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Full Length Article

Serological identification and antimicrobial resistance of *Salmonella* isolates from broiler carcasses and human stools in Beni-Suef, Egypt

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ABSTRACT

The present study was designed in order to estimate the prevalence of *Salmonella* spp. in broiler carcasses and human stools in Beni-Suef province (Egypt). Also, the serological identification and testing of the antimicrobial resistance/susceptibility of the isolates have been done. The obtained results revealed that the prevalence of *Salmonella* in broiler meat, skin, and pooled giblets (liver, gizzard, and heart) was 76, 80, and 64%, respectively, while in the case of human stools the percentage of positive samples represented 4%. The predominant serotype in broiler carcasses was *Salmonella* Infantis (56.36%) followed by *Salmonella* Kentucky (25.45%), and then *Salmonella* Enteritidis with a percentage of 5.45%. However, two serotypes of each of *Salmonella* Ferruch, *Salmonella* Kottbus, and *Salmonella* Virchow were identified out of 55 *Salmonella* isolates, while the only isolate found in human stool samples was serotyped as *Salmonella* Infantis. The results of antimicrobial resistance/susceptibility highlighted the existence of multiple antibiotic resistance (MAR) by several strains of *Salmonella*.

1. Introduction

Foodborne illnesses including food poisoning radically affect public health worldwide. They lead to uncountable premature deaths, several health complications, and massive losses in productivity, implying costs of several billions of dollars to cover healthcare and other consequent expenses. In this regard, it was estimated that one in three people worldwide suffers annually from a foodborne disease and 1.8 million die from severe foodborne diarrhea (WHO, 2007).

Among all known foodborne illnesses, *Salmonella* is identified as a chief cause of foodborne disease in humans, resulting in 16 million cases of typhoid fever, 1.3 billion cases of gastroenteritis

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and 3 million deaths around the world annually (Bhunia, 2008). *Salmonella* outbreaks have been associated with varieties of foods, especially those of animal origin (Hernandez et al., 2005) such as meat, poultry, and eggs (Bouchrif et al., 2009; Eblen et al., 2006). However, poultry meat is considered one of the major sources of *Salmonella* food poisoning in humans and has been implicated in many outbreaks of human salmonellosis. In the light of its public health significance, FAO and WHO have already undertaken risk assessments on *Salmonella* in broiler chickens (FAO/WHO, 2002).

Poultry meat is contaminated by *Salmonella* not only by infected poultry, but also by cross-contamination with feces, water, instruments and workers' hands during the slaughtering, scalding, defeathering, and preparation processes, especially in low hygienic poultry retail outlets (Saeed et al., 2013). Chicken might thus provide the main source of human infection by *Salmonella*, especially with the increasing consumer demand for this food item all over Egypt, including Beni-Suef.

The routine practice of using antimicrobials in livestock breeding for preventive and therapeutic purposes, as well as growth promoters, is a significant factor in the appearance of antibiotic resistant bacteria that are subsequently passed to human bodies through the food chain (Tollefson et al., 1997).

According to the study of Brenner and McWhorter-Murlin (1998), the genus *Salmonella* includes two species: *S. enterica* and *S. bongori*. *S. enterica* is subdivided into six subspecies, which are nominated by name into subsp. *enterica*, *salamae*, *arizonae*, *diarizonae*, *houtenae* and *indica*. The majority of human *Salmonella* food poisoning outbreaks are caused by *S. enterica* subspecies *enterica*. Between the two species of *Salmonella*, over 2500 unique serotypes have been defined and new serotypes are designated regularly.

Previous literatures on the prevalence, serological identification, and antimicrobial resistance of *Salmonella* isolates from chicken carcasses and human stools in Beni-Suef province (Egypt) are scarcely found. Therefore, the present study was carried out with the aim of isolation and serological identification of *Salmonella* spp. from broiler meat, skin and giblets of freshly dressed carcasses and human stools in Beni-Suef (Egypt). Moreover, the antimicrobial susceptibility/resistance of *Salmonella* serotypes was tested.

2. Materials and methods

2.1. Collection of samples

2.1.1. Broiler carcasses

The collection of samples was done during the period from Oct. 2015 until Feb. 2016. For achieving the aims of this study, 25 freshly dressed broiler carcasses with their edible giblets (liver, gizzard and heart) were randomly collected from different poultry retail markets at Beni-Suef, Egypt, where three carcasses were collected weekly. The carcasses were identified and wrapped in sterile polyethylene bags, the giblets were wrapped separate to their carcass, and all were directly transferred immediately in an icebox to the laboratory for further preparation and examination.

2.1.2. Human stool samples

Twenty-five samples of human stools were randomly collected from patients attending Beni-Suef University Hospital for stool analysis. Each stool sample was received in a sterile plastic container and then immediately transferred in an icebox to the laboratory where further preparation and analysis were directly operated. An oral approval from the individuals, or their guardians, included in this study was taken before collection.

2.2. Preparation and subsampling

From each carcass, 25 g each of meat, skin and pooled giblets was subsampled. The meat specimen was aseptically removed from the deep tissues of thigh and/or breast, after surface sterilization using hot spatula. Then each 25 g was aseptically transferred into a sterile homogenizer flask containing 225 ml of 0.1% sterile buffered peptone water (Biolife; Italy). The contents were homogenized at 2000 rpm for 2.5 min using a sterile homogenizer (MPW 302, Universal Laboratory Aid, Poland). In the case of human stool samples, approximately 1 g of feces from each sample was aseptically transferred into a sterile test tube containing 9 ml of 0.1% sterile buffered peptone water for preparation of the original homogenate.

2.3. Isolation of Salmonella spp.

Isolation of *Salmonella* spp. from both chicken carcasses and stool samples was carried out according to the protocol of ISO 6579 (2002) with slight modifications. Briefly, the previously prepared homogenate of the sample (meat, skin, giblets, or human stool) and buffered peptone water was incubated at 36 ± 1 °C for 16–20 h as a pre-enrichment step. After that, 0.1 and 1 ml of the pre-enrichment broth were inoculated into a tube containing 10 ml of sterile Rappaport-Vassiliadis soy peptone broth (Biolife; Italy) and another one containing 10 ml sterile Müller-Kauffmann Tetrathionate broth (Biolife; Italy), respectively, for selective enrichment. Then the inoculated broths were further incubated at 41.5 ± 0.5 °C (in the case of Rappaport-Vassiliadis broth) and 36 ± 1 °C (in the case of Tetrathionate broth) for 18–24 h. A 10 µl loopful from each incubated broth was streaked onto two selective plating media, which were *Salmonella-Shigella* agar (SS) and Xylose Lysine Desoxycholate agar (XLD). All the inoculated plates were incubated at 36 ± 1 °C for 18–24 h. Colorless colonies with black centers on SS and slightly transparent red colonies with black center on XLD agar were suspected as *Salmonella*. The characteristic colonies of *Salmonellae* were further streaked on nutrient agar plates and incubated at 36 ± 1 °C for 18–24 h for purification, and then on nutrient agar slopes for further identification and biochemical characterization.

2.4. Morphological and biochemical identification

The initial identification step was done using Gram's stain smears and oxidase test; all isolates showing Gram's stain positive and/or oxidase positive were discarded. Then other isolates were biochemically tested using indole, methyl red, Voges–Proskauer, citrate utilization, triple sugar iron (TSI), and urease tests as per the protocol described by Ewing (1986). The colonies