Bacteriological Studies on *Salmonella* Isolated from Balady Chicken Meat

Eid H. M.*, Helal I. M. and Radwa H. Kouta

*Bacteriology, Immunology and Mycology Department, Fac. Vet. Med. Suez Canal University, *Animal Health research institute - Port-Said branch, ARC.*

Abstract

Two hundred freshly slaughtered of balady chicken samples were collected aseptically from markets in Port-Said city during the period from September 2016 to September 2018. Nine *Salmonella* isolates (4.5%) were isolated and therefore serologically identified. The isolated Salmonellae were *Salmonella* Typhimurium 2/9 (22.2%) and *Salmonella* Muenster 2/9 (22.2%) which was the most frequent identified. Other serotypes as *Salmonella* Enteritidis 1/9 (11.1%), *Salmonella* Blegdam 1/9 (11.1%), *Salmonella* Anatum 1/9 (11.1%), *Salmonella* Lamberhurst 1/9 (11.1%) and *Salmonella* Ayinde 1/9 (11.1%) were also identified. The antibiogram revealed highly resistant to Erythromycin and Oxytetracycline by a percentage (100%) followed by Nalidixic acid (44.4%) while it was highly sensitive to Chloramphenicol and Ciprofloxacin by a percentage (100%) followed by Gentamycin (88.9%). Polymerase Chain Reaction detected the virulence genes (***stn***, ***sop*B**, ***spv*C** and ***bcf*C**) genes were positive in all tested *Salmonella* serotypes 7/7 (100%) while **(*avr*A)** gene was positive in 6/7 (85.7%) of tested *Salmonella* serotypes.

Key words: *Salmonella*- chicken- PCR- Antibiogram sensitivity- Port-Said**.**

Introduction

Salmonella is a cause of foodborne illness worldwide with an estimated annual economic loss about 3.7 billion dollars ***United States Department of Agriculture (2015)*.** Salmonellosis was considered as an important public health problem which causes high morbidity on poultry and human. The main clinical signs of salmonellosis in human are (typhoid) enteric fever and gastroenteritis so enteric fever is a systemic illness which results from *S.* Typhi and *S.* Paratyphi ***Pegues and Miller (2000)***. Over use of antimicrobial agents in poultry for prophylaxis, treatment purposes and growth promotion got a major antimicrobial resistance and so multidrug resistance, which are

observed among many serovars of *Salmonella* ***Duong et al. (2006).*** Polymerase Chain Reaction (PCR) is a method to investigate outbreaks of foodborne and identification of pathogens ***Riyaz et al. (2004)*** while PCR is high specific, give fast results and less time-consuming than cultural technique. PCR technique considered as a rapid diagnostic tool for detection of *Salmonella* in food.

Therefore, this work was observed for isolation and identification of *Salmonella* from chicken meat, serotyping, study antimicrobial sensitivity of *Salmonella* isolates and screens the presence of virulence genes (***stn***, ***sop*B**, ***spv*C**, ***bcf*C** and ***avr*A**) in the isolated serotypes by using PCR method.

Material and Methods

A total of 200 freshly slaughtered balady chicken samples collected aseptically from markets in Port-Said city since September 2016 to September 2018, the collected samples were from chicken breast and then subjected for bacteriological examination.

- **Isolation and identification of *Salmonella* isolates**

Twenty-five grams of the test samples were added aseptically to 225 ml buffered peptone water then incubated at 37°C for 18 hours after that enriched on Rappaport-Vassiliadis soya broth by incubation at 41.5°C / 24 hours**.** Enriched samples were streaked on Xylose lysine deoxycholate agar and Hekton enteric agar and incubated at 37 °C/ 24 hrs ***Oxoid (1998)***. Suspected colonies were purified on nutrient agar, biochemically identified by triple sugar iron agar, urea hydrolysis test, lysine decarboxylation test, indole production test, citrate utilization test and oxidase test ***Oxoid (1998)***. Auto-agglutination test was made for biochemically positive isolates, then serological confirmation by poly O and poly H antisera ***ISO 6579 (2002*)**.

