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Zoonotic Importance of Salmonellosis in Chickens and Humans at Qualyobia Province

Lobna M.A. Salem[*], Maysa A.I. Awadallah[**], Nashw O.K[*]. and Marwa O.A. Salem[*]

[*]Department of Zoonosis, Faculty of Veterinary Medicine, Benha University, Egypt. [**]Department of Zoonosis, Faculty of Veterinary Medicine, Zagazig University, Egypt.

THIS study aimed to isolate and identify *Salmonella* spp. From human and chickens by using culture method, serotyping, antimicrobial sensitivity test and detection of virulence genes (*inv*A, *avr*A, *bcf*C, *stn*). A total of 245 samples and swabs including (36 cloacal swabs, 17 intestinal contents, 60 chicken product[luncheon, nuggets , kofta, pane, 15 of each], 45 chicken meat[breast, thigh, wing, 15 of each], 20 hand swabs , 27 stool (diarrheic and non-diarrheic) and 40 serum) from humans for Widal test. The results revealed that 31 samples and swabs were positive to *Salmonella*, 6 isolates were isolated from broilers cloacal swabs, 7 isolates were isolated from chicken meat (4 in thigh samples and 3 in breast samples and swabs), 2 isolates were isolated from chicken products (one isolate in kofta sample and one isolates in pane swabs), 3 isolates were isolated from intestinal swabs, 4 isolates were isolated from hand swabs from workers in farms and poultry shops and 4 isolates were isolated from stool of diarrheic and non-diarrheic persons while 5 isolates were isolated from serum. Serotyping revealed 9 strains of *S. Gallinarum*and *S.* subspp. *Salamae* mainly in cloacal swabs, while *S. Typhimurium*, *S. Enteritidis*, *S. Kentucky*, *S.Tsevie*, *S. Colindale*, *S. Papauna* and *S. Lagos* were isolated from chicken meat, its products, hand swab, intestinal swabs and stool. *S. Typhi* and *Paratyphi* A, B were isolated from serum of patients by Widal test. Nine strains were tested against 6 commercial antibiotics and revealed that all strains were sensitive to levofloxacin and amikacin while were resistant to erythromycin by 100% and ampicillin, tetracycline and cefexime shown viability in sensitivity. Widal test revealed *S. Typhi* and *Paratyphi* A, B by a titer of 1:320 for O, H antigens in *S. Typhi*, *Paratyphi* A and B. The study detected *inv* A, *avr* A, *bcf* C, *stn* genes in 11 *S.Enteritidis* and 3 *S. Typhimurium* that isolated from human and poultry and RAPD PCR revealed relationships in *S. Enteritidis* that isolated from human and poultry.

Keywords: Salmonellosis, Chickens, Humans, Zoonotic importance.

Salmonellosis is one of the most prevalent disease and major source of food-borne infections to humans as consumption of poultry products is worldwide in distribution (Marcus *et al.,* 2007). *Salmonellae* are isolated more often from

