Available online at www.ijpab.com

DOI: http://dx.doi.org/10.18782/2320-7051.6554

ISSN: 2320 – 7051

Int. J. Pure App. Biosci. **6 (2):** 455-458 (2018)

Research Article

Detection of *Salmonella spp.* Isolated from Poultry Meat and Sources of Contamination in Retail Poultry Meat Shops by Using PCR

Patil S. S., Deshmukh V.V.*, Waghmare R. N. and Yeotikar P.V.

Department of Veterinary Public Health, College of Veterinary and Animal Sciences, Parbhani, Maharashtra

*Corresponding Author E-mail: vivekdes@gmail.com

Received: 31.05.2018 | Revised: 29.06.2018 | Accepted: 13.07.2018

ABSTRACT

Among 240 samples total 11 samples shown presence of Salmonella spp. with overall 4.58 %. Dressing table exhibited higher number of isolates i.e. 4 isolates from 30 samples with 13.33 % positivity. So the dressing table found major sources responsible for Salmonella contamination of retail poultry meat sold in Parbhani city hence hygienic measures must be adopted in poultry meat shops.

Key words: *Salmonellae, Peptone, Food poisoning, Poultry meat*

INTRODUCTION

Salmonellae are among the most important food borne pathogens that cause large number of cases of infections and food poisoning worldwide. Poultry meat is more popular in consumer market because of advantages such as early digestibility and acceptance by the majority of people[10]. Epidemiological reports suggest that poultry meat is still the primary cause of human food poisoning[7]. The present study was conducted to study the prevalence of *Salmonella* spp. in poultry meat and its contamination sources at retail poultry meat shops in Parbhani city characterization by PCR by targeting genus specific *invA* and *hto* genes.

MATERIAL AND METHODS

A total of 240 samples of different sources *viz.* cutting knife, scalding tank, defeatherer, dressing table, platform, personnel, water and poultry meat were collected from five selected retail poultry meat shops in six lots of each. Sterile cotton swabs moistened with 1 % peptone water were used for sample collection from utensils, platform and personnel. Water samples were collected in sterile screw cap test tubes. Poultry meat samples were collected in sterile polyethylene sachets[6].

Isolation of the *Salmonella* was attempted by using method described by Andrews and Hammack. For isolation pre-enrichment of samples was done by using 1 % Buffered Peptone Water (pH 7.0±0.2) at 37^0 C for 24 hours. The pre-enriched samples were enriched by using Tetrathionate Broth at 42^0 C for 24 hours.

Cite this article: Patil, S.S., Deshmukh, V.V., Waghmare, R.N. and Yeotikar, P.V., Detection of *Salmonella spp.* Isolated from Poultry Meat and Sources of Contamination in Retail Poultry Meat Shops by Using PCR, *Int. J. Pure App. Biosci.* **6(2):** 455-458 (2018). doi: http://dx.doi.org/10.18782/2320-7051.6554

Enrichment of sample was followed by selective plating on Xylose Lysine Deoxycholate (XLD) (Himedia Laboratories, Mumbai) agar at 37^0 C for 24 hours. Typical *Salmonella* colonies showing black colour with dark centre were considered as positive for *Salmonella* spp. Isolates showing typical morphological characteristics subjected to biochemical tests and sugar fermentation tests. Confirmed isolates were subjected for molecular characterization by PCR by using *invA* and *hto* genes.

