RESEARCH ARTICLE
JIVA 19 (2) August 2021

JIVA
OFFICIAL ORGAN OF INDIAN VETERINARY ASSOCIATION, KERALA

Received: 10/05/2021
Accepted: 03/06/2021

PREVALENCE OF *SALMONELLA S*PP. IN RETAIL CHICKEN SOLD IN CENTRAL KERALA

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ABSTRACT

Non- typhoidal Salmonellae are one among the four key causes of foodborne diarrhoea worldwide. Poultry and poultry products are considered one of the main sources of Salmonella infection in man. Hence the study was undertaken to study the prevalence of *Salmonella* spp. in retail chicken sold in central Kerala. Two districts belonging to central Kerala, Thrissur and Ernakulam were selected for sample collection. The isolation of the organism was done using pre-enrichment in buffered peptone water, enrichment in Rappaport Vassiliadis Salmonella enrichment broth followed by selective plating using Xylose Lysine Deoxycholate (XLD) agar. The prevalence of the organism was detected in 20 per cent of the samples. The *inv*A gene which is a conserved virulence gene in Salmonella was targeted for the molecular detection using polymerase chain reaction (PCR). All the positive isolates generated amplicons for the gene.

Keywords: *Salmonella* spp., Prevalence, Conventional culture, *inv*A, PCR

INTRODUCTION

Foodborne diseases affect millions of people worldwide which accounts for one in 10 people globally. Non-typhoidal salmonella infections are one among the four key causes of foodborne diarrhea (Bintsis, 2017). Salmonellae are Gram negative, facultatively anaerobic, non-spore forming, usually motile rods belonging to the family *Enterobacteriaceae.* More than 2500 different serotypes have been identified till date. The organism is hardy and can survive in dry environments and water for several days. The infective dose varies between 10^4 and 10^6 cells but in susceptible individuals or in foods with high fat matrix the dose can be as low as

10^1 to 10^2 cells. Salmonella infection can range from mild gastroenteritis in immune-competent to very severe diarrhoea causing dehydration in children, elderly and in the immune-compromised individuals (Cosby *et al*., 2010).

Chicken and eggs are considered to be the major source of foodborne Salmonella infection (Dar *et al.*, 2017). The consumption of undercooked poultry meat is reported as one of the main sources of infections (Ruban *et al.,* 2016 and Panisello *et al.,* 2000). The conventional isolation of Salmonella is time consuming as it takes 4 to 7 days for the presumptive detection of the organism depending upon the procedure used for the isolation. The invasion A gene (*inv*A) which is located in the salmonella pathogenicity island is responsible for invasion of epithelial cells and the induction of macrophage apoptosis. The gene is conserved in most Salmonellae and is used as a molecular target for the detection of the organism (Mkangara, *et al*., 2019). The present study was designed to study the prevalence of *Salmonella* spp. in retail chicken sold in two districts of central Kerala.

MATERIALS AND METHODS

Retail chicken samples were collected from several retail outlets located in different parts of Thrissur and Ernakulam districts of central Kerala. Each chicken sample comprised of portions of neck, breast and thigh regions and the composite sample weighed 250 g. Likewise, 100 samples each were collected from Thrissur and Ernakulam districts of Kerala. Each sample was collected in sterile polythene bags and brought to the laboratory as early as possible under refrigeration temperature. The isolation of Salmonella was carried out using the method described by Andrews *et al*. (2001). Briefly, from each pooled composite chicken meat sample, a 25 g portion meat was aseptically removed using sterile scissors and forceps. The sample was added to 225 mL of buffered peptone water and pre-enriched at 37°C for 18 h. At the end of incubation, 0.1 mL was transferred to 9.9 mL of Rappaport Vassiliadis Salmonella enrichment broth and incubated at 37°C for 24 h. At the end of incubation, a loopful was plated to Xylose Lysine Deoxycholate (XLD) agar. Pink colonies with or without black centre center were presumptively selected as *Salmonella* spp. Presumptive colonies were subjected to cultural, morphological and biochemical test as per Barrow and Feltham (1993). The DNA was extracted using snap-chill method as described by Swetha *et al*. (2015).

The primers used for the detection of *inv*A gene were custom synthesised

commercially and obtained in lyophilised form. The primers (forward and reverse) used were procured from Sigma–Aldrich, St. Louis, MO. The PCR amplification was carried out in an automated thermal cycler (Eppendorf Master Cycler, Germany) using the PCR cyclic conditions as follows; initial denaturation at 94°C for 5 min. followed by 35 cycles at 94°C for 30 sec., annealing at 60°C for 30 sec., extension at 72°C for 35 sec., followed by final extension at 72°C for 5 min. The desired amplicon size of the *inv*A gene product was 244 bp. The oligonucleotide sequence of *inv*A gene is shown in Table 1.

