Prevalence of *Salmonella* Species in Minced Beef and Meat Handlers and Their Drug Resistance

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**ABSTRACT**

The objective of this study was to estimate the prevalence of *Salmonella* spp. in minced beef and meat handlers; also the antimicrobial resistance profile of the isolated strains was evaluated. A total of 85 minced beef beside 65 human samples (25 stools and 40 hand swabs) were collected randomly from different local supermarkets and butcher shops in Beni-Suef Governorate, Egypt between March and November 2014. All samples were investigated for bacteriologic and serologic identification of *Salmonella* spp., beside evaluation of antimicrobial resistance and sensitivity pattern of the isolated strains. *Salmonella* spp. could be recovered from minced beef at a rate of 9.4% and from human hand swabs and stools at a rate of 7.5% and 4% respectively. The detected serotypes were S. Kentucky O:8,20 H:Le6 (8 isolates) and S. Augestinborg O:6,H1,13 (4 isolates). As regard to antimicrobial susceptibility profile, the highest number of S. Kentucky isolates from meat showed resistance against chloramphenicol (83.3%) followed by tetracycline and ciprofloxacin (66.7%), gentamicin (50%) and finally ampicillin, cephalothin and streptomycin (16.7%). On the other hand, S. Kentucky isolates from humans exhibited 100% sensitivity against ampicillin, cephalothin and streptomycin and 50% resistance against tetracycline, ciprofloxacin, gentamicin and chloramphenicol. Regarding S. Augestinborg, it was found highly susceptible (100%) to tetracycline, ampicillin, cephalothin and streptomycin; however 1 strain (50%) from each beef and human samples showed multiple resistance against gentamicin and chloramphenicol. On conclusion, *Salmonella* spp., of the same serotypes, were isolated from both minced beef and human handlers which suggests that considerable transfer of *Salmonella* through food chain. Also, the identification of multi-drug resistant strains should be regarded as a serious thread for the public health.

**Key words:** *Salmonella* spp., minced beef, human handlers, antimicrobials.

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1. **INTRODUCTION**

*Salmonella* is one of the most important bacterial pathogens responsible for food poisoning in humans. Both in developing and developed countries, *Salmonella* infection represents a considerable burden accounting nearly 93.8 million episodes and 155,000 deaths annually. (Majowicz et al., 2010). Non-typhoidal salmonellae (NTS) as a zoonotic agent are predominantly responsible for self-limiting gastroenteritis (diarrhea, abdominal pain, and fever, with a typical duration of 4-7 days). However, serious extraintestinal complications, such as septicemia, endocarditis, meningitis, and osteomyelitis, may occur (Crump et al., 2011).

Foods of animal origin have been implicated as major vehicles associated with illnesses caused by *Salmonella* spp. (CDC, 2006). In the last decades, much of the published reports indicated that the number of salmonellosis has increased mainly associated with the consumption of raw or undercooked poultry, meat or dairy products (Braden, 2006). Of particular concern, contaminated raw or undercooked poultry and red meat are important in transmitting *Salmonella* spp. (Vindigni et al., 2007). Since this pathogen is transmitted primarily through contaminated food or water, the presence of *Salmonella* spp. in food animals and ultimately in raw meat products has important public-health implications (Butaye et al., 2006).

Animals are known to play a major role as a source of a variety of zoonotic *Salmonella* serotypes, which are often asymptomatically carried by them (WHO, 1988). Cattle are among the known reservoirs of *Salmonella*, and ground beef has been implicated as one mode of transmission in foodborne outbreaks (CDC, 2006). Contamination of meat with *Salmonella* may occur during slaughtering processes (Rivera-Betancourt et al., 2004), where such pathogen can be easily transferred to the carcass during hide removal or during evisceration, (Galland, 1997). Also, cross-
contamination from meat handlers during the processes of manufacturing, packing and marketing, may also contribute to the prevalence of salmonellosis (Al-Mutairi, 2011).

In the last two decades, multidrug-resistant (MDR) S. enterica isolates have been increasing and became a major health hazard (Butaye et al. 2006; Alcake et al. 2007). The overuse of antimicrobial agents in food-producing animals for different purposes, including infection treatment, disease prevention, and also growth promotion has resulted in emergence of antibiotic resistant zoonotic bacteria that can be transmitted to humans through the food chain (Angulo et al. 2004; Walsh and Fanning, 2008). It is generally accepted that some multidrug-resistant (MDR) Salmonella are zoonotic in origin and acquire their resistance in animals before being transmitted to humans through the food chain (White et al., 2001; Thrallfall, 2002). It is of particular concern that contamination of food with antibiotic-resistant Salmonella can be a major threat to public health, causing more difficulties in the treatment of infectious diseases (Arslan and Eyi, 2010) and were associated with an increased rate of hospitalization (Varma et al., 2005).