Molecular characterization of isolates was done by using PCR at National Centre on Veterinary Type Culture, National Research Centre on Equine (NCVTC, NRCE) Hisar, Haryana. The PCR was performed by the method described by Sambrook *et al.*[9]. For molecular characterization of isolates PCR technique *invA* and *hto* genes were targeted. The primer sequences for *invA* gene were (F:5'-GTG AAA TTA TCG CCA CGT TCG GGC AA-3') and (R: 5'- TCA TCG CAC CGT CAA AGG AAC C-3'PCR)[8]. The primer sequences for *hto* gene were (F:5'-ACTGGCGTTATCCCTTTCTCTGGTG-3') and (R:5'- ATGTTGTCCTGCCCCTGGTA AGAGA-3')[2]. The genomic DNA was extracted from pure colonies of *Salmonella* and subjected for further processing. Master mix containing 15.0µl of sterile water, 2.5µl of 10x amplification buffer, 2.5µl of deoxynucleoside triphosphates (dNTPs), 1.5µl of 25mM $MgCl_2$, 0.15µl of a 200 pM stock of each primer and 0.4µl of Taq DNA polymerase. Template DNA 2.5µl was added to a 0.5ml thin-walled PCR tube followed by the addition of 22.5µl of PCR master mix. After mixing the sample, tubes were placed in thermocycle (Mastercycler Eppendorf) for PCR reaction run by applying cycling conditions as 95ºC for 5 min followed by 35 cycles of 95°C for 1 min, 60ºC for 30 sec, 72°C for 30 sec and 72ºC for 10 min as final extension. 2 µl of PCR product were loaded in 1 % agarose gel and run at 5v/cm a horizontal electrophoresis assembly (ATTO^R Japan). Finally PCR products were exposed to UV light in gel documentation system (Alpha IMAGER 3400 HP, USA). The gel was photographed and annotated using a PC based ALPHA IMAGER HP software.

RESULTS AND DISCUSSION

A total of 11 *Salmonella* isolates were obtained from 240 samples. Out of which one isolate from 30 samples of defeatherer with 3.33 % positivity, 4 isolates from 30 samples of dressing table with 13.33 % positivity, 2 isolates from 30 samples of water with 6.67 % positivity and 4 isolates from 30 samples of retail poultry meat with 13.33 % positivity were obtained (Table 1). Cutting knife, scalding tank, platform and personnel exhibited negative results for presence of *Salmonella*. The overall *Salmonella* isolation %age was found 4.58 % (out of 240 samples). Morris and Wells,[6] observed 14.2 % *Salmonella* contamination in poultry processing plant. A total of 8 % of contamination in retail poultry meat was also observed by Meldrum *et al.*[5].

Out of total 11 biochemically confirmed isolates of *Salmonella* 3 samples were subjected to *invA* gene and 3 isolates subjected to *hto* gene amplification at random. When these PCR product samples exposed to UV light in gel documentation system, isolates were yielded product of 284bp of *invA* gene and three isolates yielded product of 495bp of *hto* gene (Fig 1). The results of molecular characterization are on similar lines observed by Jamshidi *et al.*[3]. Kumar *et al.*[4] evaluated specificity of *invA* gene PCR for detection of *Salmonella* spp. All the 6 *Salmonella* isolates have been submitted for further typing and preservation in the repository in National Centre on Veterinary Type Culture, National Research Centre on Equine (NCVTC, NRCE) Laboratory, Hisar, Haryana.

Table 1: Isolation of *Salmonella* spp. from different sources of retail poultry meat shops

Sr. No.	Source	No. of samples tested	No. of isolates	Percent positive
1	Knife	30	0	0.00
2	Scalding tank	30	0	0.00
3	Defeatherer	30	1	3.33
4	Dressing Table	30	4	13.33
5	Platform	30	0	0.00
6	Personnel	30	0	0.00
7	Water	30	2	6.67
8	Poultry meat	30	4	13.33
Total		240	11	4.58

