Isolation and Molecular Characterization of *Salmonellae* Isolated from Some Meat Products

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**ABSTRACT**

A Total of 200 random samples of meat products (minced meat, beef burger, kofta, sausage and luncheon) collected from different shops and supermarkets at El Menofiya Governorate, Egypt. The collected samples were examined for the isolation of *Salmonella* spp, serological identifications and molecular characterization by using PCR. *Salmonella* spp was isolated from the examined samples with the percentage of 25% (10), 35% (14), 27.5% (11), 30% (12) and 7.5% (3) respectively. The isolated *Salmonella* serologically identified as *S. Enteritidis*, *S. Infantis*, *S. Paratyphi A* and *S. Typhimurium*, also multiplex PCR methods were used for detection of virulence genes (invA and hilA ) genes of *Salmonella*. The PCR results revealed that invasion gene (invA) and hyper-invasive locus gene (hilA) in *S. Enteritidis*, *S. Paratyphi A* and *S. Typhimurium*. While *S. Infantis* only positive for Invasion gene (invA) could be detected.

**Keyword:** Meat products, salmonella, PCR and virulence genes

**INTRODUCTION**

Meat and meat products have not only been following convenience trends, they have been at the heart of them also meat products such as minced meat, beef burger, kofta, sausage and luncheon are highly demanded and considered more attractive for consumers than fresh meat due to their high nutritive value, reasonable price, good taste, quick easily prepared and also easily serving. In in spite of the importance of meat products to consumers, they can be contaminated with several types of food borne microorganisms from different sources during handling, preparation and storage practices. They are considered as an ideal culture medium for growth of many organisms because of the high moisture, the high percentage of nitrogenous compounds, plentiful supply of minerals, some fermentable carbohydrates (glycogen) and of a favorable pH for most microorganisms (Al-Mutairi., 2011).

According to WHO estimations, 600 million cases of diseases caused by contaminated food were noted in 2010 Pathogenic bacteria also penetrate food production areas and may remain there in the form of a biofilm covering the surfaces of machines and equipment. A common occurrence of microbes in food products, as well as their improper or careless processing, leads to common poisonings. Symptoms of foodborne infections may be mild, sometimes flu-like, but they also may be accompanied by severe complications, some even fatal (Cetinkaya et al., 2012).

*Salmonella* spp. is one of the most severe foodborne pathogens worldwide. The World Health Organization (WHO) estimated an annual global incidence of more than 60 million foodborne cases of nontyphoid *Salmonella* and 7 million cases in the Latin American and Caribbean region (Godínez-Oviedo et al., 2019). Infections with *Salmonella* species represent a major health problem and a
significant burden on food industry (Cetinkaya et al., 2012). Worldwide, foodborne diseases are a growing public health problem. Among the infectious bacteria, non-typhoidal Salmonella enterica serovars (NTS) are the major cause of hospitalization and death (Karmi et al., 2013). Salmonella, a food-borne pathogen, has a recurrent incidence in meat and poultry products. Currently, cases of salmonellosis represent very important economic losses in many countries (Dallal et al., 2019).

Polymerase Chain Reaction (PCR) based methods are powerful diagnostic tool for the detection of pathogenic microorganisms (Malorny et al., 2003). Compared to other methods of detection, these methods are rapid, highly specific and sensitive in the identification of target organisms (Guillier et al., 2013). Therefore, the aim of the present study is isolation and molecular characterization of Salmonella spp isolated from examined samples.

**MATERIAL AND METHOD**

**Collection of samples:**
A total of 200 random samples of raw meat products (minced meat, beef burger, kofta, sausage and luncheon) (40 of each) were collected from different shops and supermarkets with different sanitation levels at Menofiya Governorate. The collected samples were transferred directly to the laboratory in an ice box under complete aseptic conditions without undue delay, thawed at room-temperature to be examined bacteriologically for detection of Salmonella.

**Preparation of samples:**
According to the technique recommended by APHA (1992) as follows: 25 grams from each beef meat product samples were transferred to a sterile polyethylene bag, and 225 ml of 0.1 % sterile buffered peptone water were aseptically added to the content of the bag. Each sample was then homogenized in a blender at 2000 r.p.m for 1-2 minutes to provide a homogenate. The prepared samples were subjected to the following examination:

**Isolation and identification of Salmonella spp** according to (ISO, 2017)

**Serological identification of Salmonellae:**
Serological identification of Salmonellae was carried out according to Kauffman – White scheme (Kauffman, 1974) for the determination of Somatic (O) and flagellar (H) antigens using Salmonella antiserum (DENKA SEIKEN Co., Japan).

