SEROTYPIC CHARACTERIZATION OF SALMONELLA ISOLATES IN MEAT AND POULTRY PRODUCTS

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ABSTRACT

A total of 100 meat and poultry products samples were collected from shops and supermarkets in Aswan governorate; including 50 meat products samples and 50 poultry products. Samples were collected to determine the serotypes and antigenic structures of Salmonella isolates in meat and poultry products and to study the correlation between serotypes and type of meat. Salmonella was isolated by conventional methods and serotyping of Salmonella isolates was performed by slide agglutination method using antisera to Salmonella. It was observed that 16 % of samples were positive for Salmonella; 26% (13/50) in meat samples and 6% (3/50) in poultry samples. Serotypes of isolated Salmonella were Salmonella enteritidis 6.25% (1/16), Salmonella newland 18.75% (3/16), Salmonella Kentucky 12.5% (2/16), Salmonella heifa 6.25% (1/16), Salmonella bron 6.25% (1/16), Salmonella rissens 12.5% (2/16), Salmonella fortune 12.5% (2/16), Salmonella agona 6.25% (1/16), Salmonella hessarek 6.25% (1/16), Salmonella hoboken 6.25% (1/16), Salmonella veneziana 6. Meat and poultry products were contaminated with pathogenic Salmonella and the more prevalent serotypes were Salmonella newland, Salmonella rissens, Salmonella fortune, Salmonella Kentucky and the lesser prevalent isolates were Salmonella enteritidis, Salmonella heifa, Salmonella bron, Salmonella agona, Salmonella hessarek, Salmonella hoboken and Salmonella veneziana. Results showed that minced meat, kofta, liver and gizzard and sheeshtawook were more contaminated with Salmonellae. Meat and poultry products considered an important source of high risk serotypes of Salmonellae for human and hygienic measures should be undertaken to reduce contamination of meat and poultry products with Salmonella.

Keywords: Salmonella, serotyping, meat, poultry products.

INTRODUCTION

Salmonellae are zoonotic bacteria of public health significance, with considerable economic impact (Molina-Lopez et al., 2011). Salmonellosis is one of the most common and widely distributed food borne diseases. In Europe, Salmonellosis is the second food borne disease after campylobacteriosis, in Italy; Salmonella is still the major cause of food borne outbreaks (Botti et al., 2013; European Food Safety Authority, 2012). The genus Salmonellae consists of two species: Salmonella enteric and Salmonella bongori. The first one includes 6 subspecies (subsp): enterica, salamae, arizona, diarizona, houtenae and indica. There are over 2,500 serotypes of zoonotic Salmonella, most belonging to the subspecies enterica (Office International des Epizooties, 2010). Salmonellae belonging to the family Enterobacteriaceae are classified and identified into serotypes according to the Kauffmann-White scheme 7, which currently contains more than 2000 serotypes (Williams and Wilkins, 1984).

Serotyping using the widely accepted Kauffmann-White scheme is central to the epidemiological classification of Salmonella strains and thus to surveillance studies, to identify trends in disease transmission, and for detection of outbreaks. Nevertheless, in recent years there is a tendency to use DNA-based methods to replace or augment serological testing, and such methods may be used as a basis to define strains as serovar (4,[5],12:i) or Typhimurium (Echeita et al., 2002; Tennant et al., 2010; Barco et al., 2010).

Ninety-seven percent of food samples were contaminated with at least one enteric pathogen, Salmonella spp. is one of the most commonly isolated pathogen about 84%. Fifty-one percent of children infected with Salmonella spp, infected with the same serotypes isolated from meat samples, suggesting this pathogen is widespread in food and humans (Bodhidatta et al., 2013). In 2007 Austria reported a total of 438 food borne outbreaks,
Salmonella caused 70% of the bacterial outbreaks and the most implicated food was the poultry meat products (Much et al., 2007).

Strains of Salmonellae isolated from poultry and products are strongly associated with multi-locus sequence type ST28 and showed antimicrobial multi-resistance profiles, so that poses a health risk to consumers (Toboldt et al., 2012). Pulsed-field gel electrophoresis (PFGE) results confirmed occurrence of similar salmonella genotypes in both poultry meat products and humans, also, antimicrobial drug resistance profiles suggesting possible transmission of resistance from meat to humans (Oloya et al., 2009). In the last two decades, Salmonella enterica serotype Enteritidis has become one of the main agents causing food borne diseases worldwide. This agent is transmitted mainly by contaminated meat and poultry products (88%), suggesting strong relationships between cases of salmonella related to human illness and salmonella positive in meat and poultry products (Rios et al., 2009; Chen et al., 2008; Pang et al., 2007).

