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Prevalence, molecular identification and antimicrobial resistance profile of *Salmonella* serovars isolated from retail beef products in Mansoura, Egypt

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ABSTRACT

The present study was undertaken to determine the prevalence of *Salmonella* in 270 raw meat samples (90 each of fresh beef, ground beef, and beef burger) purchased on nine occasions from various supermarkets and butchers' shops in Mansoura city, Egypt. Using conventional biochemical identification, *Salmonella* species were recovered from 23.3% (21/90), 20% (18/90), and 12.2% (11/90) of fresh beef, ground beef and beef burger samples, respectively with an overall prevalence of 18.5% (50/270) among all the meat products examined. Higher prevalence were obtained based on molecular identification, by detecting *gyrB* and *invA* genes, which verified the presence of *Salmonella* species in 30.0% (27/90), 26.7% (24/90), and 16.7% (15/90) of fresh beef, ground beef, and beef burger samples, respectively with an overall prevalence of 24.4% (66/270) among all the meat products tested. Of the 2635 presumptive colonies tested, 228 were biochemically identified as *Salmonella*, while 272 were molecularly identified as *Salmonella*, which were all positive for the enterotoxin (*stn*) virulent gene. Of the 272 serologically tested strains, 266 were serologically identified into six *Salmonella* serovars, while 6 strains were untypable. *Salmonella* Typhimurium and *Salmonella* Enteritidis were the most prevalent serovars with an incidence of 38.2% (104/272) and 34.6% (94/272), respectively. The other four serovars identified were *Salmonella* Haifa, *Salmonella* Muenster, *Salmonella* Virchow, and *Salmonella* Anatum were detected at lower prevalences of 11% (30/272), 7.4% (20/272), 4% (11/272) and 2.6% (7/272), respectively. Interestingly, the antimicrobial susceptibility testing indicated that all of the 100 *Salmonella* serovars tested were multidrug resistant (resistant to three or more antibiotics). Our findings demonstrated that the retail beef products tested were widely contaminated with multidrug-resistant *Salmonella* and such contamination may constitute a major public health concern.

1. Introduction

The genus *Salmonella* contains two species, *Salmonella bongori* and *Salmonella enterica*. *S. enterica* is divided into six subspecies; enterica (I), salamae (II), arizonae (IIIa), diarizonae (IIIb), houtenae (IV), and indica (VI) (Grimont & Weill, 2007). Currently, over 2587 serovars have been identified in this genus, and 99% of *Salmonella* serovars responsible for diseases in warm-blooded animals are members of group I (*S. enterica*) (Chan et al., 2003). The major pathogenic serovars of *S. enterica* infected human from food of animal origin include *Salmonella* Typhimurium, *Salmonella* Enteritidis, *S.* Newport, and *S.* Heidelberg (Hur, Jawale, & Lee, 2012).

It has been estimated that there are 9.4 million cases of foodborne illness each year in the United States; of which 55,961 cases are hospitalized and 1351 deaths; over one million and twenty seven thousand cases of this foodborne illness caused by non-typhoidal *Salmonella*, which resulting in 19,336 hospitalizations and 378 deaths (Scallan et al., 2011).

Food animals harbor a wide range of *Salmonella* serovars and therefore act as a source of contamination in non-typhoid human salmonellosis (Acha & Szyfres, 2001, pp. 233–246). Contamination of meat by *Salmonella* may occur at abattoirs during the removal of

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the gastrointestinal tract, contaminated abattoir equipment, floors and personnel, while the pathogen can gain access to meat at any stage during butchering. Cross-contamination of carcasses and meat products could continue during subsequent handling, processing, preparation and distribution (Adesiyun & Oni, 1989; Hendriksen et al., 2009).

The *gyrB* gene encoding the β-subunit of DNA gyrase and invasion A (*invA*) gene have been used as the target for the detection and identification of *Salmonella* (Bülte & Jakob, 1995; Ye, Wang, & Lin, 2011). Moreover, the enterotoxin (*stn*) gene, which encodes a protein that mediates severe diarrhea, was demonstrated as a suitable PCR target for detection of *Salmonella* strains (Prager, Fruth, & Tschäpe, 1995).

The *gyrB* gene sequencing might be more useful for bacterial identification to the species level. The *gyrB* is a single-copy gene, distributed universally among bacteria, which encodes the ATPase domain of DNA gyrase, an enzyme essential for DNA replication. The amino acid sequences of *gyrB* are conservative enough to allow the comparison of taxa which are not closely related (Yamamoto & Harayama, 1996). The chromosomally located *invA* gene contains sequences unique to *Salmonella* is found in all known serovars and has been established as an international standard suitable PCR for rapid, less expensive, and sensitive detection of this genus (Rahn et al., 1992). This virulent gene encodes a protein in the inner membrane of bacteria which is responsible for invasion of *Salmonella* into the host epithelial cells (Torpdahl, Skov, Sandvang, & Baggesen, 2005).

The widespread overuse and misuse of antimicrobial agents in food animal production has contributed to the development of antimicrobial resistant pathogens such as *Salmonella* that has emerged as a major health problem worldwide.