**Polymerase Chain Reaction (PCR) of Salmonella**

1.1. Genomic DNA extraction:
Using GeneJET Genomic DNA Purification Kit DNA amplified products "PCR master Mix" (Fermentis): Gel Elecrophoresis: Sambrook et al. (1989).

**DNA ladder (molecular marker):**
100 bp (Fermentas, lot No: 00052518).

**Primer sequences of Salmonellae used for PCR system**
The primers for detection of virulence factors including Enterotoxin (stn), and hyper-invasive locus (hilA) genes of Salmonella species were synthesized as follow:

<table>
<thead>
<tr>
<th>Target gene</th>
<th>Oligonucleotide sequence (5' → 3')</th>
<th>Product size (bp)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>invA (F)</td>
<td>5' GTGAATAATTATCCCACTTCGGGCA '3</td>
<td>284</td>
<td>Shanmugasamy et al., (2011)</td>
</tr>
<tr>
<td>invA (R)</td>
<td>5' TCATCGCACCCTCAAGGAACC '3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>hilA (F)</td>
<td>5' CTGCCGCAGTGTAAAGAAG '3</td>
<td>497</td>
<td>Guo et al., (2000)</td>
</tr>
<tr>
<td>hilA (R)</td>
<td>5' CTGTCCGCTTAAATCGCATGT '3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**DNA amplification of virulence genes of Salmonella (Singh et al., 2013):**
The reaction mix (25 µl) invariably consisted of 5 µl of the bacterial lysate, 5 µl of 10x assay buffer for Taq polymerase containing 1.5 mM MgCl2, 2 µl of 10mM dNTP mix 1 µl each of forward and reverse primer (10 pmol) and 1.25 U of Taq DNA polymerase made up to 50 µl using sterile distilled water.

The PCR cycling protocol was applied as following: An initial denaturation at 94°C for 60 sec, followed by 35 cycles of denaturation at 94°C for 60 sec, annealing at 64°C for 30 sec and extension at 72°C for 30 sec, followed by a final extension at 72°C for 7 min. Finally, 5 µl of each amplicon was electrophoresed in 1.5 % agarose gel stained with ethidium bromide and...
visualized and captured on UV transilluminator.

RESULTS

Table (1): Incidence of Salmonella spp in the examined samples of meat products. (n=40)

<table>
<thead>
<tr>
<th>Meat products</th>
<th>No. of ex. samples</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minced meat</td>
<td>40</td>
<td>10</td>
<td>25</td>
</tr>
<tr>
<td>Beef burger</td>
<td>40</td>
<td>14</td>
<td>35</td>
</tr>
<tr>
<td>Kofta</td>
<td>40</td>
<td>11</td>
<td>27.5</td>
</tr>
<tr>
<td>Sausage</td>
<td>40</td>
<td>12</td>
<td>30</td>
</tr>
<tr>
<td>Luncheon</td>
<td>40</td>
<td>3</td>
<td>7.5</td>
</tr>
<tr>
<td>Total (200)</td>
<td>200</td>
<td>50</td>
<td>25</td>
</tr>
</tbody>
</table>

Table (2): Incidence of Salmonella serovars isolated from the examined samples of meat products (n=40).

<table>
<thead>
<tr>
<th>Products</th>
<th>Salmonellae</th>
<th>Minced meat</th>
<th>Beef burger</th>
<th>Kofta</th>
<th>Sausage</th>
<th>Luncheon</th>
<th>Antigenic structure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S. Enteritidis</td>
<td>3</td>
<td>2</td>
<td>5</td>
<td>12.5</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>S. Infantis</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>2.5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>S. Paratyphi A</td>
<td>2</td>
<td>5</td>
<td>4</td>
<td>10</td>
<td>3</td>
<td>7.5</td>
</tr>
<tr>
<td></td>
<td>S. Typhimurium</td>
<td>5</td>
<td>12.5</td>
<td>7</td>
<td>17.5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>10</td>
<td>25</td>
<td>14</td>
<td>35</td>
<td>11</td>
<td>27.5</td>
</tr>
</tbody>
</table>

Table (3): Acceptability of the examined meat products samples dependin on their contamination with Salmonellae (n=40).

<table>
<thead>
<tr>
<th>Meat products</th>
<th>Salmonellae /25 g*</th>
<th>Accepted samples</th>
<th>Unaccepted samples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Minced meat</td>
<td>Free</td>
<td>30</td>
<td>75</td>
</tr>
<tr>
<td>Beef burger</td>
<td>Free</td>
<td>26</td>
<td>55</td>
</tr>
<tr>
<td>Kofta</td>
<td>Free</td>
<td>29</td>
<td>62.5</td>
</tr>
<tr>
<td>Sausage</td>
<td>Free</td>
<td>28</td>
<td>70</td>
</tr>
<tr>
<td>Luncheon</td>
<td>Free</td>
<td>37</td>
<td>92.5</td>
</tr>
<tr>
<td>Total (200)</td>
<td></td>
<td>150</td>
<td>75</td>
</tr>
</tbody>
</table>


Fig. (1): Agarose gel electrophoresis of multiplex PCR of invA (284 bp) and hilA (497 bp) virulence genes for characterization of Salmonella species.

