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Public Health Importance of Entero-Toxigenic and Multi-Drug Resistant Salmonella Serotypes from Poultry Meat in Egypt

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Abstract: This work was carried out to study the occurrence of salmonella infections in poultry shops and farms in Egypt. A total of 349 fresh poultry meat samples were collected. All samples were subjected to laboratory examination under a septic conditions and bacteriological examination of samples revealed that 13 (3.7%) were positive for Salmonella species. The isolates preliminary identified biochemically as Salmonella were subjected to serological identification (*Salmonella infantis, Salmonella typhi, Salmonella kentucky, Salmonella rubislaw, Salmonella poona, Salmonella typhimurium, Salmonella virginia, Salmonella enteritidis and Salmonella montevideo*) and also were tested for their antibiotic susceptibility by agar disc diffusion method and the results showed that 3 isolates were multidrug resistance. Also, genotyping showed that 3 isolates confirmed to be enterotoxigenic strains contain salmonella enterotoxin gene (*stn*). This concluded that under cooked poultry meat might pose a public health problem for humans and special attention must be paid to resistance to antibiotics that are used exclusively in poultry farms and appropriate measures must be taken to control the spread of resistant bacteria to humans also, Molecular techniques remain the most sensitive method in detecting salmonella enterotoxin.

Key words: Salmonella species · Enterotoxin · Resistance · *stn* gene

INTRODUCTION

Poultry meat is still the more popular in the consumer market because the easy digestibility and acceptance by the majority of people [1].

Salmonellosis is considered one of the anthropozoonotic diseases of a serious medical problem and raises great concern in the food industry. Poultry is the most potential source of salmonella food poisoning in man [2] and become infected with many different types of salmonella; about 10 percent of all salmonella species have been detected in poultry. The most important are Salmonella *Typhimurium* and Salmonella *Enteritidis*.

Salmonella infection in poultry generally causes no clinical symptoms, but nevertheless it can cause severe disease. This microorganism can pass through food chain from feed to poultry and finally human causing salmonellosis [3]

Salmonella enteritidis (SE) prevalence in chickens and the human population rose abruptly during the 1980s and quickly became pandemic. There is evidence that SE became endemic in the parental breeder flocks which lead to a rapid spread of the infection to most parts of the world [4].

Non-typhoidal salmonella species is one of leading causes of foodborne illness, with an estimated 1 million cases of foodborne salmonella infection annually in the U.S from 2000 to 2008 [5].

Salmonella infections are usually acquired by ingestion of contaminated food or water. In systemic (typhoid-like) disease, following ingestion, the bacteria survive the acid pH of the stomach, colonize the Peyer's patches of the intestine and penetrate the gut barrier via Mcells (specialized epithelial cells). From there, they disseminate to the local mesenteric lymph nodes and then to the spleen and liver via phagocytic cells [6].

Salmonella induced diarrhea is a complex phenomenon involving several pathogenic mechanisms including production of enterotoxin. This enterotoxin production is mediated by the *stn* gene [7].

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The use of antimicrobials can be effective for controlling salmonella infection in poultry however; this practice may aggravate the risk of antibiotic residues in eggs and tissues or increase the emergence of multiple antibiotic-resistant bacteria [8].

Salmonellae are widespread in humans and animals worldwide. In industrialized countries, non-typhoid salmonellae are an important cause of bacterial gastroenteritis. In the Netherlands, the estimated incidence of salmonellosis is 3 cases per 1000 inhabitants per year [9]. In the United States, salmonella is estimated to cause. 1.4 million Illnesses and 600 deaths annually [10].

So, the present work is initiated to determine the occurrence of salmonella infection in poultry, study the sensitivity of the isolated organism to different types of antibiotics and detection of enterotoxigenic gene *stn* by using polymerase chain reaction (PCR).

MATERIALS AND METHODS

Collection of Samples: A total number of 349 fresh poultry meat samples collected from some poultry farms and poultry shops from different localities in Egypt.