- **Antimicrobial susceptibility testing**

By using disc diffusion technique ***Finegold and Martin (1982)*** and isolates were classified as sensitive, intermediate and resistant according to ***CLSI (2011).***

- **PCR of *Salmonella* serotypes**

For detection of the different virulence genes in *Salmonella* serotypes **(Table 1 and Table 2)** were performed according to ***Sambrook et al. (1989).***

Results And Discussion

The prevalence of *Salmonella* in chicken samples was 9/200 (4.5%) as shown in **Table (3)** which is nearly the same result with ***Adelino et al. (2018)*** who detected *Salmonella* in 3/850

(3.7%) in chicken samples from Brazil**.** The results on this study are higher than the reports of ***FSAI (2004)*** who detected 245 (3.2%) *Salmonella* isolates in Ireland from 7,616 raw poultry meats samples. On the other hand, the results on this study was lower than ***Dhary (2019)*** who surveyed the prevalence of *Salmonella* in retail outlets was 16/225 (7.1%) and ***Elkenany et al. (2019)*** who isolated *Salmonella* from chicken samples 50/170 (29.4%) in Egypt.

Variations in the prevalence of *Salmonella* from chicken meat in many studies could be due to the differences in type and number of samples, sensitivity of detection methods, time of sampling and storage conditions. *Salmonella* isolates were serotyped in this study into *Salmonella* Typhimurium 2/9 (22.2%) and *Salmonella* Muenster 2/9 (22.2%) which was the most frequent identified. Other serotypes as *Salmonella* Enteritidis 1/9 (11.1%), *Salmonella* Blegdam 1/9 (11.1%), *Salmonella* Anatum 1/9 (11.1%), *Salmonella* Lamberhurst 1/9 (11.1%) and *Salmonella* Ayinde 1/9 (11.1%) were also identified as shown in **Table (4).** These results nearly the same result with ***Hee et al. (2007)*** who detected *Salmonella* Typhimurium 15/64 (23.4%) which was the most common serotype in broiler chicken isolates. These results were higher than ***Narapati (2007)*** who isolated *Salmonella* Typhimurium 8/52 (15.38%). While it was lower than ***Chaiba et al. (2008)*** who isolated *Salmonella* Typhimurium (40.35%) from chicken samples at markets as the most frequent serotype isolated out of 57 *Salmonella* isolates in Morocco.

According to the results concerning antimicrobial susceptibility tests in **Table (5)**, *Salmonella* isolates showed high resistance to Erythromycin and Oxytetracycline by a percentage (100%) followed by Nalidixic acid (44.4%) while it was highly sensitive to Chloramphenicol and Ciprofloxacin by a percentage (100%) followed by Gentamycin (88.9%), Colistin (77.8%) and finally Ceftoxin and Trimethoprim+Sulfamethaxzole by a percentage (66.7%) for each which is in agreement with ***Martha et al.*** **(2006)** who observed all tested *Salmonella* Enteritidis strains showed resistance to Erythromycin and Tetracycline 80/80 (100%). These results were disagreed with ***Ulaya et al. (2012)*** who showed that, *Salmonella* Enteritidis revealed sensitivity to Amoxicillin (95.7%), Tetracycline (82.6%) and Gentamicin (17.4%).

In present study 7 *Salmonella* serotypes were examined by PCR for determination the virulence genes (***stn***, ***spv*C**, ***sop*B**, ***bcf*C** and ***avr*A**) as shown in **Table (6)**. We found ***stn*** and ***sop*B** genes were positive in all tested serotypes 7/7 (100%) as shown in **Figure (1)** which is parallel with ***Vivek et al. (2015)*** who observed that, *Salmonella* Typhimurium and *Salmonella* Enteritidis were positive in ***stn*** gene and ***Prager et al. (1995)*** who showed *S*. Typhimurium and *S*. Enteritidis were found to carry ***stn*** gene. ***spv*C** gene was positive in all tested serotypes 7/7 (100%) as shown in **Figure (2)** and ***bcf*C** gene was positive in all tested *Salmonella* isolates 7/7 (100%) as shown in **Figure (3)** while ***avr*A** present in 6/7 (85.7%) of the isolated *Salmonella* which is present in all *Salmonella* serotypes except *Salmonella* Lamberhurst as shown in **Figure (4)** and it is in agreement with ***Borges et al. (2013)*** who showed ***avr*A** gene present in 100% in *Salmonella* Enteritidis strains.

