

## **SEROTYPIC CHARACTERIZATION OF SALMONELLA ISOLATES IN MEAT AND POULTRY PRODUCTS**

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### **ABSTRACT**

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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.

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**Keywords:** *Salmonella*, serotyping, meat, poultry products.

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### **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 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 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 adding to upper portion of each slide. One drop of saline solution was adding to lower part of the slide. One drop of *Salmonella* monovalent somatic (O) antiserum was adding 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) antisera instead of Somatic (o) antisera (AOAC, 2000). A Complete serological identification of salmonella isolates were done according to Kauffman-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 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) 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 bron*, *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.

Sample	Number	Positive	Incidence
Meat Products	50	13	26
Poultry Products	50	3	6
<b>Total</b>	100	16	16

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

Salmonella Serotypes	Incidence of Serotypes		Type of meat or poultry product
	Number	Rate (%)	
<i>Salmonella newland</i>	3/16	18.75	Minced meat, meat burger, Meat kofta
<i>Salmonella kentuckyn</i>	2/16	12.5	Livers & gizzards, sheeshtawook
<i>Salmonella fortunes</i>	2/16	12.5	Minced meat, Meat kofta
<i>Salmonella rissens</i>	2/16	12.5	Minced meat, Meat kofta
<i>Salmonella enteritidis</i>	1/16	6.25	Meat kofta
<i>Salmonella haifa</i>	1/16	6.25	Meat burger
<i>Salmonella hessarek</i>	1/16	6.25	Minced meat
<i>Salmonella agona</i>	1/16	6.25	Meat kofta
<i>Salmonella hoboken</i>	1/16	6.25	Sheeshtawook
<i>Salmonella veneziana</i>	1/16	6.25	Meat luncheon
<i>Salmonella bron</i>	1/16	6.25	Minced meat

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

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

## 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 polymorphism 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-acetylglucosamine (GlcNAc) 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; Dziadziszko *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 (Wasył 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 (Achtman *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 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 *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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### التصنيف السيرولوجي لعترات السالمونيلا في منتجات اللحوم والدواجن

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تم تجميع عدد ١٠٠ عينة من منتجات اللحوم والدواجن من المحال والسوبر ماركت منها ٥٠ عينة من اللحوم و ٥٠ عينة من الدواجن وتم عزل ميكروب السالمونيلا منها وتم تحليل هذه العزلات لتحديد التركيب السيرولوجي والأنتيجيني كما تم ربط هذه النتائج بأنواع العينات. تم استخدام اختبار التلازن بالشريحة باستخدام المصل المضاد للسالمونيلا. أسفرت النتائج عن أن النسبة العامة للعينات الأيجابية ١٦% والعينات الإيجابية في عينات اللحوم ٢٦% وفي عينات الدواجن ٦% وكانت نسب الأنواع السيرولوجية للسالمونيلا كالآتي: ٦.٢٥% سالمونيلا انترتيديز و ١٨.٧٥% سالمونيلا نيولاند و ١٢.٥% سالمونيلا كنتاكي و ٦.٢٥% سالمونيلا هايفا و ٦.٢٥% سالمونيلا برون و ١٢.٥% سالمونيلا ريسينس و ١٢.٥% سالمونيلا فورتيون و ٦.٢٥% سالمونيلا أجونا و ٦.٢٥% سالمونيلا هيساريك و ٦.٢٥% سالمونيلا هوبوكين و ٦.٢٥% سالمونيلا فينيزيانا. أوضحت النتائج أن منتجات اللحوم والدواجن ملوثة بأنواع مختلفة من ميكروب السالمونيلا الممرضة وأن أكثر المنتجات تلوثا هي اللحوم المفرومة والكفتة وكبد وقانصة الدواجن والشيش طاووك لذلك تعتبر هذه المنتجات مصدر للسالمونيلا الممرضة للإنسان ويجب اتخاذ الإجراءات الصحية اللازمة لتقليل ومنع التلوث بهذه الميكروبات الخطيرة.