Isolation and Molecular Identification of Salmonella typhimurium from Chicken Meat in Iraq

Aseel A. Saeed¹, Mayada F. Hasoon² and Majed H. Mohammed³, ⁴*

¹ College of Veterinary Medicine, University of Qadisiyah, Iraq
² Scientific Research Centre, Faculty of Science, Duhok University, Iraq
³ College of Veterinary Medicine, Iraq, Baghdad, University, Iraq
⁴ Faculty of Veterinary Medicine, Universiti Putra Malaysia 43400 UPM Serdang, Selangor, Malaysia

*Corresponding author’s email: majed_mohammed@putra.upm.edu.my

ABSTRACT

This study was conducted to determine the prevalence of Salmonellae contamination of chicken meat imported from different origin to local markets in south of Iraq (Diwaniya). The bacteria were cultured, isolated and biochemically characterized by the analytical profiling index (API 20E system). The 16s rRNA and invA gene primers were selected specifically for the detection of Salmonella to amplify a 406 and 558 bp DNA fragments, respectively. The results of this study showed that 22 Salmonella isolates were detected by polymerase chain reaction (PCR) from 100 chicken meats and only 7 isolates out of 22 were identified as S. typhimurium, the highest percent of isolates were 83.8% for India origin and the lowest percent were 25% from Jordan origin.

Key words: Chicken meat, Salmonella typhimurium, PCR

INTRODUCTION

Food borne diseases are main problems, particularly in developing countries and cause the majority of illnesses and death around the world. Food is the most important vehicle that transmits the microorganisms to human (Varnam, 1991), among these microorganisms Salmonellae still a major cause of food-borne human disease in most parts of the world (Soultose et al., 2003; Carraminana et al., 2004). Poultry and poultry products are frequently contaminated with Salmonellae that can be transmitted to humans through the handling of raw poultry carcasses and products, or through consumption of undercooked poultry meat (Bailey and Cosby, 2003; Kimura et al., 2004).

Poultry meat is contaminated with Salmonellae not only by infected poultry, but also by cross-contamination with faeces, water, instruments and worker’s hands during the slaughter process and handling. Chicken might thus provide the main transmission route of infection, especially with the increasing consumer demand for this food. This study was undertaken as a prelude to exposure assessment to determine Salmonellae spp. contamination associated with chicken meats was imported from different sources in the markets of south Iraq (Diwaniya).

MATERIALS AND METHODS

Sample collection

Chicken samples were collected from different market in al-diwania city with different origin include different trademark (al-kafeel , al murad , thighs U.S.A, Turkish Chicken, Chicken JD) about 25 g of meat sample were placed in enrichment medium tetrathionate broth and then transported to microbiology laboratory at the College of Veterinary Medicine / Diwaniya University, for 18-24 hr at 37°C.. This study was occurred during the period from December 2011 and carry on June 2012.

Isolation and identification of Salmonella spp.

The samples were cultivated on selective media such as bismuth sulphate agar, chromogenic agar and incubate at 37°C for 18-24 hr. Samples were subjected to biochemical tests such as (TSI), Sulfide-Indole-(SIM), (MRVP), Urea, and Api20-e system.

Specific Primers Sequence Used for PCR Amplification

The primers used for the detection specific sequence of 16s rRNA gene ribosomal genes of Salmonella spp (White et al., 2002). And invA gene encoding proteins of a type (T3SS) III secretion system (Baay et al 1993).These primers are specific for designed in this study by using NCBI Gene Bank and Primer: online and provided by (Bioneer company, Korea) as following Table 1.
Table 1. Specific primers used for the detection specific sequence of 16s rRNA gene and invA gene

<table>
<thead>
<tr>
<th>Sequences</th>
<th>Orientation</th>
<th>Position</th>
<th>Size of PCR product(bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CGG,.ACG,GGT,GAG,TAA,TGT,CT</td>
<td>Forward</td>
<td></td>
<td>16s rRNA 406</td>
</tr>
<tr>
<td>GTT,AGC,GCG,TGC, TTC, TTC, TG</td>
<td>Reverse</td>
<td></td>
<td>invA 558</td>
</tr>
<tr>
<td>ATG,CCC,GGT,AAA,CAG,ATG,ATG,AG</td>
<td>Forward</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CTC,GCC,TTT,GTC,GGT,TTT,AG</td>
<td>Reverse</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

DNA extraction
The bacterial DNA was extracted by using Genomic DNA kit according to the manufacturer’s instruction (USA).