poultry and poultry products than from any other food animals (Braden, 2006). Chickens can be infected with many different serovars of paratyphoid *Salmonella.* Among these paratyphoid salmonellae, infections due to *S. Typhimurium, S. Enteritidis* and *S. Heidelberg,* are of worldwide in distribution with wide host range and are of major economic and public health significance (Yanfen *et al.,* 2010). Salmonella is mostly transmitted to humans, through contaminated food and water. In hospitals, person to person transmission may also happen. Among veterinarians and farm workers, transmission may occur by contact with infected animals. Cross contamination of poultry can occur in slaughter houses as well as during preparation of poultry products (Olsen *et al.*, 2003). Most of the salmonella serotypes are pathogenic to humans and the common symptoms of Salmonellosis in human are abdominal pain, diarrhea, nausea, vomiting, muscle pain, prostration, drowsiness and fever. Symptoms may be varied due to variation in the dose of inoculation, mechanisms of pathogenicity, virulence factors, age and immune response of the host (Andino and Hanning, 2015).Salmonellosis is more prevalent in developing parts of the world in Africa, Asia, and South America. South Asia are at highest risk for infections that are nalidixic acid-resistant or multidrug-resistant (*i.e.,* resistant to ampicillin, chloramphenicol, and trimethoprim - sulfamethoxazole). In humans, *Salmonellosis* causes two kinds of fever. Enteric fever which can be typhoid or paratyphoid and gastroenteritis which is non-typhoidal fever. Typhoid fever is an acute, life-threatening febrile illness caused by *S. typhi* and *paratyphi*, and there are estimated 20 million cases and 200,000 deaths worldwide each year (Crump *et al.,* 2004). The epidemiology and pathogeneses of *Salmonellosis* are dictated by any array of factors that act in tandem and ultimately manifest in the typical symptoms of *Salmonellosis* virulence genes encode products that assist the organism in expressing its virulence in the host cells. Nucleic acid based techniques are being employed for the detection of various gene-encoded virulence factors *viz*, *inv* A and *avr* A genes that associated with *Salmonella* pathogenicity islands (SPIs), the fimbrial related gene *bcf* C and *stn* involved in enterotoxin production, the distribution of these genes among various isolates obtained from biological sources is yet to be elucidated (Muthu *et al.,* 2014).

Material and Methods

A total of 245 samples and swabs that included 36 cloacal swabs, 17 intestinal contents, 45 chicken meat{breast, thigh, wing, 15 of each}, 60 chicken products {luncheon, nuggets, kofta, pane, 15 of each}, 20 hand swabs, 27 stool (diarrheic and non- diarrheic) and 40 serum from humans for Widal test. Samples and swabs were collected from farm, poultry shops, supermarkets, hospital and private labs in Qualyobia governorate.

Methods for isolation and identification of Salmonella Species

- The procedures for isolation of Salmonella from previously mentioned samples were done according to procedures of ISO 6579 (2002). All samples were incubated at 37 ± 1 ºC for 18 ± 2 hrs for pre-enrichment. For enrichment, 0.1 ml of each pre-enriched sample was transferred to 10 ml of Rappaport Vasiliadis and incubated at 41.5±0.5ºC for 24±3 hrs. One loopful of each enriched broth was streaked aseptically onto Xylose Lysine Deoxycholate agar and incubated at 37±1ºC for 24±3 hrs.

- Biochemical identification was done according to the procedures as described by Murray (2003).
- Serotyping was done according to Kauffmann white Scheme (Kauffman, 1974).
- The antimicrobial sensitivity phenotypes of Salmonella were determined by agar disc diffusion method (Finegold *et al.,* 1982).
- Widal test was done on human sera as described by Felix, 1944.
- Molecular identification of *stn*, *bcf* C, *avr* A and *inv* Avirulence associated genes in 11 *S. enteritidis* and 3 *S.typhimurium* isolates from different sources was done according to QIAamp DNAmini kit instructions. (Catalogue no. 51304). The QIAamp DNA Mini Kit provides silica-membrane-based nucleic acid purification from different types of samples. The spin-column procedure does not require mechanical homogenization, so total hands-on preparation time is only 20 minutes and RAPD-PCR for nine *S. enteritidis* described by Hunter and Gaston (1990).

Results

Occurrence and serotyping of Salmonella isolates from chickens and humans:

Highest rate of *Salmonella* isolated from hand swabs, intestinal swabs, cloacal swabs, chicken meat, stool then chicken products as in Table 1.

Seroprevalence of Salmonella infection in human

Anti-*Salmonella* antibodies were recorded in 5 out of 40 serum samples examined (12.5%). *S.typhi, S.paratyphi* A represented by 100% from positive Widal while 60% paratyphi B as in Table 2.

Sensitivity of Salmonella serotypes to antibiotics.