Lane M: 100 bp ladder as molecular size DNA marker.
Lane C+: Control positive strain for invA and hilA genes.
Lane C-: Control negative.
Lane 2 (S. Infantis): Positive Salmonella strain for invA gene.
Table (4): Incidence of virulence genes of Salmonella strains isolated from the examined samples of meat products (n= 4 strains).

<table>
<thead>
<tr>
<th>Salmonella strains</th>
<th>invA</th>
<th>hilA</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. Enteritidis</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>S. Infantis</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>S. paratyphi</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>S. Typhimurium</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

*invA*: Invasion gene.  *hilA*: hyper-invasive locus gen

**DISCUSSION**

Meat products such as minced meat, beef burger, kofta, sausage and luncheon are highly demanded than fresh meat due to their high nutritive value, reasonable price, good taste, quick easily prepared and also easily serving but they can be contaminated by several types of food borne microorganisms from different sources during handling, preparation and storage practices (Mrema et al., 2006). The results obtained in table (1) revealed that the incidence of isolation of Salmonella in the examined samples of meat products (minced meat, beef burger, kofta, sausage and luncheon) were 25% (10), 35% (14), 27.5% (11), 30% (12) and 7.5% (3) respectively.

Incidence of isolation of Salmonella in the examined minced meat was (25% (10). Comparatively lower results were obtained by Karmi et al., (2013) who found number of positive samples in minced meat 23/160 (14.4%). Also, lower incidence from minced meat reported by Mrema et al., (2006) isolated Salmonella with prevalence rate (20%). On the other hand higher incidence in minced meat reported by Karmi et al., (2013) isolated Salmonella from minced meat by the percentage of 40% (4/10) and by Abd El Tawab et al., (2015) who isolated Salmonella from minced meat by the percentage of (7 = 35%).

Incidence of isolation of Salmonella in the examined samples of beef burger was 35% (14), lower incidence from beef burger reported by Hassanin et al., (2014) isolated Salmonella with the percentage of (13.13%), also, lower incidence from beef burger reported by Sallam et al., (2014) and Abd El Tawab et al., (2015) isolated Salmonella with the percentage of 12.2% (11/90), 10% (2) respectively. While results nearly agree with Essa et al., (2009) isolated Salmonella from beef burger by the percentage of 7 (23.3%) and Karmi et al., (2013) who isolated Salmonella by the percentage of 20% while Ed-dra et al., (2017) who isolated Salmonella by the percentage of 34 (21.79%).

Incidence of isolation of Salmonella in the examined samples of kofta was 27.5% (11) these results nearly agree with Hassanin et al., (2014) who isolated Salmonella with the percentage of 26.67%. lower incidence from kofta obtained by Cetinkaya et al., (2012) isolated Salmonella by the percentage of (2%) and by Eldesouky, et al., (2016) isolated Salmonella by the percentage of (8.5%).

The higher rate of microbial contamination of the examined samples of kofta may be attributed to Karmi et al., (2013) isolated Salmonella by the percentage of 60% (6/10) and Hassanin et al., (2014) isolated Salmonella by the percentage of (33.3%).

Incidence of isolation of Salmonella in the examined samples of sausage was 30% (12). Comparatively lower results were obtained by M Osman et al., (2018) isolated Salmonella by the percentage of (4%). also, by Bingol et al., (2013) and Samax et al., (2012) isolated Salmonella by the percentage of 1.18%, 21.7% respectively. while Abd El Tawab et al., (2015) isolated Salmonella from sausage by the percentage of (20%).

Incidence of isolation of Salmonella in the examined samples of luncheon was 7.5% (3). These results nearly agree with Eldesouky, et al., (2016) isolated Salmonella with the percentage of 8.5% but El-Dosoky et al., (2013) failed to isolate Salmonella from luncheon. On the other hand higher incidence of luncheon reported by Karmi et al., (2013) who isolated Salmonella by the percentage of 10% (1/10) and Essa et al., (2009) isolated Salmonella by the percentage of 16.7% (5).

Lower incidence of Salmonella spp. in luncheon, could be due to heat treatment during manufacture and presence of chemical preservatives. Cutting boards, surfaces used for preparation of meat, equipments like meat...
grinders, mincers and blenders are important sources of Salmonella contamination, other studies stated that trucks, lairages, slaughter line, quartering, knives and surface of table are sources of Salmonella contamination of meat products, also contaminated water used to clean equipment and cutting/slicing machines leading to cross-contamination especially if used with raw foods, handlers not practising proper sanitation and monitoring devices, Survival of Salmonella in ready-to-eat products has the potential to cause illness. (Karmi, 2013).

