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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*.

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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

showing *Salmonella* specific IMViC pattern (– + – +) were further inoculated on TSI slants, and colonies that produced alkaline slant (pink) and acidic butt (yellow) with or without H_2S production (blackening) were tested for urea hydrolysis on urea agar slants. All the urease negative isolates were considered as biochemically confirmed *Salmonella* isolates.

2.5. *Serological identification of* Salmonella *isolates*

All biochemically confirmed *Salmonella* isolates were serologically identified on the basis of somatic (O) and flagellar (H) antigens by slide agglutination using commercial antisera (SISIN, Berlin) following Kauffman–White scheme (Popoff et al., 2004). The serological identification was carried out at the Serology Unit, Animal Health Research Institute, Dokki, Egypt, and the Bacteriology Laboratory, Central Laboratories of Ministry of Health, Egypt.

2.6. *Antimicrobial sensitivity testing*

All *Salmonella* isolates were tested for their antimicrobial resistance/susceptibility pattern by disc diffusion technique according to Clinical and Laboratory Standards Institute (CLSI, 2014). Antibiotic discs of 5.5 mm diameter impregnated with amikacin (30 μg), ciprofloxacin (5 μg), ampicillin (10 μg), amoxicillin-clavulanic acid (20/10 μg), piperacillin-tazobactam (100/10 μg), cefotaxime (30 μg), ceftazidime (30 μg), aztreonam (30 μg), nalidixic acid (30 μg), and tetracycline (30 μg) (Oxoid, UK) were used. The diameter of the zones of complete inhibition was measured and compared with the zone size interpretation chart provided by the supplier and was graded as susceptible (S), intermediate (I), and resistant (R). Moreover, the multiple antibiotic resistance (MAR) index was calculated for all *Salmonella* isolates according to the protocol designated by Krumperman (1983), using the formula a/b where "a" is the number of antimicrobials to which an isolate was resistant and "b" is the total number of antimicrobials to which the isolate was exposed.

2.7. *Statistical analysis*

Statistical analysis of the results was done according to Knapp and Miller (1992) using the SPSS Statistics 17.0 software program.

3. Results and discussion

The prevalence of *Salmonella* spp. in examined broiler carcasses (meat, skin and pooled giblets) and human stools was outlined in Table 1. The highest level of *Salmonella* spp. was found in broiler skin, where 20 out of 25 samples were positive (80%), followed by broiler meat with a percentage of 76% (19 out of 25 samples), and then pooled giblets (liver, gizzard and heart), where 16 out of 25 samples had *Salmonella* (64%). It could be concluded that a total of 55 isolates of *Salmonella*e were detected in 75 samples of broiler carcasses, with a percentage of 73.3%.

The presence of such high levels of *Salmonella*e in examined broiler carcasses (meat, skin and pooled giblets), which were collected from Beni-Suef province than previous reports, was surprising. They were much higher than that previously reported by Gharieb et al. (2015) in Egypt, who detected *Salmonella* spp. with a prevalence of 14% in chicken meat. Additionally, the current findings were also much higher than reports from other countries, such as 14.5% from Nepal (Maharjan et al., 2006), 14% from Canada (Arsenault et al., 2007), 19.2% from South Africa (Nierop et al., 2005), and 12% from Turkey (Ozbey and Ertas, 2006). On the contrary, nearly similar high prevalence of *Salmonella* spp. in broiler carcasses was previously recorded in studies from Senegal (62.5%) (Bada-Alambedji et al., 2006) and Thailand (66%) (Jergklinchan et al., 1994).

Table 1 – The prevalence of *Salmonella* spp. in examined broiler meat, skin, and giblets, and human stools.

Sample	Number of examined samples	Number of positive samples	%
Broiler meat	25	19	76
Broilers' skin	25	20	80
Pooled giblets	25	16	64
Broilers (total)	75	55	73.3
Human stools	25	1	4

The high prevalence of *Salmonella* spp. in our study could be attributed to the low hygienic measures observed in the poultry retail markets at Beni-Suef (Egypt) during slaughtering, scalding, defeathering, evisceration, carcass cutting and handling. These procedures allow cross contamination from diseased bird or contaminated carcass to healthy and clean ones. Besides, the lack of veterinary supervision inside these markets may lead to slaughtering of diseased birds.

