PREVALENCE OF SOME ANTIBIOTIC RESISTANT STRAINS OF *SALMONELLAE* IN COMMERCIAL TABLE EGGS IN PORT-SAID CITY.

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ABSTRACT

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Multi-drug resistant *Salmonella* strains have emerged, presumably due to the extensive use of antimicrobial agents both in human and animals. In veterinary practice, antibiotics are used in livestock production, disease prevention and as growth-promoting feed additives. In the present study, the occurrence of *Salmonella* in local chicken eggs and their pattern of antibiotic resistance were determined. One hundred and forty chicken egg samples - collected from different locations of Port-Said City - were analyzed and *Salmonella* spp. detected in 3.6% of the samples. Among all presumptive *Salmonella* isolates, the isolates were confirmed as *Salmonella* Typhimurium (40%), *Salmonella agona* (20%) and *Salmonella derby* (40%) on the basis of serotyping and biochemical analysis. These isolates were subjected to susceptibility test against 9 antimicrobial disks. All the isolates were sensitive (100%) to doxycycline hydrochloride, chloramphenicol and trimethoprim-sulphamethoxazole. The highest percentage of resistance (100%) was found to erythromycin. Resistance against, streptomycin (20%), and penicillin G. (40%) was also found. One isolate of the *Salmonella* Typhimurium serovars (50%) showed resistance for streptomycin, nalidixic acid, oxytetracycline and penicillin G., while the other *Salmonella* typhimurium isolate (50%) showed high susceptibility for the same 4 antimicrobial drugs. Nearly similar results were shown for the isolated *Salmonella derby*, were one isolate (50%) showed high susceptibility for neomycin and oxytetracycline, while it was resistant to penicillin G. The other *Salmonella derby* isolate (50%) showed intermediate susceptibility for neomycin and oxytetracycline, while it was highly sensitive for penicillin G. *Salmonella* isolated from egg shell surfaces had more antimicrobial resistance than that of the egg contents. The present study suggested that, poultry eggs are potential reservoir of antibiotic resistant *Salmonellae*. The public health hazard of this microorganism, as well as recommended measures to improve quality status of table eggs were discussed.

تواجد بعض عترات السالمونيلا المقاومة للمضادات الحيوية في بيض المائدة التجاري في مدينة بورسعيد

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يعتبر البيض من أهم العناصر الغذائية التي تدخل في العديد من أصناف الطعام مثل الحلويات. ولما كان البيض النيئ يستخدم في تحضير بعض أصناف الطعام مثل المايونيز، المارنج والأيس كريم وبذلك قد يؤدي إلي العدوى بميكروب السالمونيلا في بعض الأحيان، خاصة وأن معظم الدراسات تشير أن هناك ارتباط وثيق بين تناول البيض النيئ والغير مطهو جيدا والإصابة بذلك الميكروب. لذلك تم تجميع ١٤٠ عينة من بيض المائدة المعد للتسويق بمدينة بورسعيد بهدف عزل وتصنيف عترات السالمونيلا المقاومة للمضادات الحيوية التي تظهر نتيجة للاستخدام العشوائي للمضادات الحيوية والوصول لأفضل مضاد حيوي للتخلص من ميكروب السالمونيلا مما يشكل استخدام آمن للبيض النيئ الذي يدخل في العديد من الأطعمة وما له من أثر علي الصحة العامة. وقد أفضت النتائج إلي أن نسبة العينات الايجابية لبكتيريا السالمونيلا كانت 3.6% وتم تصنيف عترات السالمونيلا المعزولة من بيض المائدة سيرولوجيا وقد أوضحت النتيجة بأنها سالمونيلا تيفيميوريم (٤٠%)، سالمونيلا أجونا (٢٠%) وسالمونيلا ديربي (٤٠%). أما بالنسبة لنتائج اختبار الحساسية، فقد تبين أن كل العترات في غاية الحساسية لكل من دوكسي سيكلين هيدروكلورايد، كلورامفينكول وترايميثوبريم سالفميثاكسازول. بينما أظهرت مقاومة عالية للإيريثروميسن. وقد كان ٥٠% من معزولات سالمونيلا تيفيميوريم مقاومة لكل من ستربتوميسين، ناليدكسيك أسيد، أوكسي تتراسيكلين وبنسيلين-ج على العكس من ذلك، كان باقي معزولات سالمونيلا تيفيميوريم حساسة لهذه المضادات الحيوية. كذلك كانت سالمونيلا ديربي حساسة لكل من نيومايسين وأوكسي تتراسيكلين على العكس من ذلك، كان باقي معزولات سالمونيلا ديربي متوسطة الحساسية لهذه المضادات الحيوية. هذا وقد تم مناقشة الأهمية الصحية وكذلك الاحتياطات التي يجب مراعاتها للحد من خطورة ميكروب السالمونيلا.