DNA Amplification:
The amplified DNA products from Salmonella spp. specific-PCR were analyzed with electrophoresis on 1% agarose gel stained with ethidium bromide and visualized by UV illumination depending on DNA marker (2000 bp DNA ladder).

Preparation master mix for Detection of 16s rRNA and invA genes
For the detection of Salmonella spp. and S. typhimurium by PCR. The PCR amplification mixture (20μl) which was used for the detection each gene includes 5 μl of PCR PreMix Lyophilized, which provided by Bioneer (Korea) include: bacterially derived Taq DNA polymerase; dNTPs which include: 400 μM of each dATP, dGTP, dCTP, dTTP; 3mM of MgCl₂. Yellow and blue dyes as loading dye, 5 μl of template DNA, 1.5 μl of each forwarded and reversed primers and 7. μl pcr water to complete the amplification mixture to 20 μl. The PCR tubes containing an amplification mixture were transferred to thermocycler and started the program for amplification of the 16s rRNA and invA genes. 30 cycles of PCR, with initial denaturation 1 cycle 95°C for 1 min then 5 min at 95°C (denaturation), 30 s at 55°C (annealing), and 45s at 72°C (extension), and 1 cycle for 7 min at 72°C.

RESULTS

Culture methods
The total percentage of isolation on tetrathionate broth, bismuth sulphate agar, chromogenic agar was 55% (55/100), 60 % (33/55), 87.8% (33/29) respectively, the highest percent of isolation was India origin. The colonies of salmonella spp.on chromogenic agar were variable in size convex and mauve in color.

Confirmatory isolation of salmonella spp. and S.typhiurum by using Api20-E
Salmonella isolates were showed positive productive results to H₂S, TSI, SIM and gives negative for indole, vo-gs Proskauer and ureas. The total percentages of these tests were 89.6% (29/32). While the result of API 20-E showed that 25 isolated positive to API20-E system from 26 with percentage 96.1% (Table 3).

Single plex PCR
The total percentage was 92 % (23/25) for chicken meat and the higher percent for isolation salmonella spp. by 16s rRNA gene were al-kafeel and U.S.A thighs 100% while the lower percent was Turkish origin 75%. The total percentage for detect invA gene for S.typhiurum serotype was 30.4 % (7/23). And the highest percent of isolation of S.typhiurum was 50% from India origin while the lower was 0 % from Turkish origin. (Table 4), (Figure 2 and 3).

Figure 1. The Results of isolation Salmonella spp. using cultural methods. Colonies of salmonella spp. on chromogenic salmonella agar (The arrow shows variable size and mauve in color).
Table 2. Results of salmonella spp. Isolation by using culture methods from chicken meat sample.

<table>
<thead>
<tr>
<th>Culture medium</th>
<th>Sample origin</th>
<th>Tetrahionate broth</th>
<th>Bismuth sulphate agar</th>
<th>Chromogenic agar</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of tested sample</td>
<td>No. of positive</td>
<td>%</td>
<td>No. of tested sample</td>
</tr>
<tr>
<td>Jordan</td>
<td>chicken JD</td>
<td>20</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Turkish</td>
<td>casken oglo</td>
<td>20</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>Brazil</td>
<td>al-kafeel</td>
<td>20</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>India</td>
<td>al-murad</td>
<td>20</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>U.S.A.</td>
<td>thighs</td>
<td>20</td>
<td>13</td>
<td>13</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>100</td>
<td>55</td>
<td>55</td>
</tr>
</tbody>
</table>

Table 3. Results of Biochemical test and API20-E system

<table>
<thead>
<tr>
<th>Test Sample origin</th>
<th>Biochemical test</th>
<th>API20-E system</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of tested sample</td>
<td>No. of positive</td>
</tr>
<tr>
<td>Jordan</td>
<td>chicken JD</td>
<td>6</td>
</tr>
<tr>
<td>Turkish</td>
<td>casken oglo</td>
<td>5</td>
</tr>
<tr>
<td>Brazil</td>
<td>al-kafeel</td>
<td>6</td>
</tr>
<tr>
<td>India</td>
<td>al-murad</td>
<td>6</td>
</tr>
<tr>
<td>U.S.A.</td>
<td>thighs</td>
<td>6</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>29</td>
</tr>
</tbody>
</table>

Figure 2. DNA amplification of a 406 bp of salmonella spp. detecting 16S rRNA gene using singleplex PCR lane 1 control, lane 2,11 negative results, lane 3,4,5,6,7,8,9,10,12,13,14,15 positive results as salmonella spp. Lane M 2000bp marker (ladder).