Table (1): *Oligonucleotide primers sequences:*

Primer	Sequence	Amplified product	Reference
stn	TTG TGT CGC TAT CAC TGG CAA CC	617 bp	***Murugkar et al. (2003)***
	ATT CGT AAC CCG CTC TCG TCC		
***avr*A**	CCT GTA TTG TTG AGC GTC TGG	422 bp	***Huehn et al. (2010)***
	AGA AGA GCT TCG TTG AAT GTC C		
***sop*B**	tca gaa gRc gtc taa cca ctc	517 bp	
	tac cgt cct cat gca cac tc		
***bcf*C**	acc aga gac att gcc ttc c	467 bp	
	ttc tgc tcg ccg cta ttc g		
***spv*C**	acc aga gac att gcc ttc c	467 bp	
	ttc tga tcg ccg cta ttc g		

Table (2): *Cycling conditions of the different primers for virulence genes during PCR:*

Gene	Primary denaturation	Secondary denaturation	Annealing	Extension	No. of cycles	Final extension
stn	94˚C 5 min.	94˚C 30 sec.	59˚C 40 sec.	72˚C 45 sec.	35	72˚C 10 min.
***avr*A**	94˚C 5 min.	94˚C 30 sec.	58˚C 40 sec.	72˚C 45 sec.	35	72˚C 10 min.
***sop*B**	94˚C 5 min.	94˚C 30 sec.	58˚C 40 sec.	72˚C 45 sec.	35	72˚C 10 min.
***bcf*C**	94˚C 5 min.	94˚C 30 sec.	53˚C 40 sec.	72˚C 45 sec.	35	72˚C 10 min.
***spv*C**	94˚C 5 min.	94˚C 30 sec.	58˚C 40 sec.	72˚C 45 sec.	35	72˚C 10 min.

Table (3) *Number and percentage of of Salmonella isolates:*

Total number of chicken samples (n)	*Salmonella* isolates	
	Number	%
200	9	4.5

Table (4): *Number and percentage of different serotypes of the isolated Salmonella from chicken samples n=9:*

Serotype	Number of isolates	% of *Salmonella* serotypes
***S.* Typhimurium**	2	22.2
***S.* Enteritidis**	1	11.1
***S.* Blegdam**	1	11.1
***S.* Anatum**	1	11.1
***S.* Muenster**	2	22.2
***S.* Lamberhurst**	1	11.1
***S.* Ayinde**	1	11.1
Total no.	9	100

Table (5): *Number and percentage of Salmonella serotypes exhibiting resistance and sensitivity to various antimicrobial agents (n=9):*

Antimicrobial agents	Sensitive *Salmonella* isolates		Intermediate		Resistant *Salmonella* isolates	
	No	%	No	%	No	%
Levofloxacine (5 µg)	5	55.6	1	11.1	3	33.3
Amoxicillin (10 µg)	2	22.2	4	44.4	3	33.3
Cefotaxim (30 µg)	6	66.7	1	11.1	2	22.2
Chloramphnicol(30µg)	9	100	0	0	0	0
Colistin (10 µg)	7	77.8	0	0	2	22.2
Erythromycin (15µg)	0	0	0	0	9	100
Gentamycin (10µg)	8	88.9	1	11.1	0	0
Nalidixic acid (30 µg)	5	55.6	0	0	4	44.4
Ciprofloxacin (5 µg)	9	100	0	0	0	0
Oxytetracycline (30 µg)	0	0	0	0	9	100
Trimethoprim+Sulfamethaxzole (1.25+23.75 µg)	6	66.7	1	11.1	2	22.2

Table (6): *Results of Polymerase Chain Reaction technique for different virulence genes of Salmonella serotypes:*