The goal of this study was to determine the incidence of *Salmonella* spp. in fresh beef, ground beef, and beef burger samples sold in Mansoura city, Egypt using both conventional method, through biochemical and serological means, and a specific PCR detection technique through detecting and sequencing of *gyrB*, *invA*, and *stn* genes among the isolated strains of *Salmonella*; also, to determine the antimicrobial sensitivities of the isolated *Salmonella*.

2. Materials and methods

2.1. Collection of samples

Two hundred seventy meat product samples (90 each of fresh beef, ground beef, and beef burger) were purchased from different supermarkets and butchers' shops distributed at Mansoura city, Egypt. Fresh beef samples were obtained from cattle carcasses slaughtered and dressed at Mansoura central province traditional abattoir, while ground beef and beef burger samples were obtained in a frozen packaged form (400–500 g) from various supermarkets. Samples were individually packed into a polyethylene bag then marked and transferred in ice box to the laboratory of Food Hygiene and Control Department, Faculty of Veterinary Medicine, Mansoura University, wherein the conventional bacteriological analyses were done.

2.2. Isolation of Salmonella

The preparation of meat samples and the detection of *Salmonella* were done according to techniques recommended by the International Organization for Standardization (ISO 6579: 2002). Briefly, 25 g from each of beef samples were weighed, and homogenized with 225 ml of sterile buffered peptone water (BPW; Oxoid CM0509) in a stomacher for 1 min. The pre-enrichment broth was then incubated at 37 °C for 18 h. An inoculum from pre-enrichment broth was inoculated into each of Rappaport Vassilliadis broth (RV; Oxoid, CM0669) and Muller-Kauffmann tetrathionate/novobiocin broth (MKTTn; Oxoid, CM1048). The RV broth is incubated at 41.5 °C for 24 h, while the MKTTn broth at 37 °C for 24 h. From these two cultures, 0.1 ml was streaked onto two selective solid media; Xylose-Lysine-Desoxycholate (XLD) agar (Oxoid, CM0469) and Brilliant Green Agar w/Sulfadiazine (BGA; Neogen, 7310). XLD plates were incubated at 37 °C for 24 h, while BGA plates were incubated at 35 °C for 24 h. Presumptive colonies on XLD and BGA were picked up from each positive sample. A total of 2635 presumptive *Salmonella* colonies were collected and cultured onto non-selective media for further identification by both conventional and molecular methods.

2.3. Identification of suspect Salmonella isolates by conventional method

Conventional identification of the 2635 presumptive *Salmonella* colonies was performed using classical biochemical tests. The molecularly verified *Salmonella* isolates (272 strains) were serologically typed by slide agglutination test according to Kauffmann white scheme (Kauffmann, 1974) with the use of rapid diagnostic *Salmonella* antisera sets (Wellcome Diagnostic, a Division of the Wellcome Foundation Limited, Dartford DA15 AH, UK), which typically utilize polyvalent antisera for somatic (O) and flagellar (H) antigens.

Isolates were subcultured on nutrient slope for 24 h at 37 °C for application of slide agglutination technique. Two homogenous suspensions were made on a slide by suspending a piece of suspected colony in a drop of sterile physiological saline. A drop of each of separate O and H *Salmonella* factors were added separately to each of the suspensions with standard loop thoroughly mixed to bring the microorganisms in close contact with antisera. Positive agglutination occurred within a minute and could be easily seen with the naked eye. A delayed or partial agglutination was considered as negative or false result. Isolates with a typical biochemical profile, which agglutinate with both H and O antisera, were identified as *Salmonella* spp.

2.4. Molecular identification of presumptive Salmonella isolates by PCR

Molecular identification of the 2635 presumptive *Salmonella* isolates was carried out at Bio-production Research Institute, National Institute of Advanced Industrial Science and Technology (AIST), Hokkaido, Japan. Genomic DNA of *Salmonella* isolates was prepared by the method reported by Choo et al. (2007). *S.* Typhimurium (RIMD 1985009) was used as a positive control strain, while *Escherichia coli* K12 DH5α was used as a negative control strain. Primer sets for PCR amplification have been previously described for *gyrB* (Ye et al., 2011), *InvA* (Chiu & Ou, 1996) and *stn* (Lee et al., 2009) genes that produce an amplified band size of 366, 244, and 179 bp, respectively. The oligonucleotide primers were synthesized by Hokkaido System Science Co. Ltd. (Hokkaido, Sapporo, Japan). PCR was operated with the use of GeneAmp PCR system 2700 thermal cycler (Applied Biosystems, Foster City, CA, USA). PCR was carried out with a 12-μl reaction mixture consists of 0.75 μl *Salmonella* DNA template, 1.25 μl each of forward and reverse primers (3 pmol each), 2.5 μl dNTPs (2 mM), 6 μl of 2 × PCR Buffer for KOD FX, and 0.25 μl KOD FX DNA Polymerase (Toyobo Co., Ltd., Osaka, Japan). After an initial denaturation at 94 °C for 2 min, 35 cycles (98 °C for 10 s, 58 °C for 30 s, and 68 °C for 45 s) were performed followed by a final extension at 68 °C for 5 min. PCR products of each reaction mixture were separated by subjecting 3 μl aliquots to agarose (1.5%) gel electrophoresis for 30 min at 100 V