Results given in table (2) revealed that incidence of isolated serotypes of Salmonella in examined samples of minced meat were S. Enteritidis 7.5%(3) S. Paratyphi A 5%(2) and S. Typhimurium 12.5%(5). These results agree with Stock and Stolle (2001) who isolated S. Typhimurium and Mrema et al., (2006) who isolated S. Typhi, S. Enteritidis, S. Typhimurium and S. Paratyphi. 

Results given in table (2) revealed that incidence of isolated serotypes of Salmonella in examined samples of beef burger were S. Enteritidis 5% (2), S. Infantis 2.5% (1), S. Paratyphia A 10% (4), S. Typhimurium 17.5% (7). These results agree with Abd El Tawab et al., (2015) who isolated S. Typhimurium, S. Enteritidis and S. Typhi and Essa et al., (2009) who isolated 4 strains of Salmonella typhimurium and 3 strains of Salmonella enteritidis and dis agree with El-Dosoky et al., (2013) who failed to isolate Salmonella from beef burger.

Moreover the results given in table (2) revealed that incidence of isolated serotypes of Salmonella in the examined samples of kofta were S. Enteritidis 12.5% (5), S. Infantis 7.5% (3) and S. Paratyphi A 7.5%(3). These results agree with Eldesouky, et al., (2016) THE isolated S. enteritidis was the predominant one followed by S. infantis and dis agree with Cetinkaya et al (2012) who isolated (S. Typhimurium).

Results given in table(2) revealed that incidence of isolated serotypes of Salmonella in the examined samples of sausage were S. Enteritidis 5% (2), S. Infantis 5% (6), S. Paratyphi A 7.5% (3) and S. Typhimurium 2.5%(1), these results disagree with Samax et al.,(2012) isolated Salmonella enterica subsp, salamae II and agree with Mrema et al., (2006) who isolated S. Typhi, S. Enteritidis, S. Typhimurium, S. Paratyphi and S. Infantis.

Finally the results given in table (2) revealed that incidence of isolated serotypes of Salmonella in the examined luncheon samples were S. Enteritis 2.5% (1) and S. Typhimurium 5% (2), these results agree with Eldesouky, et al., (2016) who isolated S. enteritidis was the predominant one (47%) followed by S. typhimurium (23.52%).and dis agree with Essa et al., (2009) who isolated 3 Salmonella paratyphi-B and 2 Salmonella newport strains. The results showed that the examined beef burger were the more contaminated than other meat products and this may be due to destruction of the thermal system during freezing. the very high initial contamination level in frozen beef burgers is the primary cause of this large outbreak and bad cooking practices (Guillier, et al., 2013). there is no reason for concern in consuming ground beef burgers. In case of raw samples, microbes could originate from the vendors. Vendors have to be educated on hygienic practices which could help to reduce risks of foodborne infection. These data indicate that food handlers may contribute to contamination and that there are some handling practices that require more attention. (Soltan, et al., 2009).

The results given in table (3 ) revealed that the accepted samples of Salmonella in minced meat (30) with the percentage of (75%) and the unaccepted samples (10) with the percentage (25) , while in beef burger (26) with the percentage (55%) and the unaccepted samples (14) with the percentage (35%).kofta (29) with the percentage (62.5%) and the unaccepted samples (11) with the percentage (27.5%), sausage (28) with the percentage (70%) and the unaccepted samples (12) with the percentage (30%) and luncheon (37) with the percentage (92.5%) and the unaccepted samples (3) with the percentage (7.5%).

This study was directed to recognize some virulance genes that play an important role in virulence of Salmonella strains by using one of the resent development molecular biological techniques (PCR). The genes were invasion gene (invA) and hyper –invasive locus gene(hila).

PCR result show that invasion gene (invA) and hyper –invasive locus gene (hila) were detected in S. Enteritis, S. Paratyphi and S.Typhimurium, but S. Infantis only positive for invasion gene (invA). These results agree with Ed-dra, et al., (2017) who found that all Salmonella strains (34) were positive for
invasion gene invA and negative for the virulence gene spvC, and agree with Sallam, et al., (2014) detected gyrB and invA genes in all isolated serotypes while Karmi. (2013) All Salmonella isolates were positive for the invA gene, also Eldesouky, et al., (2016) detected invA gene and hyper-invasive locus (hilA) in isolated serotype of Salmonella and dis agree with Salehi et al., (2010) who isolated spvA, spvB and spvC genes from S. enteritidis. (Table 4 and phase 1)

REFERENCES