Sample	Results				
	stn	***spv*C**	***sop*B**	***bcf*C**	***avr*A**
***S.* Typhimurium**	+	+	+	+	+
***S.* Enteritidis**	+	+	+	+	+
***S.* Blegdam**	+	+	+	+	+
***S.* Anatum**	+	+	+	+	+
***S.* Muenster**	+	+	+	+	+
***S.* Lamberhurst**	+	+	+	+	-
***S.* Ayinde**	+	+	+	+	+

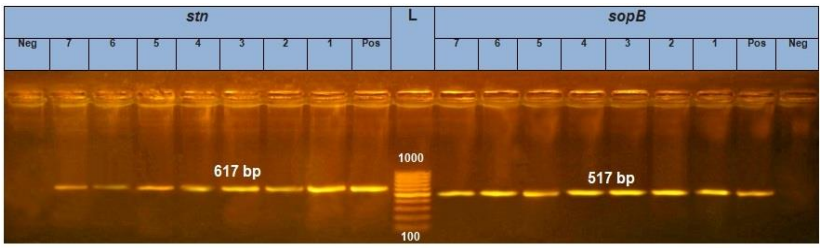

Figure (1): PCR of (*stn*) and (*sop*B) in *Salmonella* serotypes:
Primer set for genes (***stn*** and ***sop*B**) (617 bp and 517 bp) respectively. Neg= negative control*, Pos=positive control* and L= ladder (100-1000 bp). All lanes showed positive results: Lane (1): *S.* Typhimurium, Lane (2): *S.* Enteritidis, Lane (3): *S.* Blegdam, Lane (4): *S.* Anatum, Lane (5): *S.* Muenster, Lane (6): *S.* Lamberhurst and Lane (7): *S.* Ayinde.
*(Positive and or negative controls were represented by field sample that were previously confirmed to be positive or negative by PCR for the related genes in the Reference laboratory for veterinary quality control on poultry production, Animal health research institute).

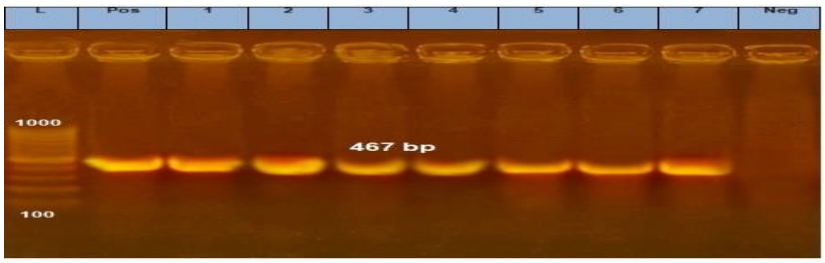

Figure (2): PCR of (*spv*C) in *Salmonella* serotypes:
Primer set for ***spv*C** gene (467 bp). Neg= negative control*, Pos=positive control* and L= ladder (100-1000 bp). All lanes showed positive results: Lane (1): *S.* Typhimurium, Lane (2): *S.* Enteritidis,

Lane (3): *S*. Blegdam, Lane (4): *S*. Anatum, Lane (5): *S*. Muenster, Lane (6): *S*. Lamberhurst and Lane (7): *S*. Ayinde.
*(Positive and or negative controls were represented by field sample that were previously confirmed to be positive or negative by PCR for the related genes in the Reference laboratory for veterinary quality control on poultry production, Animal health research institute).

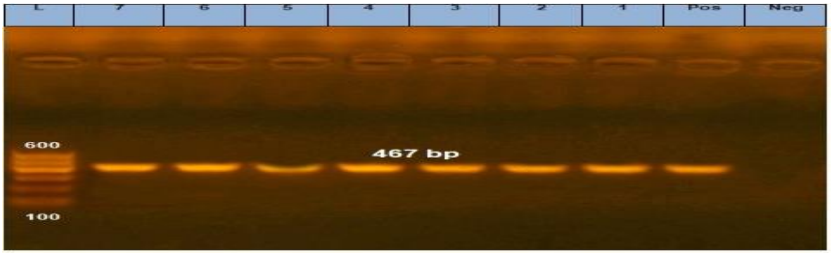

Figure (3): PCR of (*bcf*C) in *Salmonella* serotypes:
Primer set for ***bcf*C** gene (467 bp). Neg= negative control*, Pos=positive control* and L= ladder (100-600 bp). All lanes showed positive results: Lane (1): *S*. Typhimurium, Lane (2): *S*. Enteritidis, Lane (3): *S*. Blegdam, Lane (4): *S*. Anatum, Lane (5): *S*. Muenster, Lane (6): *S*. Lamberhurst and Lane (7): *S*. Ayinde.
*(Positive and or negative controls were represented by field sample that were previously confirmed to be positive or negative by PCR for the related genes in the Reference laboratory for veterinary quality control on poultry production, Animal health research institute).