Isolation and Identification of Salmonellae: Samples collected for salmonella isolation were taken to the laboratory on the day of collection, were pre-enriched in sterile buffered peptone water (Oxoid) and incubated at 37°C for 18 h. then enriched in Rappaport-Vassiliadis broth (RV) (Oxoid). The RV broth was incubated for 24 h at 42°C and 0.1 ml of the RV broth samples were streaked onto Xylose Lysine Desoxycholate agar (XLD). Colonies suspected of being salmonella subculture onto nutrient agar plates and tentatively identified according to morphological features, gram staining and biochemical characters. Serotyping of the isolates of salmonella species by biochemical identification (RAPID ONE Panel -Oxoid - remel USA) and by serological identification of somatic (O) and flagellar (H) antigens with commercial antisera according to the Kauffman - White serotyping scheme [11].

Antibiotic Sensitivity Test: The isolated salmonella species were tested for their *In vitro* antimicrobial susceptibility using the disk diffusion technique on Mueller-Hinton agar. The results were recorded after 24 h of incubation at 37°C. The zone of inhibition of each antibiotic disc was recorded and interpreted referring to zone diameter interpretive standards of [12]. The following antibiotics were tested in this study including qAmoxycillin+clavulanic acid (*AMC*), Azithromycin (*AZM*) Chloramphenicol (*C*), Colistin sulphate (*CT*), Doxycycline (*DO*), Enrofloxacin (*ENR*), Erythromycin (*E*), Flumequin (*UB*), Ofloxacin (*OFX*), Oxytetracyclin (*OT*), Rifampicin (*RD*), Spectinomycin (*SH*) and Vancomycine (*VA*).

DNA Extraction: A rapid boiling procedure was used to prepare template DNA from bacterial strains according to [13]. Two to 5 loops of salmonella isolates taken from the nutrient agar plate were collected and suspended in 200 µl of RNA DNA free water. After boiling for 10 min, the suspension was centrifuged for 2 min. to sediment bacterial debris. The supernatant was aspirated and from which 5 µl was used directly for PCR amplification. A strain of *Salmonella enterica* serovar Typhimurium (stn+) was used as positive control for *stn* gene and *Escherichia coli* were used as negative controls.

Primers: Primers used for PCR amplification were synthesized in Bio Basic Inc. (Canada). Details of primer sequences, their specific targets and amplicon sizes according to [14] were *Stn* P1 5 - TTG TGT CGC TAT CAC TGG CAA CC - 3 (forward primer) and *Stn* M13 5 - ATT CGT AAC CCG CTC TCG TCC - 3 (revers primer).

Polymerase Chain Reaction (PCR): A PCR assay targeting *stn* gene (salmonella enterotoxin gene) was developed and used in our study. The PCR mixture (25 µl) included 12.5 µl master mix (QIAGEN, USA) containing 2.5 U Taq DNA polymerase, 20µM each of dATP, dCTP, dTTP and dGTP and PCR buffer, 5 µl (1µM) each of upper and lower primers and 2.5µl of template DNA (bacterial cell suspension). PCR incubation was performed in a thermocycler (Perkin-Elmer, USA) in 25 cycles of denaturation (94°C for 1min), primer annealing (59°C for 1 min) and primer extension (72°C for 1 min) followed by incubation at 72°C for 10 min. Amplification products were electrophoresed in 1.5% agarose gel containing 0.5X TBE at 70 volts for 60 min. and visualized under ultraviolet light. To assure that the amplification products were of the expected size, a 100 bp DNA ladder was run simultaneously as a DNA marker. Amplification of 617 bp bands indicated the isolate to be enterotoxigenic salmonella strain.

RESULTS AND DISCUSSION

Salmonella infection is one of the most serious problems that affect poultry industry causing high economic losses not only due to high mortality in young chickens but also for the debilitating effect which predisposes for many other diseases [15].

Epidemiological reports suggest that poultry meat is still the primary cause of human food poisoning [1].