MATERIALS and METHODS

Collection of samples: A total of 100 meat and poultry products samples were collected from shops and supermarkets in Aswan governorate; including 50 meat products samples and 50 poultry products. Samples were collected in sterile polyethylene bags, put in ice box under low temperature and transported to the laboratory for bacteriological examination.

Preparation of the samples: Twenty five g meat was taken from each poultry meat product sample in sterile stomacher bag, mixed with 225 ml buffered peptone water (BPW) (Oxoid Limited, Hampshire, England) and homogenized by using Stomacher® 400 Circulator (Seward Ltd., UK).

Isolation and Identification: The samples mixtures were incubated at 37 °C for 18 hours, 0.1 ml mixture was transferred to 10 ml Rappaport-Vassiliadis (RV) medium, vortexed and incubated for 24 ± 2 h at 42 ± 0.2°C (circulating, thermostatically-controlled, water bath). 3 mm loopful (10 µl) of each incubated tube was streaked on Xylose Lysine Desoxycholate (XLD) agar and incubated for 24 ± 2 h at 35°C. Typical colonies of Salmonella were pink colonies with or without black centers. Many cultures of Salmonella may produce colonies with large, glossy black centers or may appear as almost completely black colonies. Salmonella isolates were confirmed by biochemical tests as Triple Sugar Iron agar (TSI), Lysine decarboxylase (LIA), Urease, Indole, Methyl red, Voges-Proskauer and Simmons citrate (Ewing, 1986; June et al., 1995; Hammack et al., 1999; June et al., 1999; Hammack et al., 2001).

Serotyping of bacterial isolates: Positive Salmonella isolates were tested for presence of somatic (O) and flagellar (H) antigens using slide agglutination test. 3 mm loopful of culture from 24-48 h tryptose blood agar base (without blood) was emulsified with 2 ml 0.9% saline. One drop of culture suspension was added to upper portion of each slide. One drop of saline solution was added to lower part of the slide. One drop of Salmonella monovalent somatic (O) antiserum was added to other section only. With clean sterile transfer loop or needle, mix culture suspension with saline solution for one section and repeat for other section containing antiserum. Tilt mixtures in back-and-forth motion for 1 min and observe against dark background in good illumination. Consider any degree of agglutination a positive reaction. Classify monovalent somatic (O) test results as follows: (a) Positive — agglutination in test mixture; no agglutination in saline control (b) Negative — no agglutination in test mixture; no agglutination in saline control (c) Nonspecific — agglutination in test and in control mixtures. For the flagellar (H) antigen test, repeat the previous steps and test with Salmonella monovalent flagellar (H) antiser instead of Somatic (o) antiser (AOAC, 2000). A Complete serological identification of salmonella isolates were done according to Kaufman-White classification Scheme (Murray et al., 1995; Grimont, 2013; Chiou et al., 2006; Popoff, 2001).

RESULTS

It was observed that 16% of samples were positive for Salmonellae; 26% (13/50) in meat samples and 6% (3/50) in poultry samples (Table 1). The identified Serotypes of isolated Salmonellae were Salmonella enteritidis 6.25% (1/16), Salmonella newland 18.75% (3/16), Salmonella Kentucky 12.5% (2/16), Salmonella heifa 6.25% (1/16), Salmonella hessarek 6.25% (1/16), Salmonella rissens 12.5% (2/16), Salmonella fortune 12.5% (2/16), Salmonella agona 6.25% (1/16), Salmonella hessarek 6.25% (1/16), Salmonella hoboken 6.25% (1/16) and Salmonella veneziana 6.25% (1/16) (Table 2). Results showed that higher rates of Salmonellae contamination were found in minced meat, kofta, liver and gizzard and sheeshtawook, while lower rates were found in luncheon, burgers, and smoked turkey. The most isolated serotype was Salmonella newland, then Salmonella rissens, Salmonella fortune, Salmonella Kentucky and the lesser prevalent isolates were Salmonella enteritidis, Salmonella heifa, Salmonella brun, Salmonella agona, Salmonella hessarek, Salmonella hoboken and Salmonella veneziana (Table 2). Antigenic structures of isolated Salmonellae were determined (Table 3).
Table 1: Incidence of Salmonellae in meat and poultry products.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Number</th>
<th>Positive</th>
<th>Incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meat Products</td>
<td>50</td>
<td>13</td>
<td>26</td>
</tr>
<tr>
<td>Poultry Products</td>
<td>50</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>100</td>
<td>16</td>
<td>16</td>
</tr>
</tbody>
</table>

Table 2: Incidence rate of Salmonella serotypes isolated from meat and poultry products.

