

## Prevalence and Molecular detection of some *virulence* genes among *Salmonella* species Isolated from Poultry

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### Abstract:

The current study aimed to isolate the *salmonella* species from different organs of broilers with complete phenotypic identification of the recovered isolates. After that all recovered isolates were tested for detection of some genes responsible for pathogenicity. A total of 250 samples from broilers farms were examined bacteriologically for isolation of *salmonella*. All recovered isolates were identified phenotypically, biochemically and serologically. PCR technique was used for detection of *invA*, *hilA* and *avrA* genes. Bacteriological examination of samples revealed that *Salmonella* was recovered in 34 samples out of 250 samples (13.6%). Twelve *S. Typhimurium*, 8 *S. Kentucky*, 5 *S. Newport*, 2 *S. Tamale*, 2 *S. Enteritidis*, 1 *S. Molade*, 2 *S. Takoradi*, 1 *S. Virchow* and 1 *S. Inganda* were isolated from broilers with percentage of (35.29%), (23.5%), (14.7%), (5.88%), (5.88%), (2.94%), (5.88%), (2.94%) and (2.94%) respectively. All tested *Salmonella* serovars showed positive amplification at 284 bp, 150 bp and 422 bp fragments which specific for the *invA* gene (common gene), *hilA* gene and *avrA* gene respectively, with total percentage (100%) from examined samples.

**Keywords:** *Salmonella* species, *Salmonella* virulence genes, broilers, PCR.

### Introduction:

Salmonellosis is an important health problem and a major challenge worldwide. *Salmonella* spp. are Gram negative and rod shape which

have been divided into over 2700 serotypes based on somatic, flagellar and capsular antigens (*Gallegos et al., 2008*). Many authors (*Bailey and Maurer, 2001*;

*Mølbak et al., 2006*) have outlined that bacteria of genus *Salmonella* are important causes of foodborne infections in humans, and the most frequent etiological bacterial agents of foodborne disease outbreaks. In particular, two *Salmonella* serotypes, *S. Enteritidis* and *S. Typhimurium* became major causes of human illness in the 1980s and 1990s, with important impact on public health and the economy in industrialized countries.

The genus *Salmonella* is divided into two species *Salmonella* Enterica and *Salmonella* Bongori; *Salmonella* Enterica itself is comprised of 6 subspecies. They are *S. Enterica subsp. Enterica*, *S. Arizonae*, *S. Diarizonae*, *S. Indica*, *S. Salamae* & *S. Houtenae or I, II, IIIa, IIIb, IV and VI*, respectively (*Popoff and Minor, 1997; Brenner, et al., 2000*).

*Salmonella* pathogenicity island (SPI) is a genetic element that occurs as a distinct and separate unit in the bacterial chromosome. There are approximately 200 genes including those on the five *Salmonella* chromosomal pathogenicity islands (SPI-1 to SPI-5) on *Salmonella* chromosomes that are essential for their virulence *Hensel (2004)*.

To fulfill the aim of the work, the following were done:

1- Isolation of *Salmonella* using global standard methods (from internal organs) in different poultry farms.

2- Identification of the isolated organism (biochemical and serological identification).

3- Detection of common *Salmonella* virulence genes using cPCR.

#### **Material and methods:**

**Samples:** A total of 250 chicken samples from broilers farms were collected for *Salmonella* isolation and these samples include liver, caecum, spleen and heart blood. All samples were put in sterile plastic bags in ice box and transported directly to Mansoura laboratory (Animal Health Research Institute).

**Identification of the isolates:** Each sample was inoculated separately in selenite F broth and incubated at 37°C for not more than 18 hours and rappaport-vassiliadis soya broth incubated at 42°C for 24 hours. A loopful from the enrichment culture was streaked onto the surface of Xylose Lysine Deoxycholate (XLD), *Salmonella* Shigella agar (S-S agar) and MacConkey's plates then incubated at 37°C ± 1°C for 24hrs ± 2hrs. MacConkey's plates were used to detect the presence of coliform lactose fermenters. Each colony was identified morphologically and biochemically according to *Quinn et al. (2002)*.

#### **Serological identification:**

The preliminarily identified isolates biochemically as *Salmonella* were subjected to serological identification according to Kauffman-White Scheme (*Kauffman, 1974*) for

determination of somatic (O) and flagellar (H) antigens using slide agglutination test.