Key words: *table chicken eggs,* ***Salmonella*** *species, antibiotic resistance, public health hazard.*

INTRODUCTION

Table eggs are one of the most economic and balanced source of protein, with a relatively lower cost than chicken or meat (per kg) in most countries. In addition, eggs contain unsaturated fatty acids, iron, phosphorus, trace minerals, and vitamins (Watkins, 1995). Due to its exceptional nutritive value, eggs remain a potential host for pathogens like *Salmonella* spp. Gast and Beard (1992) suggested that, outbreaks of human salmonellosis, related to consumption of eggs, occurred as a consequence of three independent events; (i) contamination of eggs with *Salmonella* spp. by infected hens, (ii) improper handling of eggs or products allowing proliferation of the microorganisms to infectious levels, and (iii) ingestion of raw or undercooked contaminated eggs. It is apparent from the voluminous reports that *Salmonella* spp. were more oftenly associated with food borne disease outbreaks than other pathogens particularly those associated with egg and egg products (Shirota et al., 2001). Unlike other pathogens, *Salmonella* serovars can infect the egg before it is laid or may contaminate the shell during the laying process (Humphrey, 1999). More than 90% of food borne salmonellosis, were due to contaminated egg shells (Woodward *et al.,* 1997). Food borne *Salmonellae* were estimated to cause 1.3 million illnesses, 15,000 hospitalizations and 500 deaths per year (Schroeder *et al.,* 2005).

Generally, *Salmonella* spp. are the causative agent of human gastroenteritis, an infection that results in a clinical syndrome generally known as salmonellosis which may manifested by severe abdominal pain, non-bloody diarrhea, myalgia, chills, nausea, headache, fever, vomiting, and prostration. In addition, other medical conditions such as pericarditis, neurological and neuromuscular diseases and reactive arthritis may result in some individuals after the infection (D' Aoust, 1989). Clinical signs occur 12-72 h

after consumption of *Salmonella* spp. the microorganism multiplies and colonizes the small intestine, producing an enterotoxin that causes inflammatory reaction and diarrhea, and in some cases it can invade the blood stream to cause more severe illness (Poppe, 1999; D' Aoust, 2001 and Bell and Kyriakides, 2002). Duration of gastroenteritis syndrome generally varied from 4 -10 days; during this period, microbial invasion of the small intestine and colon could affect absorption of nutrients in the patient (Poppe, 1999). Moreover, susceptibility of humans to salmonella infections depend on a series of factors that include the dose of the pathogen, the type of contaminated food, and the age and immune condition of the host (D' Aoust, 1989 and Poppe, 1999). The newborn, elderly, and immune compromised patients are more susceptible to infection by *Salmonella* spp. In these risk groups, salmonellosis could result in serious systemic infections with sporadic cases of death (D' Aoust, 2001). On the other hand, healthy individuals rarely die from salmonellosis, and they normally recover from the disease after treatment with fluid and electrolyte replacement, while antibiotic therapy is not usually recommended in developing countries (Bell and Kyriakides, 2002). Eggs are one of the major animal foods that mostly marketed raw and frequently consumed raw. Many of the dishes like caesar salad, mayonnaise, eggnog, mousse and home-made ice cream, which form an important part of meals, contain raw eggs as an essential ingredient. These dishes are not heated up to the Food and Drug Administration recommended temperatures of 155°C for at least 15 seconds (Mermelstein, 2001).

Salmonellae are gram negative, non-spore forming rod-shaped bacteria belonging to the family *Enterobacteriaceae*. However, *Salmonella* is not included in the group of organisms referred to as coliforms. *Salmonella* is one of the principal causes of foodborne gastroenteritis worldwide and is also an important zoonotic pathogen of livestock (Scallan *et al.,* 2011). Salmonella food poisoning is one of the most common and widely distributed diseases in the world (Gomez *et al.,* 1997). Outbreaks were usually associated with ingestion of contaminated food of animal origin like, poultry, meat and milk (Swartz, 2002). Although the majority of infections resulting in asymptomatic or self-limited disease; however, in immuno-compromised patients, neonates and elderly, it requires antibiotic treatment (Van *et al.,* 2007). Recently, multi-drug resistant (MDR) strains have emerged, presumably due to the extensive use of antimicrobial agents both in human and animals. In veterinary practice, antibiotics are used in livestock production, disease prevention and as growth-promoting feed additives (Swartz, 2002). The use of antibiotics in animals disrupts the normal flora of intestine, resulting in the emergence of antibiotic-resistant salmonellae and their prolonged faecal shedding into the environment (Threlfall, 2002). The fatality rate in people infected with antibiotic-resistant salmonellae is 21 times greater than that infected with non-antibiotic resistant *Salmonella* strains (Altekruse *et al.,* 1999).