All *Salmonella* strains were sensitive to levofloxacin and amikacin (100%), while all isolates were resistant to erythromycin (100%). In contrast, ampicillin had the basic effect on viability of Salmonella strains followed by cefexime and tetracycline. *S.kentucky* isolated from chicken meat showed 100% resistance to erythromycin and ampicillin as in Table 3.

TABLE 1. Occurrence and serotyping of *Salmonella* isolates from chickens and humans.

Samples	No. of examined samples	Positive		Serovars	Isolates		Type
		No.	%		No.	%*	
Cloacal swabs	36	6	16.66	*S. gallinarum*	3	50	Swab
				S. tsevie	1	16.6	Swab
				S. typhimurium	1	16.6	Swab
				S. subspp. *salamae*	1	16.6	Swab
Chicken meat	45	7	15.5				
Breast	15	3	20	*S. papauna*	1	14.28	Sample, swab
				S. colindale	1	14.28	Sample, swab
				S. enteritidis	1	14.28	Swab
Thigh	15	4	26.6	*S. enteritidis*	3	42.85	Sample
				S. kentucky	1	14.28	Sample
Wing	15	0	0				
Chicken products	60	2	3.33				
Luncheon	15	0	0				
Nuggets	15	0	0				
Kofta	15	1	6.66	*S. Enteritidis*	1	25	Sample
Pane	15	1	6.66	*S. Enteritidis*	1	25	Swabs
Intestinal swabs	17	3	17.64	*S. Kentucky*	1	33.3	Swab
				S. papauna	1	33.3	Swab
				S. enteritidis	1	33.3	Swab
Hand swabs	20	4	20	*S. papauna*	1	25	Swab
				S. lagos	1	25	Swab
				S. tsevie	1	25	Swab
				S. enteritidis	1	25	Swab
Stool	27	4	13.8				
Diarrheic	17	3	17.6	*S. kentucky*	1	25	Swab
				S. typhimurium	2	50	Swab
Non-diarrheic	12	1	8.33	*S. enteritidis*	1	25	Swab

TABLE 2. Occurrence and percentage of *S.Typhi* and *Paratyphi* A,B in human serum.

Samples	No. of examined samples	Positive		Isolates					
		No.	%	*S.typhi*		*S. paratyphi A*		*S. paratyphi B*	
				No.	% *	No.	%*	No.	%*
Serum	40	5	12.5	5	100	3	60	5	100

5 positive samples were *S. typhi* and *S. paratyphi* B (100%) and 3(60%) of them were mixed with *S. paratyphi* A.

TABLE 3. Sensitivity of *Salmonella* serotypes to antibiotics.

Types and concentration of Antibiotics	Antibiotic sensitivity		
	S	I	R
Ampicillin (10 mg)	6 (66.6 %)	1 (11 %)	2 (22 %)
Tetracycline (30)	5 (55.5 %)	4 (44 %)	-
Erythromycin (15)	-	-	9 (100 %)
Levofloxacin (5 mg)	9 (100 %)	-	-
Amikacin (30)	9 (100 %)	-	-
Cefexime (5 mg)	3 (33 %)	5 (55.5 %)	1 (11 %)

S: sensitive R: resistant I: intermediate

Distribution of virulence genes in Salmonella enteritidis and typhimurium

The occurrence values of the investigated virulence associated genes (*inv*A, *avr*A, *bcf*C *and stn)* were 100% in the examined S. *typhimurium* and *S. enteritidis* isolates as in Table 4 and photos 1, 2, 3, 4.

TABLE 4.Distribution of virulence genes in *Salmonellaenteritidis* and *typhimurium*.