**PCR detection of *Salmonella* isolates virulence genes:** Ten *Salmonella* serovars were examined for detection of *invA*, *hilA* and *avrA* genes. QIAamp DNA Mini Kit used for extraction of DNA. Primers used were shown in table (2). Temperature and time conditions of the primers during PCR are shown in table (3). Electrophoresis grade agarose (1 g) was prepared in 100 ml TBE buffer in a sterile flask, it was heated in microwave to dissolve all granules with agitation, and allowed to cool at 70°C, then 0.5µg/ml ethidium bromide was added and mixed thoroughly according to (*Sambrook et al., 1989*)

### Results:

A total of 250 chickens (70 apparently healthy, 90 diseased chickens and 90 freshly dead broiler chickens) were collected from different farms in Dakahlia Governorate and the clinically examined birds showed signs of septicemia, retarded growth, depression, profuse watery white diarrhea and accumulation of fecal matter around the vent. While the freshly dead birds show bronze discoloration and enlargement of liver, splenomegaly, inflammation of intestine and caecum and unabsorbed yolk sacs in young chicks. Bacteriological examination of samples all over seasons of the

year revealed that *salmonella* was recovered in 34 samples with an incidence rate 13.6 % (34 out of 250). It was clear that *Salmonella* was recovered from 4 samples out of 70 examined samples from apparently healthy chicken (5.7%), 18 samples out of 90 diseased chickens (20%) and 12 samples out of 90 freshly dead broiler chickens (13.33%) were found to be positive for *Salmonella* as shown in table (1). After culturing on XLD, *Salmonella* appeared as smooth red coloured colonies with black center. While on Hektole enteric it appeared as deep blue colonies but on MacConkey's agar appeared as pale, colorless smooth, transparent and raised colonies and on *Salmonella* Shigella (S-S) agar *Salmonella* produce colourless colonies with black centers due to H<sub>2</sub>S production. The staining characters appeared as Gram negative, non-spore forming short rod shaped. Serologically identified into twelve *S. Typhimurium*, 8 *S. Kentucky*, 5 *S. Newport*, 2 *S. Tamale*, 2 *S. Enteritidis*, 1 *S. Molade*, 2 *S. Takoradi*, 1 *S. Virchow* and 1 *S. Inganda* were isolated from broilers with percentage of (35.29%), (23.5%), (14.7%), (5.88%), (5.88%), (2.94%), (5.88%), (2.94%) and (2.94%) respectively.

All 10 *Salmonella* serovars showed positive amplification at 284 bp, 150 bp and 422 bp fragments which specific for the *invA* gene (common gene), *hilA* gene and *avrA* gene

respectively, with total percentage in figure 1, 2 and 3. (100%) from examined samples as

**Table (1)** Incidence of *Salmonella* infection in examined chickens

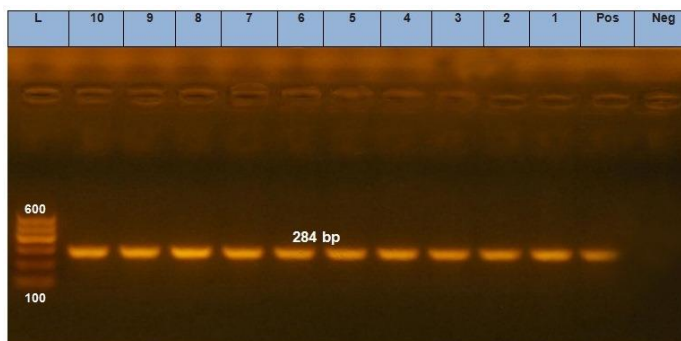
Examined chicken	Number of examined chicken	Number of positive	%
Apparently healthy chicken	70	4	5.7
Diseased chicken	90	18	20
Freshly dead chicken	90	12	13.33
<b>Total</b>	250	34	13.6

**Table (2):** Primers of some virulence genes.

Primer	Sequence (5-3)	Amplified product (size)	Reference
<i>invA</i>	GTGAAATTATCGCCACGTTCTGGGCAA	284 bp	Oliveira et al., 2003
	TCATCGCACCGTCAAAGGAACC		
<i>Hila</i>	CATGGCTGGTCAGTTGGAG	150 bp	Yang et al., 2014
	CGTAATTCATCGCCTAAACG		
<i>avrA</i>	CCT GTA TTG TTG AGC GTC TGG	422 bp	Huehn et al. 2010
	AGA AGA GCT TCG TTG AAT GTC C		

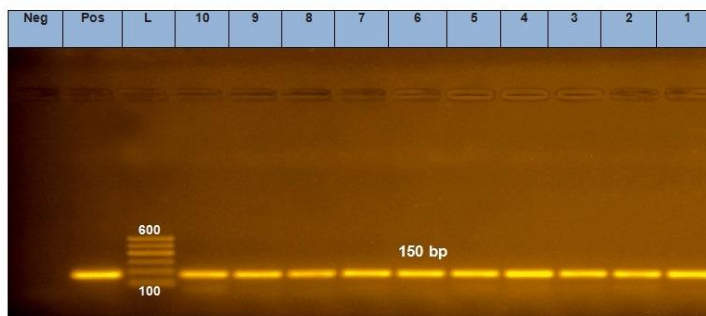
**Table (3):** Cycling conditions of the different primers during cPCR

