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

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**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\(^6\). 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\(^\circ\) C for 24 hours. The pre-enriched samples were enriched by using Tetrathionate Broth at 42\(^\circ\) C for 24 hours.

Enrichment of sample was followed by selective plating on Xylose Lysine Deoxycholate (XLD) (Himedia Laboratories, Mumbai) agar at 37°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 GTG CAA AGG AAC C-3'PCR)9. The primer sequences for hto gene were (F:5'-ACTGCGGTATCCCTTCTCTGTTG-3') and (R:5'- ATGTTGTCCCTGCCCCCTGGTA 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 MgCl2 , 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 95°C for 5 min followed by 35 cycles of 95°C for 1 min, 60°C for 30 sec, 72°C for 30 sec and 72°C 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® 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

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Source</th>
<th>No. of samples tested</th>
<th>No. of isolates</th>
<th>Percent positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Knife</td>
<td>30</td>
<td>0</td>
<td>0.00</td>
</tr>
<tr>
<td>2</td>
<td>Scalding tank</td>
<td>30</td>
<td>0</td>
<td>0.00</td>
</tr>
<tr>
<td>3</td>
<td>Defeatherer</td>
<td>30</td>
<td>1</td>
<td>3.33</td>
</tr>
<tr>
<td>4</td>
<td>Dressing Table</td>
<td>30</td>
<td>4</td>
<td>13.33</td>
</tr>
<tr>
<td>5</td>
<td>Platform</td>
<td>30</td>
<td>0</td>
<td>0.00</td>
</tr>
<tr>
<td>6</td>
<td>Personnel</td>
<td>30</td>
<td>0</td>
<td>0.00</td>
</tr>
<tr>
<td>7</td>
<td>Water</td>
<td>30</td>
<td>2</td>
<td>6.67</td>
</tr>
<tr>
<td>8</td>
<td>Poultry meat</td>
<td>30</td>
<td>4</td>
<td>13.33</td>
</tr>
<tr>
<td></td>
<td><strong>Total</strong></td>
<td><strong>240</strong></td>
<td><strong>11</strong></td>
<td><strong>4.58</strong></td>
</tr>
</tbody>
</table>

Fig. 1: Agarose gel showing PCR amplified product of *invA* 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.

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REFERENCES