***Salmonella*s erovars**	**Source**	**No. of examined isolates**	**Virulence gene**			
			***inv*A No (%)**	***avr*A No (%)**	***bcf*C No (%)**	***stn* No (%)**
Salmonella Enteritidis	3 samples thigh 1swab breast 1sampleskofta 3 swabs pane 1 hand swab 1 intestinal swab 1 stool (diarrheic)	9	9(100%)	9(100%)	9(100%)	9(100%)
Salmonella Typhimurium	2 stools (non-diarrheic) 1 cloacal swab	3	3(100%)	3(100%)	3(100%)	3(100%)
Total		12	12(100%)	12(100%)	12(100%)	12(100%)

All examined *Salmonella enteritidis* and *Salmonella typhimurium* showed positive amplification of 284bp fragment specific for the *inv*A gene as in photo 1.

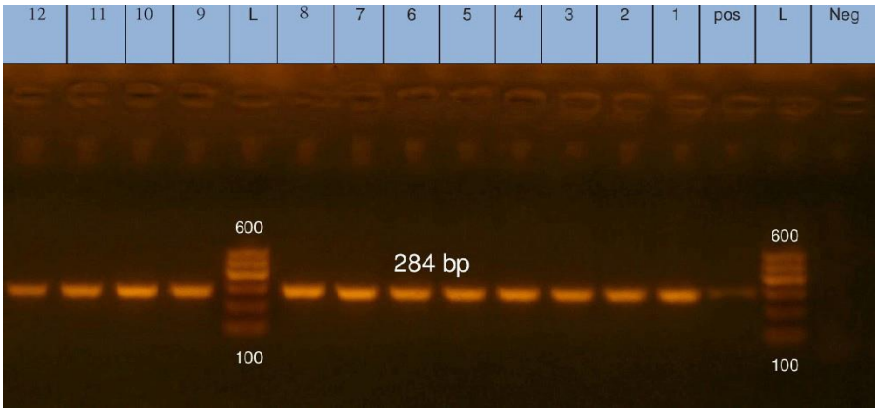

Photo1. Agarose gel electrophoresis showing amplified bands of *Salmonella* isolates using primer set for the *inv*A (284bp) gene in human and poultry samples and swabs.

-Negative control-DNA leader 100-600bp
-Positive control-Lanes1-9*S. enteritidis*
-Lanes10-12*S. typhimurium*

All examined *Salmonella enteritidis* and *Salmonella typhimurium* showed positive amplification of 422bp fragment specific for the ***avr*A**gene as in photo 2.

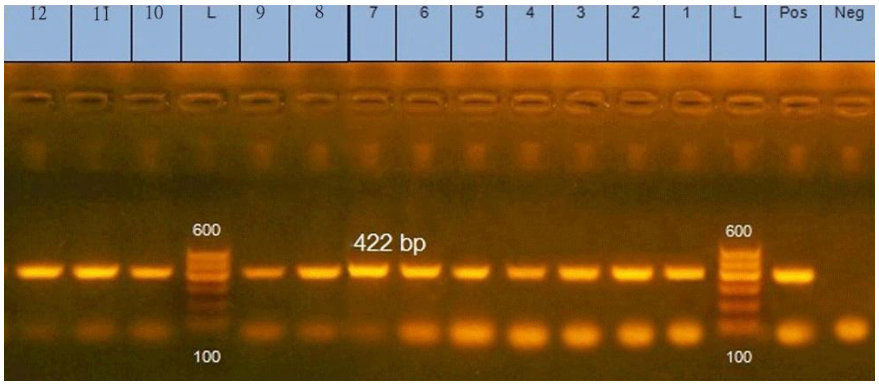

Photo 2. Agarose gel electrophoresis showing amplified bands of *Salmonella* isolates using primer set for the *avr*A (422bp) gene in human and poultry samples and swabs.

-Negative control-Positive control - DNA leader 100-600bp -Lanes 1-9*S. enteritidis* -Lanes10-12*S. typhimurium*

All examined *Salmonella enteritidis* and *Salmonella typhimurium* showed positive amplification of 467bp fragment specific for the *bcf*C gene as in photo 3.