Salmonellosis is an important socioeconomic problem in several countries, mainly in developing countries, where this etiological agent is reported as the main responsible for foodborne disease outbreaks [16].

In the present study, the results in Table (1) show that the incidence of salmonella in poultry meat was 3.7% (13out of 349 chickens). This is in agreement with the results obtained by [17] who found that 2.5% of chicken meat was positive for salmonella. However, [18] reported that salmonella present in (62.5%) in examined chickens with higher incidence. The difference in the incidence rates may be due to socioeconomic factors. Also the results show that 3 out of 13 salmonella isolates carrying the *stn* gene (23%). This heat-labile salmonella enterotoxin (*stn*) serves as effector proteins, which are involved in the pathogenesis of salmonellosis [19].

This confirmed that poultry and poultry products are the major sources of salmonella contaminated food products that cause human salmonellosis [20] and salmonella is one of the most abundant causative agents of gastroenteritis in humans, with poultry meat being the main sources of this pathogen [21].

Infections with Salmonella in poultry meat may be due to contamination during its production, handling, packing and storage. These diseases have been associated with significant economic losses to poultry producers [22].

Zoonotic *Salmonella enterica* serovars are among the most important agents of food – borne infections throughout the world. Poultry, pigs and cattle rank as the major sources of salmonella contaminated food products that cause human salmonellosis [23].

In Table (2) *S. Infantis* and *S. Kentucky* showing resistance to the majority of antibiotics tested in this study. However *S. Infantis* sensitive to *AMC*, *ENR* and *OFX* while *S. Kentucky* sensitive to *AZM*, *C*, *CT* and *OT*.

While the result of the antibiotics sensitivity tests of *S.* Poona and *S.* Montevideo revealed that sensitivity to all antibiotics shown in table (2) but resist to *AMC*, *AZM*, *OT* and *RD* for *S.* Poona and *E* for *S.* Montevideo.

While *S. typhi*, *S. typhimurium*, *S. virginia*, *S. enteritidis*, *S. rubislaw* and *S. santiago* shown sensitivity to all antibiotic tested in this study.

In this concern, Chaslus-Dancla & Martel [24] recommended that the fluoroquinolones are drug of choice for treatment of invasive salmonellae and some antibiotics namely enrofloxacin, danofloxacin and marbofloxacin are also specifically approved for therapeutic veterinary use. Furthermore, [25] concluded that *S. typhimurium* isolates were susceptible to chloramphenicol, tetracycline and quinolones.

At slaughter, resistant strains from the gut readily soil poultry carcasses and as a result poultry meats are often contaminated with multi resistant salmonella. Hence, resistant fecal salmonella from poultry can infect humans both directly and via food. These resistant bacteria may colonize the human intestinal tract and may also contribute resistance genes to human endogenous flora [26].

The addition of broad spectrum antibiotic to poultry feed has raised the question of whether such sub therapeutic doses might select resistant salmonella strains which then can be transmitted to humans. Antimicrobial resistance genes are often located on transmissible plasmids in *Escherichia coli* and other normal flora; antibiotic pressures can promote the transfer of these plasmids to other enteric pathogens such as *Salmonella* spp. [27].

Salmonella species with high level of drug resistance have been continuously reported and the exact location and changes of genes associated with a mutation of these species have been searched [28].

Multidrug resistant salmonella serovars cause septicemic salmonellosis more frequently than those that are not resistant [29, 30].

Most salmonella infection is limited to uncomplicated gastroenteritis that seldom requires antimicrobial treatment [31]. In fact, antimicrobial treatment does not reduce the duration or severity of gastroenteritis and instead may result in prolonged fecal excretion and emergence of resistant strains which increase the risk of development of complications of extra-intestinal salmonellosis such as meningitis, septic arthritis and osteomyelitis [32]. All salmonella can cause extra-intestinal infections, but *S. Typhi, S. Paratyphi, S. Choleraesuis* and *S. Dublin* are the major serotypes which cause invasive salmonellosis [33]. The other serotypes are associated with a relatively low proportion of invasive infections and in the view of the