Gene	Primary denaturation	Secondary denaturation	Annealing	Extension	No. of cycles	Final extension
<i>invA</i>	94°C 5 min.	94°C 30 sec.	55°C 30 sec	72°C 30 sec	35	72°C 7 min.
<i>hila</i>	94°C 5 min.	94°C 30 sec.	60°C 30 sec	72°C 30 sec	35	72°C 7 min.
<i>avrA</i>	94°C 5 min.	94°C 30 sec.	58°C 30 sec.	72°C 30 sec.	35	72°C 10 min.

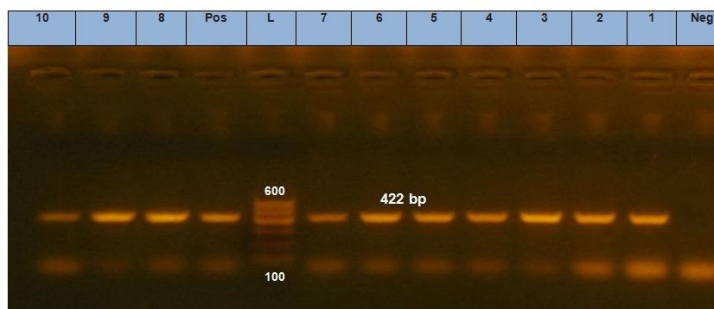


**Figure (1):** PCR photo showing *Salmonella invA* gene at 284 bp. Lane L: 100-600pb DNA ladder. Pos.: Positive control (reference strain from Animal

Health Research Institute, Dokki, Egypt). Neg.: Negative control. Lane 1: *S. Kentucky*. Lane 2: *S. Tamale*. Lane 3: *S. Inganda*. Lane 4 and 7: *S. Typhimurium*. Lane 5: *S. Newport*. Lane 6: *S. Molade*. Lane 8: *S. Enteritidis*. Lane 9: *S. Takoradi*. Lane 10: *S. Virchow*.



**Figure (2):** PCR photo showing *Salmonella hila* gene at 150 bp. Lane L: 100-600pb DNA ladder, Pos.: Positive control (reference strain from Animal Health Research Institute, Dokki, Egypt). Neg.: Negative control. Lane 1: *S. Kentucky*. Lane 2: *S. Tamale*. Lane 3: *S. Inganda*. Lane 4 and 7: *S. Typhimurium*. Lane 5: *S. Newport*. Lane 6: *S. Molade*. Lane 8: *S. Enteritidis*. Lane 9: *S. Takoradi*. Lane 10: *S. Virchow*.



**Figure (3):** PCR photo showing *Salmonella avrA* gene at 422 bp. Lane L: 100-600pb DNA ladder. Positive control (reference strain from Animal Health Research Institute, Dokki, Egypt). Neg.: Negative control. Lane 1: *S. Kentucky*. Lane 2: *S. Tamale*. Lane 3: *S. Inganda*. Lane 4 and 7: *S. Typhimurium*. Lane 5: *S. Newport*. Lane 6: *S. Molade*. Lane 8: *S. Enteritidis*. Lane 9: *S. Takoradi*. Lane 10: *S. Virchow*.

**Discussion:**

In this study 250 broiler chickens from different poultry farms in Dakahlia governorate were examined for the presence of *Salmonella*, 34 out of 250 chickens

(13.6%) were found positive while 216 (86.4%) chickens were negative for *Salmonella* isolation. The incidence of *Salmonella* in broilers was 13.6% (34 out of 250 chickens) and these results agree with

**Rehan (2004)** who isolated *Salmonella* species from broiler chickens with an incidence of 12%, **Ammar et al. (2010)** who identified the most common *Salmonella* serovars in broilers and laying breeding reproducers in Eastern Algeria and isolated *S. Typhimurium* with incidence of (12%). **Chon et al. (2015)** investigated the occurrence of *Salmonella* spp. isolated from 120 chicken carcasses produced in 6 poultry slaughterhouses in South Korea were 11 samples (9.2%). **Osman (1992)** isolated 45 *Salmonella* strains with an incidence of 30% out of 150 random samples from different broiler farms. On the other hand, lower incidence was recorded by **Magwedere et al. (2015)** was 5%. **Ibrahim et al. (2018)** discussed that the prevalence of *Salmonella* was 6.8% in broilers, 42.3% in layers, 0% in breeders and cloacal swabs, 6.5% in litter and 15.6% in eggs. In this study *Salmonella* were rapidly detected by targeting *invA* gene, giving PCR product of 284 bp size. Therefore, this technique can be used for the screening of *Salmonella* in the routine testing. **Darwin and Miller (1999)** reported that the *invA* gene encodes a protein in the inner membrane of bacteria, which is necessary for invasion to the epithelial cells of the host. **Lampelet al. (2000)** observed that *Salmonella* specific PCR with primers for *invA* is rapid, sensitive, and specific for detection of