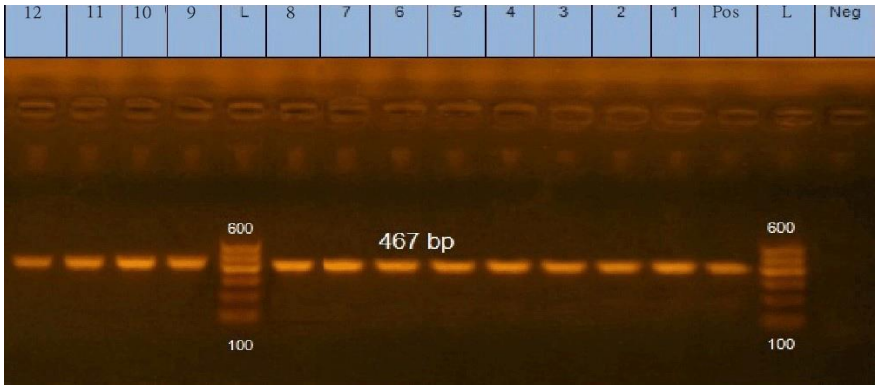

Photo 3. Agarose gel electrophoresis showing amplified bands of *Salmonella* isolates using primer set for the *bcf*C (467bp) gene in human and poultry samples and swabs.

-Negative control-DNA leader 100-600bp
-Positive control - Lanes1-9*S. enteritidis*
- Lanes10-12*S. typhimurium*

All examined *Salmonella enteritidis* and *Salmonella typhimurium* showed positive amplification of 617bp fragment specific for the *stn gene* as in photo 4.

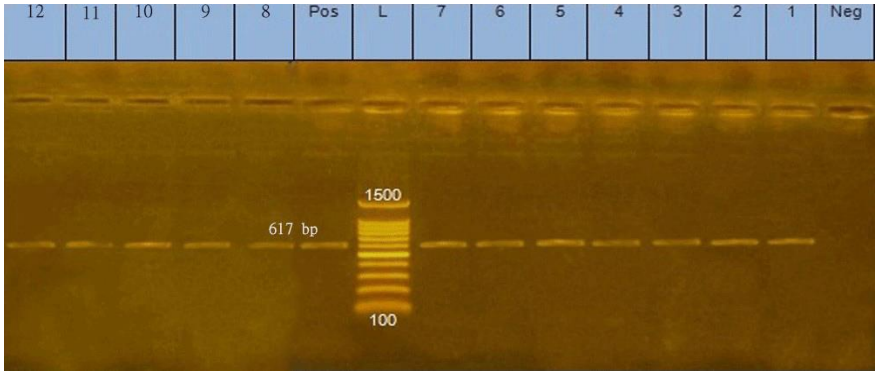

Photo 4. Agarose gel electrophoresis showing amplified bands of *Salmonella* isolates using primer set for the *stn* (617bp) gene in human and poultry samples and swabs.

-Negative control -DNA leader 100-600bp -Positive control

-Lanes1-9*S. enteritidis* -Lanes 10-12*S. typhimurium*

RAPD-PCR profiles and associated clusters

The RAPD-PCR patterns of 9 *S.enteritidis* isolates from different sources were investigated using a single amplification profile. The profiles were discriminated by the number and position of the amplified fragments. Visual comparison of the banding patterns revealed multiple DNA fragments ranged in size between 2300 bp and 700 bp (Photo5). The primer sets produced 6 profiles (referred to as E1 to E6) as in Table 5. The discriminatory power of the RAPD-PCR was calculated by Simpson's index of diversity and *D* value was 0.9 indicating high discriminatory power. The dendrogram analysis of the examined isolates showed two clusters and one separate isolates (Fig.1). Cluster I contained isolates from kofta, intestinal content, breast swab and thigh while cluster II contained isolates from thigh, swab pane, hand swab and a single isolate from stool.