Egg-associated salmonellosis is a public health problem. *Salmonellae* infect ovaries of healthy hens and contaminate eggs before shell is formed and illness sometimes occurs, if such eggs are consumed raw or undercooked. The present study aimed for isolation and identification of different species of *Salmonellae* in chicken eggs of Port-Said City in addition to the resistance pattern to different antibiotics.

MATERIALS and METHODS

1-Collection of samples:

A total of 140 samples (70 egg shells and 70 egg contents) were collected from different markets in Port-Said City**.** Each individual sample was placed separately into sterile plastic bag, identified and delivered to laboratory in a refrigerated container. All specimens were processed within 24 hours of collection.

2- Bacteriological examination:

2-1 Preparation and enrichment of the samples:

Enrichment of egg samples was done according to Official Methods of Analysis of AOAC International (2005). Egg shells were washed with 10ml sterile Ringer's solution. Both egg shells and Egg contents (yolk and white) 10 milliliter were enriched in 90ml Buffered peptone water and homogenized in a stomacher for two min. and incubated at 37°C ± 1°C for 18±2 h. After incubation 0.1 ml was inoculated into tube containing sterile 10 ml Rappaport-Vassiliadis Soy broth (RVS), and 1 ml of the same culture was inoculated into a tube containing 10 ml sterile Muller-Kauffmann tetrathionate/ novobiocin broth (MKTTn). The RVS broth was incubated at 41.5 °C ± 1°C for 24h±3h, and MKTTn broth at 37°C ± 1°C for 24h±3h**.** Isolation and identification was done according to the method recommended by ISO (2002).

2-2 Serotyping of *Salmonella* isolates:

The antigenic formula of *Salmonella* serotypes are defined and maintained by the World Health Organization.

3-Antimicrobial susceptibility test:

The antimicrobial susceptibility test was performed using agar disc diffusion assay as described by National Committee for Clinical and Laboratory Standards Institute (NCCLS, 2004). Antimicrobials used were streptomycin 10 µg, neomycin 30 µg, penicillin 10 U, chloramphenicol 30 µg, erythromycin 15 µg, nalidixic acid 30 µg, oxytetracycline 30 µg, trimethoprim/sulphamethoxazole (1.25+23.75***)*** and doxycycline hydrochloride 30 µg. Pure colonies of isolated *Salmonellae* were emulsified in normal saline and turbidity was matched with 0.5 McFarland turbidity standards. Selected antimicrobial discs were placed on Mueller Hinton Agar plates seeded with bacteria. These plates were incubated at 37°C for 24 hours. The organisms were observed for antimicrobial sensitivity based on diameters of zones of inhibition on petridishes. Susceptible and resistant isolates were defined according to the criteria suggested by the NCCLS (2004).

RESULTS

Table 1: Prevalence of different *Salmonella* serovars isolated from chicken commercial table eggs:-

Type of sample	No.	*Salmonella* positive		Isolated serovars
		No.	%	
Egg-shells	70	3	4.3%	*Salmonella* Typhimurium
				Salmonella agona
				Salmonella derby
Egg -contents	70	2	2.9%	*Salmonella* Typhimurium
				Salmonella derby
Total	140	5	3.6%	

Table 2: Prevalence of different *Salmonella* serovars in the total isolates (5):-

Salmonella serovars	*Salmonella* isolates	
	No.	%
Salmonella Typhimurium	2	40%
Salmonella agona	1	20%
Salmonella derby	2	40%
Total	5	100%

Table 3: Antigenic structure of different *Salmonellae* isolated from chicken commercial table eggs:-

Salmonella serovars	Sero-group	Antigenic structure		
		[O]	[H]	
			Phase (1)	Phase(2)
Salmonella Typhimurium	B	1,4,[5],12	i	1,2
Salmonella Typhimurium	B	1,4,[5],12	i	1,2
Salmonella agona	B	1,4,12	f,g,s	-
Salmonella derby	B	1,4,[5],12	f,g	[1,2]
Salmonella derby	B	1,4,[5],12	f,g	[1,2]

Table 4: Antibiotic resistance pattern of *Salmonellae* (5) isolated from chicken commercial table eggs:-

Salmonella serovars	Antibiotic agent								
	streptomycin	doxycycline hvdrochloride	nalidixic acid	chloramphenicol	erythromycin	neomycin	oxytetracycline	penicillin G	trimethoprim-sulphamethoxazole
Salmonella Typhimurium	S	S	S	S	R	S	S	S	S
Salmonella Typhimurium	R	S	R	S	R	S	R	R	S
Salmonella agona	S	S	S	S	R	I	I	S	S
Salmonella derby	S	S	S	S	R	I	I	S	S
Salmonella derby	S	S	S	S	R	S	S	R	S