Such findings coincide with that obtained by Humphrey et al. (1988), who reported that the existence of *Salmonella* in the intestinal tract, on the skin and above the feathers of broilers, could cause carcass contamination during slaughtering, evisceration and processing. Thus, it is responsible for introducing *Salmonella* in the slaughterhouses/slaughter areas, where it will multiply along the processing area and endanger the consumers' health. Furthermore, it was reported that the cross contamination from workers' hands, equipment and utensils used during carcass preparation, subsequent handling of the raw poultry carcasses and ready-to-eat products together with the consumption of improperly cooked poultry meat could act as the most frequent sources of infection by *Salmonella* reported in humans (Saeed et al., 2013; Yildirim et al., 2011).

Regarding the prevalence of *Salmonella* in examined human stools (Table 1), it was found that only one out of 25 samples had *Salmonella* spp. with a percentage of 4%. In this regard, Gharieb et al. (2015) reported a similar prevalence (4%) of *Salmonella* in human stools in Egypt. Moreover, Murugkar et al. (2005) reported nearly similar prevalence in India. Such low prevalence in human stools reported in this study could be attributed to the samples that were not collected from only diarrheic patients, but randomly from 25 patients who attended the hospital for stool analysis regardless of the reasons.

Concerning the distribution of *Salmonella* serotypes in examined broiler samples, the obtained results as shown in

Table 2 – Distribution of *Salmonella* serotypes in examined broiler meat, skin and giblets (n of isolates = 55).

Serotypes	Antigenic formula	Meat			Skin			Pooled giblets			Total	
		No	%*	%**	No	%*	%**	No	%*	%**	No	%
S. Enteritidis	O: 1,9,12; H_1: g, m; H_2: –	0	0	0	2	10	3.63	1	6.25	1.81	3	5.45
S. Colindale	O: 6,7; H_1: r; H_2: 1,7	1	5.26	1.81	0	0	0	0	0	0	1	1.81
S. Infantis	O: 6,7,14; H_1: r; H_2: 1,5	10	52.6	18.18	12	60	21.81	9	56.25	16.36	31	56.36
S. Kentucky	O: 8, 20; H_1: i; H_2: Z_6	7	36.8	12.72	4	20	7.27	3	18.75	5.45	14	25.45
S. Ferruch	O: 8; H_1: e, h; H_2: 1,5	0	0	0	1	5	1.81	1	6.25	1.81	2	3.63
S. Kottbus	O: 6, 8; H_1: e, h; H_2: 1,5	0	0	0	1	5	1.81	1	6.25	1.81	2	3.63
S. Virchow	O: 6,7,14; H_1: r; H_2: 1,2	1	5.26	1.81	0	0	0	1	6.25	1.81	2	3.63

%* represents the percentage of positive in relation to the number of positive Salmonella in broiler meat, skin or giblets, while %** represents the percentage of positive in relation to the total positive Salmonella in examined broiler samples.

Table 2 revealed that the predominant serotype was *Salmonella* Infantis followed by *Salmonella* Kentucky, where 31 out of 55 isolates of *Salmonella* in broiler carcasses (56.36%) were serotyped as *Salmonella* Infantis. While *Salmonella* Kentucky was detected in 14 out of 55 serotypes (25.45%), *Salmonella* Enteritidis came in the third position with three isolates out of 55 (5.45%). However, two serotypes were detected for each of *Salmonella* Ferruch, *Salmonella* Kottbus and *Salmonella* Virchow. Finally, *Salmonella* Colindale was detected in only one sample of broiler meat, while it was failed to be detected in skin or giblets.

With regard to the predominant serotypes of *Salmonella* isolates from chicken meat in other studies, Gharieb et al. (2015) and Kaushik et al. (2014) reported the predominance of *S. typhimurium* in chicken meat in Egypt and India, respectively. Nevertheless, the predominant serotypes found in this study were previously reported as predominates in other several reports, such as S. Infantis (Cetinkaya et al., 2008) and S. Kentucky (Saad et al., 2015).