TABLE 5. RAPD-PCR profiles and associated clusters.

Profile	Number of isolates	Sources (Isolate code)	Cluster
E1	2	(sample kofta) S5	I
		(intestinal content) S6	
E2	1	(swab breast) S1	
E3	1	(sample thigh) S8	
E4	3	(sample thigh) S3	II
		(sample pane) S4	
		(hand swab) S2	
E5	1	(sample thigh) S7	
E6	1	(stool) S9	Single isolate

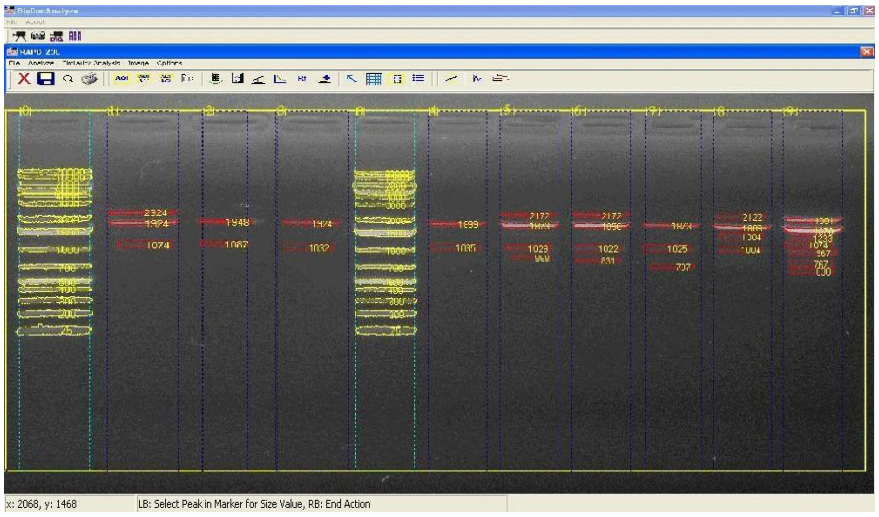

Photo 5. Amplification of nine RAPD products by *Salmonella enteritidis* isolates.

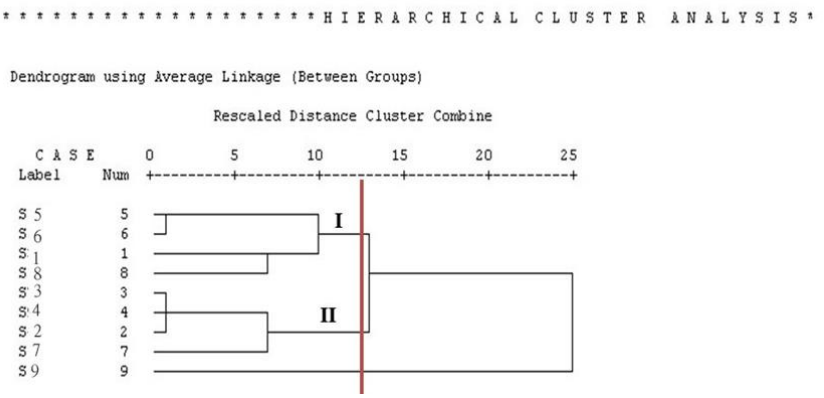

Fig. 1. Dendrogram among nine isolates of *Salmonella enteritidis*generated through RAPD data using UPGMA method.