S= susceptibility, I= Intermediate, R= Resistant

Table 5: Antibiotic resistance pattern of different *Salmonella* isolates from chicken commercial table eggs:-

Antibiotic agent	Disc potency	No. of ***Salmonella*** isolates					
		Sensitive		Intermediate		Resistant	
		No.	%	No.	%	No.	%
streptomycin "S"	(10mg)	4	80%	-	-	1	20%
doxycycline hydrochloride "Do"	(30mg)	5	100	-	-	-	-
nalidixic acid "NA"	(30mg)	4	80%	-	-	1	20%
chloramphenicol "C"	(30mg)	5	100	-	-	-	-
erythromycin "E"	(15mg)	-	-	-	-	5	100%
neomycin "N"	(30mg)	3	60%	2	40%	-	-
oxytetracycline "OT"	(30mg)	2	40%	2	40%	1	20%
penicillin G "P"	(10 units)	3	60%	-	-	2	40%
trimethoprim-sulphamethoxazole "SXT"	(1.25+23.75)	5	100%	-	-	-	-

DISCUSSION

Little attention has been given to antimicrobial resistance of bacteria isolated from commercial shell eggs or the egg-processing environment. *Salmonella* may colonize the ovaries and peri-ovarian tissue of laying hens, and thus it has the potential for vertical transmission from breeders to layers and then to eggs sold for human consumption. The results of examination of 140 commercial chicken eggs showed that, *Salmonella* spp. was observed in (3.6%), (Table1).

Serological identification of the isolated *Salmonella* serovars obtained from commercial chicken table eggs, (Table 2), revealed the identification of *Salmonella* Typhimurium (40%), *Salmonella agona* (20%) and *Salmonella derby* (40%). The antigenic structures of the isolated serovars were shown in Table (3). Akhtar *et al.* (2010) isolated *Salmonella* Typhimurium in a percentage of (14.58%) from Egg-shell, while Selvaraj *et al.* (2010) could detect *Salmonella* Typhimurium in a percentage of (5.88%) from egg wash. The difference in the percentage for isolation of *Salmonella* Typhimurium may attributed to difference in localities and methods of sampling.

Salmonella antibiogram results were summarized in Tables (5) & (6). Highest resistance was observed for erythromycin (100%) then penicillin G. (40%). Resistance was also noted for streptomycin (20%), oxytetracycline (20%), and nalidixic acid (20%). Akhtar *et al.* (2010) demonstrated very high level of resistance to erythromycin by *Salmonella enteritidis*. *Salmonella* resistance at varying concentrations of penicillin, streptomycin, spectinomycin and erythromycin has also been reported by Sultana *et al*. (1995). High susceptibility was shown to chloramphenicol, doxycycline hydrochloride and trimethoprim-sulphamethoxazole by all isolated serovars. One isolate of the *Salmonella* Typhimurium serovars (50%) showed resistance for streptomycin, nalidixic acid, oxytetracycline and penicillin G., while the other *Salmonella* Typhimurium isolates (50%) showed high susceptibility for the same 4 antimicrobial drugs. Nearly similar results were shown for the isolated *Salmonella derby*, one isolate (50%) showed high susceptibility for neomycin and oxytetracycline, while it was resistant to penicillin G. The other *Salmonella derby* isolates (50%) showed intermediate susceptibility for neomycin and oxytetracycline, while it was highly sensitive for penicillin G. This difference - for the same serovar - may be due to the empirical use of different antimicrobial drugs during the chicken production. Few studies have reported on antimicrobial resistance of *Salmonella* isolates collected from eggs or the egg-processing environment. In a study conducted in India, Bajaj *et al.* (2003) indicated that all of the 66 *Salmonella* isolates were susceptible to at least one of the compounds tested however, they did not report on the serotypes of *Salmonellae.* Verma and Gupta (1992) demonstrated the susceptibility of various *Salmonella* serovars to several antimicrobial drugs and reported high resistance to kanamycin, followed by trimethoprim-sulphamethoxazole and tetracycline. High susceptibility was shown to chloramphenicol, ampicillin and tetracycline.

CONCLUSION

This study focused on the indirect danger of the empirical use of antimicrobial agents in poultry industry, which leads to the release of resistant *Salmonella* serovars to the environment thus, creating a major health hazard problem. It also proved the importance of periodical antibiotic sensitivity tests for the *Salmonella* serovars from chicken to find the proper antibiotic which could be used in treatment of salmonellosis.

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