<table>
<thead>
<tr>
<th>Salmonella Serotypes</th>
<th>Incidence of Serotypes</th>
<th>Type of meat or poultry product</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>Rate (%)</td>
</tr>
<tr>
<td><em>Salmonella newland</em></td>
<td>3/16</td>
<td>18.75</td>
</tr>
<tr>
<td><em>Salmonella kentucky</em></td>
<td>2/16</td>
<td>12.5</td>
</tr>
<tr>
<td><em>Salmonella fortunes</em></td>
<td>2/16</td>
<td>12.5</td>
</tr>
<tr>
<td><em>Salmonella rissens</em></td>
<td>2/16</td>
<td>12.5</td>
</tr>
<tr>
<td><em>Salmonella enteritidis</em></td>
<td>1/16</td>
<td>6.25</td>
</tr>
<tr>
<td><em>Salmonella haifa</em></td>
<td>1/16</td>
<td>6.25</td>
</tr>
<tr>
<td><em>Salmonella hessarek</em></td>
<td>1/16</td>
<td>6.25</td>
</tr>
<tr>
<td><em>Salmonella agona</em></td>
<td>1/16</td>
<td>6.25</td>
</tr>
<tr>
<td><em>Salmonella hoboken</em></td>
<td>1/16</td>
<td>6.25</td>
</tr>
<tr>
<td><em>Salmonella veneziana</em></td>
<td>1/16</td>
<td>6.25</td>
</tr>
<tr>
<td><em>Salmonella bron</em></td>
<td>1/16</td>
<td>6.25</td>
</tr>
</tbody>
</table>

Table 3: Antigenic Structures of Salmonella serotypes isolated from meat and poultry products.

<table>
<thead>
<tr>
<th>Salmonella Serotypes</th>
<th>Antigenic Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Salmonella newland</em></td>
<td>3,10 (15, 34) e, h; e, n, x</td>
</tr>
<tr>
<td><em>Salmonella kentucky</em></td>
<td>8, 20, l, z6</td>
</tr>
<tr>
<td><em>Salmonella fortune</em></td>
<td>1,4,12,27; z10, z6</td>
</tr>
<tr>
<td><em>Salmonella rissens</em></td>
<td>6,7;14; f, g</td>
</tr>
<tr>
<td><em>Salmonella enteritidis</em></td>
<td>1,9,12; g, m</td>
</tr>
<tr>
<td><em>Salmonella haifa</em></td>
<td>1,4 (5); 12, z10; 1,2</td>
</tr>
<tr>
<td><em>Salmonella hessarek</em></td>
<td>4,12,27; a; 1,5</td>
</tr>
<tr>
<td><em>Salmonella agona</em></td>
<td>1,4 (5); 12; f, g, s; (1,2)</td>
</tr>
<tr>
<td><em>Salmonella hoboken</em></td>
<td>3,10; l; l, w</td>
</tr>
<tr>
<td><em>Salmonella veneziana</em></td>
<td>11; l; e, n, x</td>
</tr>
<tr>
<td><em>Salmonella bron</em></td>
<td>13,22; g, m; (e,n,z15)</td>
</tr>
</tbody>
</table>

**DISCUSSION**

The overall incidence of salmonella were 16 % of samples; 26% (13/50) in meat products samples and 6% (3/50) in poultry products samples. The most common serotypes of salmonella were *Salmonella newland*, *Salmonella Kentucky*, *Salmonella rissens* and *Salmonella fortune*. These serotypes were isolated mainly from minced meat, kofta, meat burgers and sheeshtawook which have higher incidence of salmonella; 60% in kofta, 40% in minced meat, 20% in meat burgers and 20% in sheeshtawook samples. The most famous pathogenic serotypes *Salmonella enteritidis* was found in kofta samples. It is noticed that meat samples (26%) were more contaminated with salmonella than poultry samples (6%), so we can say that meat products are more risky for consumers. Also, results showed that meat and poultry products contain high variety of salmonella serotypes, about 11 different serotypes.
which can cause different pathogenic effects for human.