Therefore, the objective of this study was to reveal the presence of Salmonella spp. in minced beef and meat handlers, beside evaluation of the antimicrobial resistance and sensitivity pattern of the isolated strains.

2. MATERIALS AND METHODS

2.1. Sample collection

A total of 85 minced beef beside 65 human samples (25 stools and 40 hand swabs) were collected randomly from different local supermarkets and butcher shops in Beni-Suef Governorate, Egypt between March and November 2014. Each meat sample was packed in sterile plastic bag and labeled. Human samples were collected from workers at supermarkets and butcher shops from which meat samples were obtained. All samples were transferred in an ice-box to the laboratory of Hygiene, Management and Zoonoses Department, Faculty of Vet. Med. Beni-Suef University, where bacteriologic analysis was carried out within 2 h after collection.

2.2. Processing of samples:

2.2.1. Meat samples:

Meat samples were prepared according to the technique recommended by ICMSF (1978). Briefly, 10 g from each sample were transferred under aseptic condition to a sterile polyethylene bag containing 90 ml of sterile buffered peptone water (BPW). The content of the bag was then homogenized using stomacher (Lab. Blender 400, Seward Lab, London) and incubated at 37 °C for 24 h.

2.2.2. Human samples:

Each stool sample was received in a sterile plastic container from which a loopful was inoculated into a sterile tube containing BPW. Also, each hand swab was inoculated into a sterile tube containing BPW and incubated at 37 °C for 24 h.

2.3. Isolation, identification and serotyping of Salmonella:

Salmonella cultures from all samples were performed according to Collee et al. (1996) and Walsman (1999). 0.1 ml from each BPW tube (after incubation) was transferred into a 10 ml Rappaport-Vassiliadis broth (RV broth, Difco, USA) and incubated at 42 °C for 24-48 h. The RV broth samples were streaked onto Xylose-Lysine-Desoxycholate agar (XLD, oxoid) plates and incubated overnight at 37 °C. Typical colonies were picked and further tested by standard biochemical methods. Full identification of the Salmonella suspect isolates were done after matching the achieved morphological, biochemical and serological results against standard methods reported by Kerig and Holt (1986) and Garrity (2001). The serological identification of the strains was carried out with Salmonella polyvalent O and H antisera in the Clinical Microbiology Department, Central Health Laboratories of Ministry of Health on Egypt.

2.4. Determination of antimicrobial susceptibility:

All strains were subjected to antimicrobial susceptibility testing against Tetracycline (30 µg), Ampicillin (10 µg), Cephalothin (30µg), Ciprofloxacine (5 µg), Gentamicin (10 µg), Chloramphenicol (30 µg) and Streptomycin (10 µg) (Oxoid, UK). The disk diffusion assay was carried out following the guidelines of Clinical and Laboratory Standard Institute (CLSI., 2008) to determine the antimicrobial susceptibility pattern of Salmonella isolates. The pre-incubated 24 h cultures of Salmonella were diluted in sterile buffer peptone water and matched with the 0.5 MacFarlane turbidity standards to get 1×10³ CFU/ml as total count. Bacterial suspensions were spread on
Mueller-Hinton agar (Oxoid, UK). The antibiotic disks were placed over the lawn and incubated at 37 °C for 18-24 h. The degree of sensitivity was determined by measuring the clear zone around each antibiotic disk in millimeter.

3. RESULTS

Table (1) showed that Salmonella spp. were isolated from minced beef at a rate of 9.4% and from hand swabs and stool of the examined humans at a rate of 7.5% and 4% respectively. S. Kentucky O:8,20 H:J:z6 could be detected from all samples at an overall prevalence of 5.3% whereas S. Augustinborg O:6,7 H:J:1,3 was only isolated from minced beef and human hands at a rate of 2.3% and 5% respectively.

Of the 6 serotypes of S. Kentucky identified in meat samples, 5 isolates (83.3%) exhibited resistance for chloramphenicol, 4 isolates (66.7%) for both ciprofloxacin and tetracycline and 3 isolates (50%) for gentamicin, whereas only one isolate (16.7%) was found resistant to each ampicillin, cephalothin and streptomycin. Regarding human samples, the 2 isolates of S. Kentucky exhibited 100% sensitivity against ampicillin, cephalothin, streptomycin, while one isolate was found to resist each tetracycline, ciprofloxacin, gentamicin and chloramphenicol. On the other hand, S. Augustinborg, identified in beef and human samples was found highly susceptible (100%) to tetracycline, ampicillin, cephalothin and streptomycin; however 1 strain (50%) from each beef and humans showed multiple resistances against gentamicin and chloramphenicol.