Table 1: Occurrence of Salmonella species recovered from fresh poultry meat samples

Type of samples	Number of samples	Salmonella serotypes			Stn gene		
Fresh poultry meat	349	Serotypes	No	(%)*	Serotypes	No	(%)*
		S. typhi	2	15.38	*S. poona*	1	7.6
		S. poona	2	15.38	*S. virginia*	1	7.6
		S. typhimurim	2	7.6	*S. infantis*	1	7.6
		S. virginia	1	7.6			
		S. entritidis	1	7.6			
		S. montevideo	1	7.6			
		S. kentucky	1	7.6			
		S. rubislaw	1	7.6			
		S. santiago	1	7.6			
		S. ifantis	1	7.6			

(%)* related to positive number of salmonella isolates.

Table 2: Result of antibiotic sensitivity test of 13 salmonella species isolates.

No.	Antimicrobial agents	*S. infantis*	*S. typhi*	*S. kentucky*	*S. poona*	*S. typhimurium*	*S. virginia*	*S. entritidis*	*S. montevideo*	*S. rubislaw*	*S. santiago*
1	*AMC*	S	S	R	R	S	S	S	S	S	S
2	*AZM*	R	S	S	R	S	S	S	S	S	S
3	*C*	R	S	S	S	S	S	S	S	S	S
4	*CT*	R	S	S	S	S	S	S	S	S	S
5	*DO*	R	S	R	S	S	S	S	S	S	S
6	*ENR*	S	S	R	S	S	S	S	S	S	S
7	*E*	R	S	R	S	S	S	S	R	S	S
8	*UB*	R	S	R	S	S	S	S	S	S	S
9	*OFX*	S	S	R	S	S	S	S	S	S	S
10	*OT*	R	S	S	R	S	S	S	S	S	S
11	*RD*	R	S	R	R	S	S	S	S	S	S
12	*SH*	R	S	R	S	S	S	S	S	S	S
13	*VA*	R	S	R	S	S	S	S	S	S	S

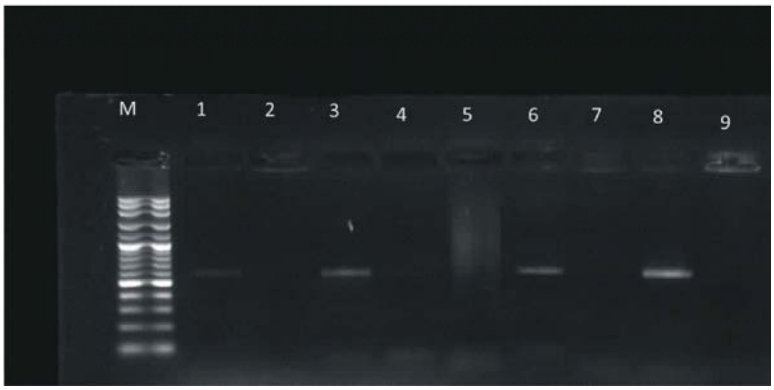

Fig. 1: Detection of *stn* gene by polymerase chain reaction (PCR). Lane M - DNA Ladder; Lane 1, 3 and 6 -Test isolates (*S. poona, S. virginia* and *S. infantis* respectively); Lane 8- Positive control (*S. typhimurium*); Lane 9- Negative control (*E. coli*)

data illustrated in Table 1 and 2 it was clear that both *S. Poona* and *S. infantis* were multi-drug resistant carrying enterotoxin gene this current results support the report of [31] who concluded that the total number of invasive cases caused by these serotypes appears to be high, because they are relatively prevalent among the whole salmonella population.

In conclusion, salmonellosis is one of the most important reportable diseases. Molecular-biological methods and the determination of resistances to antibiotics play an important role in the elucidation of epidemiological questions. The antibiotics resistance found in the present study can be explained by the spread use of antibiotics agents given to poultry in Egypt as prophylaxis, growth promoters or treatment.

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