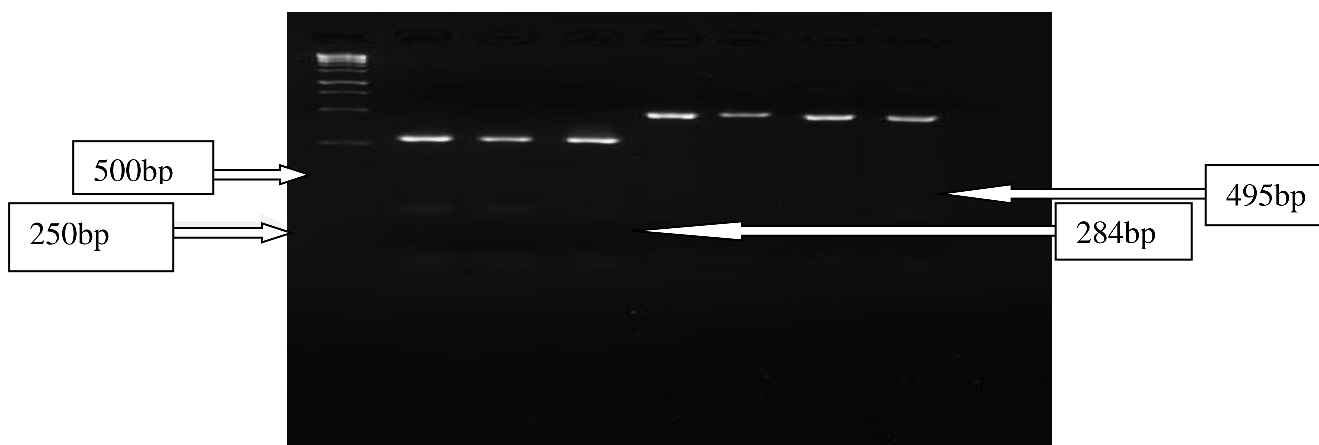

Fig. 1: Agarose gel showing PCR amplified product of *inv*A gene and *hto* gene of *Salmonella* spp.

L1: 1kb DNA marker

L2-L4: PCR amplification of *invA* gene of *Salmonella* spp.

L5-L7: PCR amplification of *hto* gene of *Salmonella* spp

L8: Positive control *Salmonella* spp.

Acknowledgments

Authors are thankful to Dr. R. K. Singh, Director, NRCE & NVTCC, Hisar for helping in molecular characterization of the isolates.

REFERENCES

1. Andrews, W. H. and Hammack, T. S., *Salmonella*. In Bacteriological Analytical Manual, Chapter 5, 8th edn. *U.S. Food and Drug Administration*, AOAC International (2001).
2. Geng, S. Z., Jiao, X. A., Pan, Z. M., Chen, X. J., Zhang, X. M. and Chen, X. J., *Biomed Biotechnol,* 1-8. (2009).
3. Jamshidi, A., Bassami, M. R., Afshari, N. S., *Int.J.Vet.Res*. **3:** 43-48. (2009).
4. Kumar, K., Saklaini, A. C., Singh, S. and Singh, V. P., Evaluation of specificity for *invA* gene PCR for detection of *Salmonella* spp. Proceeding of VIIth Annual Conference of Indian Association of Veterinary Public Health Specialists (IAVPHS) (2008).

5. Meldrum, R. J., Tucker, D. and Edwards, C. J., *Food Prot*. **67:** 1226-1228. (2004).
6. Morris, G. K. and Wells, J. B., *Salmonella* contamination in poultry processing plant. *Appl. Microbiol*., **19:** 795 – 799, (1970).
7. Mulder, R. W., Hygiene during transport, slaughter and processing. In: Poultry Meat Science. Poultry Science Symposium Series. Richardson and Mead (Edn.). **25:** 277-285 (1999).
8. Rahn, K., De Grandis, S. A., Clarke, R. C., McEwen, S. A., Galan, J. E., Ginocchio, C., Curtiss, R. and Gyles, C. L., *Mol. Cell. Probe*. **6:** 271-279. (1992).
9. Sambrook, J., Fritsch, E. F. and Maniatis, T. A., Molecular Cloning: A Laboratory Manual.

a. 2nd Edn. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York. 136-147, (1989).

10. Yashoda, K. P., Sachindra, N. M., Sakhare, P. Z. and Rao, D. N., *J. Food Quality*. **24:** 249-259 (2001).