4. DISCUSSION

Salmonella is one of the most important zoonotic bacterial pathogen and remains a major public-health concern all over the world, responsible for several foodborne outbreaks in many countries (Majowicz et al., 2010). They contaminate a wide range of animal products including meat.

Table (1) Prevalence of Salmonella spp. in minced beef and human samples

<table>
<thead>
<tr>
<th>Types of samples</th>
<th>No. of samples</th>
<th>+ve samples (%)</th>
<th>S. Kentucky O:8,20 H:J:z6</th>
<th>S. Augustinborg O:6,7 H:J:1,3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minced beef</td>
<td>85</td>
<td>8 (9.4%)</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>Hand swabs</td>
<td>40</td>
<td>3 (7.5%)</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Human stool</td>
<td>25</td>
<td>1 (4%)</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>150</td>
<td>12 (8%)</td>
<td>8</td>
<td>4</td>
</tr>
</tbody>
</table>

Table (2): Antimicrobial susceptibility profile of Salmonella serotypes recovered from meat and human samples

<table>
<thead>
<tr>
<th>Antimicrobials</th>
<th>Minced beef (n=6)</th>
<th>Humans (n=2)</th>
<th>Minced beef (n=2)</th>
<th>Humans (n=2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S (%)</td>
<td>R (%)</td>
<td>S (%)</td>
<td>R (%)</td>
</tr>
<tr>
<td>Tetracycline (30 µg)</td>
<td>33.3</td>
<td>66.7</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Ampicillin (10 µg)</td>
<td>83.3</td>
<td>16.7</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>Cephalothin (30 µg)</td>
<td>83.3</td>
<td>16.7</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>Ciprofloxacin (5 µg)</td>
<td>33.3</td>
<td>66.7</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Gentamicin (10 µg)</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Chloramphenicol (30 µg)</td>
<td>16.7</td>
<td>83.3</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Streptomycin (10 µg)</td>
<td>83.3</td>
<td>16.7</td>
<td>100</td>
<td>0</td>
</tr>
</tbody>
</table>
Salmonella spp. were the only serotypes from several isolates in minced meat. Globally, approximately 1.3% of chilled beef carcasses and 3.8% of raw (mostly minced) beef have been found to be contaminated with Salmonella (Rhoades et al., 2009). Also, combining data from European countries indicated that Salmonella prevalence in minced beef ranged from 0.0% to 3.6% (Anon, 2006). It is worth mentioning that meat products were the second most common food group contributing to human salmonellosis in the European Union in 2005 (Norrung and Buncic, 2008). In addition, several studies in the United States, Northern Ireland, Australia, and Belgium have reported prevalence of Salmonella on cattle carcasses varying between 0.2% and 4.1% (Vanderlinde et al., 1998; Madden et al., 2001; Li et al., 2004; Ghaifar et al., 2005). However, Salmonella spp. are not naturally present in beef carcasses; they can be present as the direct result of cross-contamination during the slaughter process. The presence of the pathogen in hides and feces has been found to correlate with carcass contamination (Elder et al., 2000). Subsequent grinding may introduce the pathogen into the interior of the meat (De Boer and Heuvelink, 2001). Also, processing and packaging of meat at the wholesale or retail levels are likely to contribute to high level of contamination of minced meat. Even if ground meat is originally contaminated at a low level with Salmonella, growth and/or cross-contamination may occur during storage and handling under poor hygienic conditions (Erol, 1999).

Salmonella spp. could be recovered from the hands of meat handlers at a rate of 7.5% (Table, 1). This result is higher than that obtained by Abd-Allah (2003) who isolated Salmonella spp. at the rate of 3.1% from human hand swabs.

The present study revealed that S. Kentucky and S. Augustinborg were the only serotypes isolated from minced beef and humans (Table, 1). S. Kentucky had been ranked among the top ten serovars isolated from humans in Europe (Gill and Threlfall, 2007) and frequently isolated from poultry, cattle and seafood (Majtan et al., 2006; Boyle et al., 2010; Melendez et al., 2010). In contrast, in the USA, S. Kentucky is the most frequent serovar in poultry and poultry carcasses but has little relevance for human morbidity (Fricke et al., 2009; Melendez et al., 2010).