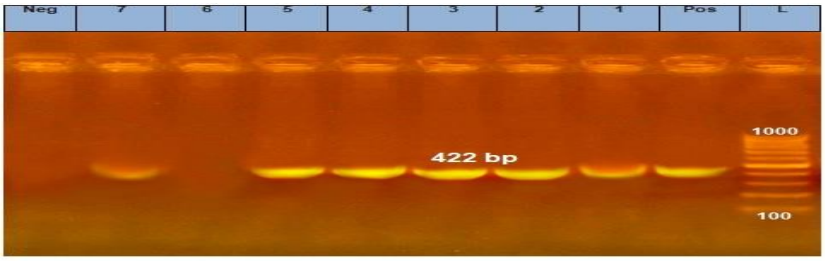

Figure (4): PCR of (*avr*A) in *Salmonella* serotypes:
Primer set for ***avr*A** gene (422 bp). Neg= negative control*, Pos=positive control* and L= ladder (100-1000 bp). Lanes (1-2-3-4-5-7) showed positive results and lane (6) *S*. Lamberhurst showed negative result.
*(Positive and or negative controls were represented by field sample that were previously confirmed to be positive or negative by PCR for the related genes in the Reference laboratory for veterinary quality control on poultry production, Animal health research institute).

Conclusion

Salmonellosis is very important public health problem which has a negative effect on human health all over the world. Antibiogram considered an important tool to detect the proper antibacterial which should be used in treatment of salmonellosis and must be know the antimicrobial sensitivity to *Salmonella* to prevent the random use of antibiotic in poultry treatment and so to avoid the occurrence of antibiotic resistance. PCR is a good rapid tool for detection the virulence genes in pathogenic bacteria

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دراسات بكتريولوجيه عن السالمونيلا المعزوله من لحوم الدجاج البلدى

حمزه ابراهيم عيد ، ايهاب محمود هلال*، رضوى حسني قوطه

قسم البكتريا والمناعه والفطريات- كليه الطب البيطري- جامعه قناه السويس

*معهد بحوث صحه الحيوان- فرعى بورسعيد, مركز البحوث الزراعية**

اجريت الدراسه على 200 عينة من الدجاج البلدي المذبوح بشكل عشوائي من أسواق بورسعيد لدراسة مدى انتشار أنواع السالمونيلا. كشف الفحص البكتريولوجي لعينات لحوم الدجاج عن عزل 9 من عزلات السالمونيلا (4.5٪). وكشف التصنيف السيرولوجي عن عزل السالمونيلا تيفميريم (22.2٪)، السالمونيلا مونستر (22.2٪), السالمونيلا انتيريتيدس (11.1٪)، السالمونيلا البلجدام (11.1٪)، السالمونيلا اناتم (11.1٪), السالمونيلا لامبر هورست (11.1٪) و السالمونيلا ايبندي (11.1٪). كشف اختبار الحساسيه للمضادات الميكروبيه للسالمونيلا المعزولة، أن العزلات المختبرة كانت شديدة المقاومة للإيريثروميسين والأوكسيتتراسيكلين بنسبة (100٪) يليها حمض الناليديكسيك (44.4٪) بينما كانت حساسة للغاية للكلورامفينيكول وسيبروفلوكساسين بنسبة (100٪) تليها الجنتاميسين (88.9٪). كما كشف اختبار البلمره أن 7 عترات من السالمونيلا كانت إيجابية لوجود جينات الضراوة (***spv*C ,*stn* ,*sop*B** و***bcf*C**) و 6 عترات فقط من السالمونيلا كانت إيجابية لوجود جين الضراوة (***avr*A**).