp. in the poultry processing line could be due to cross-contamination during processing under unhygienic conditions. *Salmonella* spp. can colonise in poultry by lateral or vertical transmission and the faeces or litter of these birds can contaminate cages during transportation which in turn can be a source of contamination for other birds. Lee *et al*. (2016) from South Korea and Binsy *et al*. (2021) from Kerala reported similar occurrence of *Salmonella* spp. in 11.7 per cent of isolates from chicken carcasses and 14.33 per cent of isolates from retail chicken, respectively. On the contrary, lower occurrence of four per cent and 9.3 per cent of *Salmonella* spp. was reported by Anju *et al*. (2014) and Afsal (2021), respectively from Kerala. Raji *et al*. (2008) reported that 10 per cent of the carcass rinse samples collected after head and feet removal in poultry processing line of a slaughterhouse in Kerala were positive for *Salmonella* spp. However, significantly high prevalence of 88.46 per cent *Salmonella* spp. was reported in poultry samples from Malaysia (Nidaullah *et al*., 2017). The significant difference in occurrence of *Salmonella* spp. in cloacal swabs and caecal contents might be due to difference in source of procurement of birds for slaughter.

**Table 1**. Primers used for identification of *inv*A and *iro*B genes

| Genes | Primer sequence | Size (bp) | Ref. |
|---|---|---|---|
| *inv*A | F: 5'-ACAGTGCTCGTTTACGACCTGAAT-3' | 244 | Zadernowska and Chajęcka-Wierzchowska (2017) |
| | R: 5'-AGACGACTGGTACTGATCTAT-3' | | |
| *iro*B | F: 5'-TGCGTATTCTGTTTGTCGGTCC-3' | 606 | Bäumler *et al.* (1997) |
| | R: 5'-TACGTTCCCACCATTCTTCCC-3' | | |

**Table 2**. Overall occurrence of *Salmonella* spp. by conventional culture technique

| Sl. No. | Sample | Plant A (samples analysed) | Plant B (samples analysed) | Total | | Chi-square test | p-value |
|---|---|---|---|---|---|---|---|
| | | | | No. | % | | |
| 1. | Cloacal swabs | 19(75) | 8(75) | 27 | 18 | $5.46^{s}$ | 0.02 |
| 2. | Caecal contents | 11(75) | 5(75) | 16 | 10.66 | $2.51^{ns}$ | 0.11 |
| 3. | Carcass rinsates | 15(75) | 6(75) | 21 | 14 | $4.48^{s}$ | 0.03 |
| | **Total** | **45** | **19** | **64** | **14.22** | | |

s-significant, ns- non significant

**Table 3**. Overall occurrence of *iro*B gene in *Salmonella* spp. isolates from both of the processing plants

| Sl. No. | Sample | Plant A (samples analysed) | Plant B (Samples analysed) | Total | | Chi-square test | p-value |
|---|---|---|---|---|---|---|---|
| | | | | No. | % | | |
| 1. | Cloacal swabs | 9(19) | 4(8) | 13 | 37.14 | $2.10^{ns}$ | 0.24 |
| 2. | Caecal contents | 7(11) | 4(5) | 11 | 31.42 | $0.88^{ns}$ | 0.53 |
| 3. | Carcass rinsates | 9(15) | 2(6) | 11 | 31.42 | $4.80^{ns}$ | 0.05 |
| | **Total** | **25** | **10** | **35** | | | |

s-significant, ns- non significant

***Molecular confirmation of Salmonella spp.***

All the *Salmonella* spp. positive isolates were subjected to molecular confirmation by targeting *inv*A gene by PCR. Out of 64 positive isolates, 43 (67.19 per cent) were harbouring *inv*A gene. In a study conducted by Kadry *et al*. (2019), a similar 50 per cent occurrence of *inv*A gene in *Salmonella* spp. has been reported from Egypt. The presence of *inv*A gene in cent per cent of the *Salmonella* spp. isolates were reported by Ogunremi *et al*. (2017) from Canada and Colello *et al*. (2018) from Argentina. However, a low prevalence of 12.5 per cent *inv*A gene in *Salmonella* spp. was reported by Yanestria *et al*. (2019) from Indonesia. In the present study *inv*A gene could be detected only in 67.19 per cent of the *Salmonella* spp. isolates which may be due to the fact that some Salmonella are non-invasive or could have other invasive mechanisms (Kadry *et al*., 2019).

For the confirmation of *Salmonella enterica iro*B gene was targeted. Out of 43 samples analysed, 35 samples (81.4 per cent) were positive for *iro*B gene (Table 3). There was no significant difference ($p < 0.05$) in the occurrence of *Salmonella enterica* between the two poultry processing plants. In a study conducted by Sohail *et al*. (2021), 65.09 per cent of the *Salmonella* spp. isolates had harboured *iro*B gene which is lower than the present study. Mthembu *et al*. (2019) from South Africa reported a low occurrence of *iro*B gene in 30.18 per cent of *Salmonella* spp. A high occurrence of *iro*B gene in 98.97 per cent of *Salmonella* spp. was observed in a study conducted by Ogunremi *et al*. (2017) from Canada. Amplification of *iro*B gene by PCR is the rapid and sensitive method of detection of *Salmonella enterica* species. The present study revealed the high occurrence of *Salmonella enterica* in the processing line. These are the most pathogenic species having more than 2600 serovars which commonly cause food poisoning in humans. The rest of the eight *inv*A positive but *iro*B negative isolates may belong to *S. bongori* species, *iro*B gene is absent in this species. However, further biochemical and molecular confirmation is required to confirm the species as *S. bongori*.

**Conclusion**

The present study revealed the occurrence of *Salmonella enterica* species throughout the poultry processing lines. Occurrence of *S. enterica* in the processing line poses a potential public health risk. This study also emphasises the importance of enforcing stricter hygiene and sanitation standards in processing plants to decrease Salmonella

outbreaks. There is evidence that some of the processing procedures enhance the infection of poultry carcasses since poultry processing involves multiple phases. Containment strategies at the farm and slaughterhouses along with integrated surveillance through one health approach involving animal health, human health and food safety is required to minimise the contamination of *Salmonella* spp. along the poultry production chain.

**Conflict of interest**

The authors declare that they have no conflict of interest.

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