Discussion

The isolation rate of *Salmonella* from broilers' cloacal swabs was 6(16.66%). This agrees with those reported by Ibrahim *et al.* (2013), who isolated *Salmonella* from broilers with a percentage of 16.66% for each. However, these findings are lower than those reported by Ramya *et al.* (2012),and higher than those reported by Parvej *et al.*(2016). Salmonella species were detected in the intestinal contents of 3 slaughtered chickens (17.64%). The identified isolates were serotyped as; *S. kentucky*, *S.papauna*, *S.enteritidis* (with a percentage of 33.3% for each). Phagoo and Neetoo (2015) isolated *Salmonella* from 11% from the intestinal contents. The isolation rate of *Salmonella* from chicken meat in this study was (15.5%) that is nearly similar to those recorded by Saad *et al.* (2011), his result was (16%) in chicken meat. *Salmonella* species were isolated from 2 (3.33%) of examined chicken products including one isolate (25 %) from kofta and pane, but not isolated from luncheon and nuggets. Serotyping revealed that all isolates were *S.enteritidis*. Lower isolation rate of *Salmonella* from meat product was previously recorded by Saad *et al.* (2011) whose result was 3.75%. In contrary, Samar (2015) isolated *Salmonella* from 32% of pane samples. Moreover, Mohamed (2013) isolated *Salmonella* from kofta samples with rate of 40%. Salmonella species in hand swabs from workers in poultry shops and farms was 20% (4 isolates). They were identified serologically as *S.papauna*, *S.enteritidis, S.lagos, S.tsevie* (one isolate for each, 25%).This result was higher than that reported by Ibrahim *et al.* (2013) who found *Salmonella* in examined hand swabs with percentage of 8.88%. *Salmonella* species were isolated from human stools with 13.79 % (4 isolates) out of them 3 isolates (17.64%) were

from non-diarrheic patients and one isolate (8.3 %) was from diarrheic patients. This result was lower than those reported by Nader *et al.* (2015), his result was 10%. *Salmonella* species were detected in stool of non-diarrheic persons indicated that persons with asymptomatic infection which act as chronic carriers and source of infection to external environment. All strains were sensitive to levofloxacin and amikacin (100%), while all isolates were resistant to erythromycin (100%). In contrast, ampicillin had the basic effect on viability of *Salmonella* strains followed by cefexime and tetracycline. *S.kentucky* isolated from chicken meat showed 100% resistance to erythromycin and ampicillin. This result agrees with that reported by Al-ferdous *et al.*(2013), who found that 16(100%) of *S.typhimurium* isolates were resistant to erythromycin, but nearly similar to that reported by Mir *et al.* (2015), who found that *S.enteritidis* , *S.typhimurium*and *S. gallinarum* show resistance to Ampicilline and Tetracycline with 68.75%, 65.62% respectively, and 75% sensitive to Levofloxacin. Anti-Salmonella antibodies were recorded in 5(12.5%) out of 40examined serum samples. *S.typhi, S. paratyphi* B represented by 100% from positive Widal while 60 % paratyphi A, 3 (7.5%) of *S. typhi* and *paratyphi*A,4 (10%) *S.paratyphi* B had a titer of 1:320 while 2 (5 %) for *S. typhi* and 1 (2.5%) for *S. paratyphi* B had a titer of 1:80. This result disagree with Oluyege *et al.*(2015), who found that out of 99 samples examined, 86 were tested for Widal test while the remaining 13 were cultured directly, 42 (48.8%) were positive Widal , detected *S.typhi*only 3 (3.5%) had a titer of 1:320 and 14 (16.3%) had a titer of 1 : 80 , *S. paratyphi* A only 3 (3.5%) had a titer of 1 : 320 and 15 (17.4%) had a titer of 1 :80 also *S. paratyphi* B only 3 (3.5%) had a titer of 1 :320 and 11 (12.8%) had a titer of 1:80.