*Salmonella* in many clinical samples. The genes *hilA*, *hilC*, *hilD* and *invF* suggests that these regulators operate in a hierarchy to modulate invasion gene expression with *hilA* acting as the central regulator in this cascade. The *hilA*, is required for expression of SPI-1 invasion genes (**Schechter and Lee, 2000**). The *invF* and its putative chaperone protein, *sicA* (along with *hilA*) is responsible for activating and controlling the expression of TTS-associated proteins encoded within and outside of SPI-1. PCR assay was carried out for the detection of the *avrA* gene from isolated strains has revealed that the gene was present in all of the isolates (100%) which was demonstrated by the presence of a 422 bp PCR product. The results obtained in the present study were in corroboration with **Karen et al., (2013)**; **Ren et al., (2016)**. Moreover, **Claudete et al. (2017)** detected the *avrA* gene in all of the *S. Pullorum* and *S. Gallinarum* serotype strains (100%) and 38 (97.4%) strains of the paratyphoid group were positive. *avrA* is an effector protein of the TTSS complex that contributes to the virulence of *Salmonella* spp. by limiting the host's inflammatory responses through the inducement of cell apoptosis, especially of macrophages, and by the inhibition of IL-8 and TNF- $\alpha$  (**Collier-Hyamset al., 2002**).

**Conclusion:**

From the previous result, concluded that widespread of *Salmonella* spp. among the broilers in Dakahlia Governorate in this study may be due to insufficient hygiene, improper disinfection in hatcheries and farms. Also, there are defect in farm biosecurity. Further studies are needed to improve surveillance strategies to decrease the prevalence of *Salmonella* spp. in chicken population of Dakahlia Governorate. PCR technique is rapid and a sensitive confirmatory method detection of *Salmonella*.

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مدى تواجد والكشف الجزيئي لبعض جينات الضراوة بين أنواع السالمونيلا المعزولة من الدواجن محمود عزت<sup>1</sup>, محمود السطوحى<sup>2</sup>, أبو الخير عيسوى<sup>2</sup>, علي وهدان<sup>1</sup>  
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تهدف الدراسة الحالية إلى عزل أنواع السالمونيلا من الأعضاء المختلفة من بدارى التسمين مع التعريف الظاهري الكامل للمعزولات. بعد ذلك تم اختبار جميع المعزولات المستعادة للكشف عن بعض الجينات المسؤولة عن المسببات المرضية. تم الفحص البكتريولوجي ل ٢٥٠ عينة من مزارع دواجن بدارى التسمين لعزل السالمونيلا. تم التعريف الظاهري، والبيوكيميائي والسيرولوجي لجميع المعزولات. تم استخدام تقنية تفاعل انزيم البلمرة المتسلسل للكشف عن جينات *hila* و *invA* و *avrA*. بالفحص البكتريولوجي للعينات أظهرت وجود السالمونيلا في ٣٤ عينة من أصل ٢٥٠ عينة (١٣,٦٪). و بإجراء التصنيف السيرولوجي لعترات السالمونيلا المعزولة من الدواجن تم تحديد الانواع المصلية التالية: سالمونيلا تيفيموريوم (١٢) بنسبة (٣٥,٩٪), سالمونيلا كنتاكي (٨) بنسبة (٢٣,٥٪), سالمونيلا نيوبورت (٥) بنسبة (١٤,٧٪), سالمونيلا تامالي (٢) بنسبة (٥,٨٨٪), سالمونيلا انتريتيدس (٢) بنسبة (٥,٨٨٪), سالمونيلا مولادي (١) بنسبة (٢,٩٤٪), سالمونيلا تاكورادي (٢) بنسبة (٥,٨٨٪), سالمونيلا اينجيدي (١) بنسبة (٢,٩٤٪) وسالمونيلا فيرشو (١) بنسبة (٢,٩٤٪). أظهرت جميع عترات السالمونيلا التي تم اختبارها للكشف عن وجود جينات *invA* و *hila* و *avrA* قد تبين تواجدهم بنسبة ١٠٠٪.