Regarding the serotyping of *Salmonella* isolate from human stools illustrated in Table 3, it has been shown that the only one isolate of *Salmonella* found in human stools was serotyped as *Salmonella* Infantis. The positive stool sample was collected from a patient suffering from a severe gastroenteritis with diarrhea and abdominal pain. In this concern, it was reported that *Salmonella* Infantis belongs to the main serotypes of *Salmonellae* inducing human gastroenteritis (Najjar et al., 2012). S. Infantis foodborne infection had been reported in several areas around the world, such as Japan (Shahada et al., 2006) and India (Patil et al., 2012). As well, the UK reports affirmed that infection with *Salmonella* Infantis is responsible for 0.3% of mortalities caused by salmonellosis during 1996–2006 (Jones et al., 2008).

Therefore, *Salmonella* became a worldwide concern in public health sector and its importance as a public health issue is growing day by day all over the world. In this regard, Steven et al. (2011) reported that over the last some decades there has been a significant shift in predominant *Salmonella* serotypes associated with human illnesses.

Populations who are mostly at risk for severe complications due to *Salmonella* food infection are elderly, infants, children, pregnant women, and immunocompromised persons. One of three major syndromes, which are per acute systemic infection, acute enteritis or chronic enteritis, could manifest *Salmonella* infection clinically in all hosts (Merchant and Packer, 1967). The main symptoms commonly include headache, nausea, vomiting, gastroenteritis, fatigue, abdominal pain and bloody diarrhea with mucus, and occasionally reactive arthritis (Dworkin et al., 2001).

The aforementioned results in Table 4 illustrated the results of antimicrobial resistance/susceptibility of isolated *Salmonella* serotypes from broiler carcasses and human stools. Variable rates of resistance of *Salmonella* serotypes were found against 10 different types of antimicrobials impregnated into discs of 5.5 mm diameter. It was evident that *Salmonella* Kottbus isolates showed 100% resistance against all used antimicrobials; furthermore, all *Salmonella* Virchow isolates were resistant (100%) against all antimicrobials except that they showed intermediate resistance against piperacillin-tazobactam. Nevertheless, the obtained results highlighted the high resistance levels of *Salmonella* isolates against nalidixic acid (100% resistance) and tetracycline (89.3% resistance).

Table 3 – The prevalence and serotypes of *Salmonella* isolated from human stools.

Number of examined samples	Number of positive samples	%*	Serotype	Antigenic formula	%**
25	1	4	S. Infantis	O: 6,7,14; H_1: r; H_2: 1,5	100

%* represents the percentage of positive in relation to the number of examined human stool samples, while %** represents the percentage of serotype in relation to the total positive Salmonella in examined human stool samples.

Table 4 – The antimicrobial resistance/susceptibility of isolated *Salmonella* serotypes from broilers and human stool samples; the data are represented by number of isolates.

Antimicrobials	Strains	S. Enteritidis			S. Colindale			S. Infantis			S. Kentucky			S. Ferruch			S. Kottbus			S. Virchow		
	No. of strains	3			1			32			14			2			2			2		
		S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
AK (30 µg)		3	0	0	1	0	0	16	8	8	10	0	4	2	0	0	0	0	2	0	0	2
CIP (5 µg)		0	3	0	0	1	0	0	21	11	0	0	14	0	2	0	0	0	2	0	0	2
AMP (10 µg)		3	0	0	0	0	1	12	10	10	0	0	14	0	0	2	0	0	2	0	0	2
AMC (20/10 µg)		3	0	0	1	0	0	26	6	0	2	4	8	2	0	0	0	0	2	0	0	2
TZP (100/10 µg)		3	0	0	1	0	0	14	14	4	4	4	6	2	0	0	0	0	2	0	2	0
CTX (30 µg)		3	0	0	1	0	0	9	2	21	2	0	12	2	0	0	0	0	2	0	0	2
CAZ (30 µg)		3	0	0	1	0	0	23	5	4	0	2	12	2	0	0	0	0	2	0	0	2
ATM (30 µg)		3	0	0	1	0	0	26	3	3	2	6	6	2	0	0	0	0	2	2	0	0
NA (30 µg)		0	0	3	0	0	1	0	0	32	0	0	14	0	0	2	0	0	2	0	0	2
TE (30 µg)		3	0	0	1	0	0	2	0	30	0	0	14	2	0	0	0	0	2	0	0	2

S is susceptible, I is intermediate resistance and R is resistant. Antimicrobials: AK (amikacin), CIP (ciprofloxacin), AMP (ampicillin), AMC (amoxicillin-clavulanic acid), TZP (piperacillin-tazobactam), CTX (cefotaxime), CAZ (ceftazidime), ATM (aztreonam), NA (nalidixic acid), and TE (tetracycline).