S. Augustinborg is an uncommon isolate in human infections (CDC; 2004) and it was described as a new Salmonella species in 1966 (Pedersen and Petersen, 1966). It can be highly pathogenic and induce rare manifestations as reported by Lin et al. (2006) in a 9-month-old male infant complain hand abscess, phlebitis, and bacteremia, where the organism was isolated from their pus and blood cultures. S. Augustinborg is relatively sensitive to antibiotics and was found susceptible to ampicillin, chloramphenicol, trimethoprim/sulfamethoxazole, ceftriaxone, and ciprofloxacin (Lin et al., 2006).

The detection of Salmonella spp. in both minced beef and humans (hands and feces) in this study and correlation of the serotypes indicated either cross contamination from miss handling of beef have occurred or both meat and their handlers might exposed to the same environmental source of infection. Also it should be regarded as a serious threat to public health, because Salmonella bacteria are usually transmitted to humans via fecal-oral route, where foods could be contaminated with feces from unwashed hands of an infected food handler.

For susceptibility test, antimicrobials which were important in veterinary and human medicine were selected. In the present study the highest number of S. Kentucky isolates from meat showed resistance against chloramphenicol (83.3%) followed by tetracycline and ciprofloxacin (66.7%), gentamicin (50%) and finally ampicillin, cefalothin and streptomycin (16.7%). On the other hand, S. Kentucky isolates from humans exhibited 100% sensitivity against ampicillin, cefalothin and streptomycin and 50% resistance against tetracycline, ciprofloxacin, gentamicin and chloramphenicol. Regarding S. Augustinborg, it was found highly susceptible (100%) to tetracycline, ampicillin, cefalothin and streptomycin; however 1 strain (50%) from each beef and human samples showed multiple resistance against gentamicin and chloramphenicol. The resistance pattern of Salmonella reported herein is consistent with the studies conducted by many authors around the world; Chiù et al. (2002), Egorova et al. (2008), Forough et al. (2013), Nuananong et al. (2015), Teshome, and Anbessa (2012). The resistance of Salmonella against streptomycin and tetracycline has been already reported by others authors (Dargaz...
et al., 2003; Stevens et al., 2006). Also Murugkar et al. (2005) reported that *Salmonella* isolated from diarrheal samples collected from adult patients who consumed chicken meat, were resistance to tetracycline (71.1%), ampicillin (68.9%), cephalothin (66.7%), and chloramphenicol (46.7%). In the United States, several outbreaks of MDR *Salmonella* infection have been reported associated with consuming ground beef or dairy products and with having direct contact with cattle or cattle environments (Villar et al., 1999; CDC, 2002).

Recently, the increasing prevalence of MDR *Salmonella* and resistance to clinically important antimicrobial agents such as fluoroquinolones and third-generation cephalosporins have been an emerging problem worldwide (Chen et al., 2007). Additionally, isolated MDR *Salmonella* strains have been found to be of many serotypes such as Typhimurium, Choleraesuis, Enteritidis, Dublin, Heidelberg, Kentucky and Newport (Chen et al., 2004; Gebreyes and Thakur, 2005).

The emergence of *S. Kentucky* strain resistant to ciprofloxacin is of particular interest since fluoroquinolones are considered by the WHO in human and veterinary medicine as drugs of critical importance. The first ciprofloxacin-resistant *S. Kentucky* to be identified was isolated from Egypt in 2002 (Le Hello et al., 2011). From then until 2008, laboratory surveillance systems for salmonellosis in France, England, and Denmark detected 489 cases of infection with this strain in people who had travelled to or stayed in Africa or the Middle East. (Le Hello et al., 2011). Recently, in France, England, Wales, Denmark and the USA about 500 human cases of MDR *S. Kentucky* isolates displaying high-level resistance to fluoroquinolones (ciprofloxacin, MIC > or = 4 mg/l) have occurred (Beutlich et al., 2012).

The extensive use or misuse of antimicrobial agents, not only as treatment in humans and veterinary medicine but also as growth promoting substances in livestock production, has greatly contributed to the appearance of antimicrobial-resistant non-typhoidal *Salmonella* strains and with the dissemination and transmission of these strains to humans (Angulo et al., 2000; Araque, 2009; Gousia et al., 2011). The primary concern with the increase in MDR *Salmonella* infections is that resistant bacteria have been implicated in increased morbidity and mortality compared to pan-susceptible *Salmonella* (Varma et al., 2005). Recent studies have shown that, compared with patients infected with drug-susceptible *Salmonella* spp., patients infected with MDR *Salmonella* are at greater risk of bacteremia, hospitalization, and death (Molbak, 2005).

On conclusion, *Salmonella* spp., of the same serotypes, were isolated from both minced beef and human handlers which suggests that considerable transfer of *Salmonella* through food chain. Also, the identification of multi-drug resistant strains should be regarded as a serious thread for the public health.

**Acknowledgment**

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