The occurrence values of the investigated virulence associated genes (*inv*A, *avr*A, *bcf*C *and stn)* were 100% in the examined *S. typhimurium* and *S. enteritidis* isolates. Osman *et al.*(2014a) detected *inv*A and *bcf*Cin all *S. enteritidis* and *S. typhimurium* recovered from imported turkey poultry in Egypt. In Italy, all 13 *S. typhimurium* isolates from water buffalo calves with lethal enteritis displayed the presence of *invA* and *bcfC*genes (Boriello *et al.*, 2012). On the other hand, Karen *et al.* (2013) detected *inv*A and *avr*A genes in 100% of *S. enteritidis* isolates from poultry carcasses. However, *inv*Awas detected only in 47.3% of *S. enteritidis* and 50% of *S. typhimurium* isolates from animals and human in Egypt (Moussa *et al.*, 2013). Maysa and Abd-Elall (2015) in Sharkia province, Egypt detected *inv*A and *bcf*C in *S.typhimurium, S.enteritidis* and *S. new port* isolates from leafy greens, animals, human and waste water samples. The respective occurrence of *inv*A gene versus *bcf*C gene in the aforementioned salmonella isolates were (100% versus 88.9%), (100% versus 100%) and (50% versus 100%). Nwiyi *et al.* (2015) detected*inv*A gene in *S. enteritidis* isolated from chicken. The recorded high frequencies of *inv*A and *bcf* Cgenes in the present study confirm the previous results that little or no variation occurred for most genes incorporated in SPIs (*inv*A) and for the fimbrial markers (*bcf*C), thus these genes were present throughout most serotypes (Osman *et al.*, 2014b). The *inv*A is the *Salmonella* invasion gene which is essential for entry of bacteria into epithelial cells and is a putative inner membrane component of SPI-1 dependent

type III secretion system (TTSS-1) virulence apparatus (Hur *et al.,* 2011), whereas *bcf*Cis bovine colonization factor and fimbrial usher (Boriello *et al.,* 2012). The RAPD-PCR patterns of 9 *S. enteritidis* isolates from different sources were investigated using a single amplification profile. The profiles were discriminated by the number and position of the amplified fragments. Visual comparison of the banding patterns revealed multiple DNA fragments ranged in size between 2300 bp and 700 bp. The primer sets produced 6 profiles (referred to as E1 to E6). The discriminatory power of the RAPD-PCR was calculated by Simpson's index of diversity and *D* value was 0.9 indicating high discriminatory power. The dendrogram analysis of the examined isolates showed two clusters and one separate isolates. Cluster I contained isolates from sample kofta, intestinal content, swab breast, sample thigh, cluster II contained isolates from sample thigh, swab pane, hand swab and a single isolate from stool.

Conclusions

From this study, it could be concluded that *Salmonella* spp were highly prevalent in the examined chicken, chicken meat, chicken products, human stools and hand swabs Serotyping of recovered *Salmonella*, however, clarified predominance of *S. enteritidis*and *S. typhimurium* in examined sources, but other serovars were also encounter reflecting wide variance in the examined sources. Virulotyping of recovered *Salmonella* serovars verified widespread distribution of virulence associated genes among isolates and provided additional evidence on risk of virulent *salmonellosis* posed from chicken and their products for human.

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الاهمية الصحية لمرض السالمونيلا في الدجاج والانسان في محافظة القليوبية

لبني محمد علي سالم*، مايسة عبدالبديع ابراهيم، نشوة عثمان خليفة* و مروة عكاشة عبدالحليم سالم***

*قسم الامراض المشتركة – كلية الطب البيطري – جامعة بنها و **قسم الامراض المشتركة – كلية الطب البيطري – جامعة الزقازيق – مصر

اوضحت هذه الدراسة تواجد ميكروب السالمونيلا بنسبة عالية في الفراخ ولحومها ومنتجاتها و براز الاشخاص ومسحات من ايدي العمال. كما اوضحت التحاليل السيرولوجية ان السالمونيلا انتيرتييدس و السالمونيلا تيفيميريم هما العترات الاكثر شيوعا في العينات التي تم فحصها بالاضافة الي عترات اخري تم عزلها من نفس العينات. كما اوضح اختبار البلمرة الجزيئية وجود الجينات المسؤلة عن الضراوة في عترات السالمونيلا التي تم عزلها وانتقالها من الفراخ ومنتجاتها الي الانسان.