Figure 3. DNA amplification of a 558 bp of Salmonella spp. detecting invA gene using singleplex PCR lane 1 control results, lane 4, 6,7,8,9,10,12, positive results as S. typhimurium spp. Lane 2,3, 5,13 negative result, lane M 2000bp marker (ladder).
### Table 4. Results of detecting salmonella spp. by single plex PCR 16s rRNA gene and in vA gene.

<table>
<thead>
<tr>
<th>Sample origin</th>
<th>trademark</th>
<th>No. of tested sample</th>
<th>No. of positive</th>
<th>%</th>
<th>No. of tested sample</th>
<th>No. of positive</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jordan</td>
<td>chicken jd</td>
<td>5</td>
<td>4</td>
<td>80</td>
<td>4</td>
<td>1</td>
<td>25</td>
</tr>
<tr>
<td>Turkish</td>
<td>casken oglo tu</td>
<td>4</td>
<td>3</td>
<td>75</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Brazil</td>
<td>al-kafeel</td>
<td>4</td>
<td>4</td>
<td>100</td>
<td>4</td>
<td>1</td>
<td>25</td>
</tr>
<tr>
<td>India</td>
<td>al-murad i</td>
<td>6</td>
<td>6</td>
<td>100</td>
<td>6</td>
<td>3</td>
<td>50</td>
</tr>
<tr>
<td>U.S.A. thighs u.</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>100</td>
<td>6</td>
<td>2</td>
<td>33.3</td>
</tr>
<tr>
<td>Total</td>
<td>T</td>
<td>25</td>
<td>23</td>
<td>92</td>
<td>23</td>
<td>7</td>
<td>30.4</td>
</tr>
</tbody>
</table>

### Discussion

Salmonellosis is considered one of the anthropozooneotic diseases of a serious medical problem and raises great concern in the food industry. Poultry is the most potential source of Salmonella food poisoning in man (Ailsa, et al., 2003).

In the present study the prevalence of Salmonella spp. based on Tetraionat broth as enrichment media were 55% (55/100) this results came compatible with (Vera, et al., 2005) which their result (58.6%) from chicken meat when used Tetrathionat broth as pre enrichment media 42°C, and higher than those obtained by (Pietzsch, et al., 1984) (48%) and (Arroyo et al., 1995) (31.4%) the difference in the results may be attributed to difference in sampling procedure. Several bacteriological selective media have been used to isolating Salmonella sp. like bismuth sulphate agar and the results of isolation were 60% (33/55) and this finding higher than (Dhaher, et al., 2011) when use Bismuth sulphate agar isolated Salmonella sp. from a ported chicken in market of Baghdad city which his results was 24.76%. Other chromogenic agar was used as one of the latest techniques that used in recent decade to rapid isolation of pathogenic agent in water and food (Tavakoli et al., 2008), Salmonella sp. was isolated (29/33) samples with percent (87.8%) which was significantly higher what has been reached in the study (Nancy et al 2005), the reason of this variation due to the difference in the number of samples examined and health standards in the massacres.

The present study shows that the total percentage of isolation Salmonella spp. according to the reading of API 20-E system were 25 isolates from 27 with percentage 92.5% and this percentage was very closer to (Nucera et al., 2006) that was his result 99% when evaluated API 20-E as indicator for Salmonella enterica.

In this work molecular genetics study has been carried out to identify the genetic characters of Salmonella by using of 16s r RNA gene or invA gene specific PCR (White et al., 2002), the results showed that chicken meat samples were 92% (23/25). These results obtained were in corroborated with (Raafat et al., 2011; Darwin et al., 1999). The high relationship found between isolates from chicken meat and patient with food poisoning signs indicates a close genetic relationship between Salmonella isolation of Salmonella typhimurium from poultry meat compared to that isolates from human.

Chicken meat inspection for Salmonella spp should be under supervision of Public Health and Veterinary Authorities to ensure the detection of the spread of zoonosis and identify the prevalence in human to improve preventive measures and decrease contamination of poultry products.

### REFERENCES