Unfortunately, *Salmonella* Kentucky isolates exhibited high rates of resistance against the majority of the used antimicrobials, where 100% (14 of 14) were resistant to ciprofloxacin, ampicillin, nalidixic acid and tetracycline; moreover, 85.7% (12 of 14) showed resistance against both of cefotaxime and ceftazidime. However, few of them were found susceptible to some antimicrobials such as amoxicillin-clavulanic acid, piperacillin-tazobactam, cefotaxime and aztreonam, while 10 out of 14 (71.4%) isolates of *Salmonella* Kentucky were susceptible to amikacin. Concerning *Salmonella* Enteritidis, it appeared to be susceptible to all antimicrobials except nalidixic acid. Other serotypes of *Salmonella* detected in the present study showed moderate rates of resistance and susceptibility to the used antimicrobials as outlined in Table 4.

As regard to the human isolate, it was found that *Salmonella* Infantis isolated from human stool showed similar pattern to that of chicken isolates, as it exhibited a complete resistance against each of ciprofloxacin, nalidixic acid, cefotaxime, amikacin and tetracycline, and intermediate resistance against piperacillin-tazobactam, while it was susceptible to others.

Regarding the multiple antibiotic resistance (MAR) index of *Salmonella* serotypes (data not shown), the obtained results revealed that *Salmonella* Kentucky had the highest values, where 4 isolates got one MAR, 4 isolates got 0.8 MAR and 4 isolates got 0.6 MAR, followed by *Salmonella* Kottbus where the two isolates got one MAR. Then they were followed by *Salmonella* Infantis where two isolates got 0.7 MAR. The lowest values of MAR were determined in *Salmonella* Enteritidis with 0.1 MAR for the three isolates. In this concern, Butaye et al. (2006) reported that *Salmonella* isolates that are resistant to four or more separate classes of antimicrobials were defined as multidrug resistant; several researchers have attributed that resistance to different antimicrobial agents to a large plasmid they have.

The high rates of antimicrobial resistance of *Salmonella* spp. recorded in the current study are not surprising, as similar observations were reported by Ammari et al. (2009) in Morocco, Gharieb et al. (2015) in Egypt (except for ciprofloxacin, which showed high efficacy in their study), and Soomro et al. (2010) in Pakistan.

These results could be attributed to the fact that these antibiotics of low efficiency are cheap, easily affordable and frequently used for humans and poultry without medical prescription, so it could be used with incorrect doses. In poultry, these antimicrobials are used either for therapeutic purposes or as growth promoting feed additives, which lead to the development of resistance in the enteric microflora of poultry. Consequently, the pathogenic microorganisms such as *Salmonella* acquire resistance from this microflora and transfer it to the human strains through food chain, which leads to the appearance of multidrug resistant *Salmonellae* that constitute a public health hazard and potentially affect the efficacy of medications in humans. These findings are parallel to that reported by Gharieb et al. (2015) and Tollefson et al. (1997).

4. Conclusion

From the obtained results in the present study, it could be concluded that the bad hygienic measures adopted in the retail poultry markets in Beni-Suef (Egypt) during slaughtering, scalding, defeathering, evisceration and handling contributed in the high prevalence of *Salmonella* in poultry carcasses. In addition, the lack of veterinary supervision inside the markets could lead to slaughtering of diseased birds. *Salmonella* Infantis that was isolated from both broilers carcasses and human stools provides evidence that poultry meat constitutes a public health risk to consumers. The existence of multiple antibiotic resistances by several strains in this study, as a result of misuse of these antibiotics without medical prescription, potentiates the public health danger of salmonellosis and threatens the efficiency of medications in human. Further studies on the genetic characterization of *Salmonella* isolates from chicken and human samples in Beni-Suef are recommended.

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