It is noteworthy that in 2009 the great majority of strains were collected from poultry (45.7%), swine (16.02%) and turkey (4.14%) (Centro de Referenza Nazionale per le Salmonellosi, 2009). However, some genes encoding for virulence factors located on plasmids, can be transferred from one strain to another and can cause an increase in the pathogenicity of serotypes (Boyd and Hartl, 1997). Different serotypes can be present in different animal species: some of them are considered species-specific, while others are ubiquitous (Graziani et al., 2011). The presence of virulence plasmids in host-adapted serovars suggests that horizontal virulence acquisition can have expanded the host range of Salmonella (Rotger and Casadesus, 1999). In detection study 46 Salmonella strains were isolated from 42 meat samples; the positive rate was 20.1%. The majority of positive samples were fresh meat, 69.23% in duck, 37.14% in chicken, 20% in beef and 16.67% in pork. The most common serotypes were Derby (21.74%), Typhimurium (10.87%), Enteritidis (8.7%), Tshiongwe (8.7%), Indiana (8.7%) and Weltevreden (8.7%). Salmonellae present in retail meats were common and phenotypically diverse (Yang et al., 2013). Differences between serogroups are related to polymorphisms at a specific genome locus, the O antigen gene cluster, responsible for O antigen biosynthesis (Ovchinnikova et al., 2013).

In study of the structures and genetics of the 46 O antigens of Salmonellae, a major pathogen of humans and domestic animals, found great variations in structures which underpin the serological specificity of the 46 recognized serogroups. Salmonellae O antigens can be divided into two major groups: N-acetylgalactosamine (GalNAc) and N-acetylgalactosamine (GalNAc), the structures and gene clusters of the GlcNAc/GalNAc-initiated O antigens were found to be highly diverse, and 24 of them were found to be identical or closely related to Escherichia coli O antigens ((Liu et al., 2013; Dziadziusko et al., 2012). Salmonella enterica serovar Enteritidis colonises chickens usually without any gross clinical signs, however, inflammation can be recorded in the intestinal tract, caecum in particular (Matulova et al., 2012; Matulova et al., 2013-a; Matulova et al., 2013-b). In India, enteric fever is a major public health problem and Salmonella typhi is the most common aetiologic agent. Multilocus sequence typing (MLST) pattern grouped Salmonella typhi into two sequence types: ST1 and ST2. ST1 was predominantly present followed by ST2 (Dahiya et al., 2013). In study of children suffering from gastrointestinal infection, 19 Salmonella enteritidis were isolated from 34 anus swab samples of suspected cases and the detection rate was 56%. All of the 19 Salmonella enteritidis showed the same serological serotype, biochemical reaction, drug sensitivity and phage lysis pattern. The Salmonella enteritidis had the identical pulsed field gel electrophoresis (PFGE) pattern (100% similarity), and were different from the pattern of local sporadic infection cases (Yang et al., 2013). Salmonella Typhimurium is the serovar most frequently isolated from persistently infected slaughter pigs in Europe, and its pathogenesis is host species specific (Van Parys et al., 2013; Hernandez et al., 2013). Study of occurrence and characterization of serological variant Salmonella Typhimurium revealed that monophasic Salmonella Typhimurium was found at low frequency in various sources along the food chain, including feed, animals, and meat and sewage sludge (Wasyl and Hoszowski, 2012; Hopkins et al., 2012).

Sero-incidence estimation is a new method to estimate and compare the incidence of salmonella infections in human populations independent of surveillance artefacts (Falkenhorst et al., 2012; Falkenhorst et al., 2013). Some authors see that serotyping is a classic method and should be replaced by a recent techniques such as multilocus sequence typing (MLST) that can be more capable to recognize evolutionary groups and strain relatedness (Achtsman et al., 2012) and PCR assay (Beaubrun et al., 2012). Application of hygienic approaches and effectiveness of potential interventions during production, slaughtering, manufacturing, preparation and processing of poultry meat products can significantly reducing the number of salmonella positive samples in poultry meat products (Van der Fels-Klerx et al., 2008).

CONCLUSION

The results indicated that meat and poultry products contaminated with pathogenic Salmonellae and the more prevalent serotypes were Salmonella newland, Salmonella rissens, Salmonella fortunei, Salmonella Kentucky and the lesser prevalent isolates were Salmonella enteritidis, Salmonella heifi, Salmonella bron, Salmonella agona, Salmonella hessarek, Salmonella hoboken and Salmonella veneziana. Results showed that minced meat, kolha, liver and gizzard and sheeshtawook were more contaminated with Salmonella. Meat and poultry products considered an important source of high risk serotypes of Salmonella for human and hygienic measures should be undertaken to reduce contamination of meat and poultry products with Salmonellae.

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COMPETING INTERESTS

The author declares that he has no competing interests.

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