RESULTS AND DISCUSSION

All the 100 retail chicken samples collected were subjected to isolation and identification of *Salmonella* spp. followed by confirmation for the presence of *inv*A gene. The district–wise prevalence of *Salmonella* spp. is shown in Table 2. Among the 100 chicken samples from Thrissur district, only two samples were positive for the organism whereas 18 samples from Ernakulam were positive for *Salmonella* spp. There was a significant difference ($p \leq 0.01$) in the prevalence of *Salmonella* spp. between the two districts. The overall prevalence of the organism was detected in 20 per cent of the samples from central Kerala. This is in perfect tune with a study in Japan where 20 per cent of the chicken samples collected from supermarkets were positive for *Salmonella* spp. (Iwabuchi *et al*., 2011). Yet another study by Schwaiger *et al*. (2012) detected the presence of the organism in 21 per cent of the chicken drumstick collected from retail shops in Bavaria, Germany. However a lower prevalence of seven per cent was reported in chicken samples analysed in Chattisgarh (Naik *et al.,* 2015). Likewise, a study from Thailand detected the presence of the organism in 5.26 per cent of the retail chicken analysed (Akbar and Anal, 2013). A study from Kerala reported the presence of *S. enteritidis* from 4.4 per cent of the chicken samples collected from Thrissur and Ernakulam districts (Anju *et al*., 2014) Higher detection of the organism was also reported in 53.3 and 65 per cent of chicken samples from Vietnam and Thailand (Van

Table 1. Primer sequences of *inv*A

Primer	Primer sequence	Size (bp)	Reference
*inv*A F	5'- ACAGTGCTCGTTTACGACCTGAAT -3'	244	Zadernowska and Chajęcka-Wierzchowska, (2017)
*inv*A R	5'- AGACGACTGGTACTGATCGATAAT -3'		

Table 2. District – wise prevalence of *Salmonella* spp.

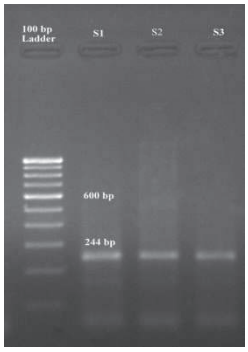
Sl. No.	District	Samples analysed	Positive samples		p- value
			Number	Per cent	
1	Thrissur	100	2 [a]	2.00	0.00
2	Ernakulam	100	18 [b]	18.00	
Total		**200**	**20**	**20.00**	

Figures bearing different superscripts between rows differ significantly ($p \leq 0.01$)

et al., 2007 and Vindigni *et al.,* 2007). The detection of the organism showed a highly significant statistical difference between the two districts which could be attributed to the different sources of procurement of the stocks of birds and another probable source of contamination of the meat with the bacteria could be the meat cutting surfaces at the butcher shops.

All the *Salmonella* spp. positive isolates were subjected to PCR for the detection of *inv*A gene as described by Zadernowska and Chajęcka-Wierzchowska, (2017). Cent per cent of the isolates were positive for *inv*A gene which generated amplicons at 244 bp (Fig.1). A representative amplicon was sequenced at SciGenome Labs Private Limited, Cochin, using Sanger's dideoxy nucleotide chain termination method and the sequence data was uploaded in GenBank and accession number was obtained (MW447835). The *inv*A is a virulence gene which is conserved in most *Salmonella* spp. (Shanmugasamy *et al*., 2011). The detection of the virulence factor is an indicator of the threat posed by the organism. The presence of the organism in cent per cent of the samples is in accordance with studies from Egypt and Iran (Abdel-Aziz, 2016 and Fardsanei *et al*., 2017). However researchers from Canada and Turkey (Diarra *et al*., 2014 and Baran *et al.,* 2019) could only detect the gene in 97.9 and 58.3 per cent of the Salmonella isolates.

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



Lane1- 100 bp ladder, Lane 2-4- *inv*A positive samples

SUMMARY

The present study detected the presence of *Salmonella* spp. in 20 per cent of the retail chicken samples sold in central Kerala. All the isolates harboured the virulence gene *inv*A. This shows that chicken can act as a potential source of

Salmonella infection in man especially when food is improperly cooked. The contaminated meat can also cause cross contamination with other foods in instances where kitchen hygiene is compromised.

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