Journal of Current Veterinary Research

ISSN: 2636-4026

Journal homepage: http://www.jcvr.journals.ekb.eg

Molecular Characterization of Virulence Genes Associated with *Salmonella* spp. Isolated From Poultry

Gehad Helal[1], Reda Tarabees[2], Gamal Younis[3]

(1) Veterinarian at the Directorate of Veterinary Medicine, Sadat province

(2) Bacteriology, Mycology and Immunology Department, Faculty of Veterinary Medicine, University of Sadat City

(3) Microbiology Department, Faculty of Veterinary Medicine, Mansoura University, Mansoura, Egypt

*** Corresponding Author:** reda.tarabees@vet.usc.edu.eg ***Submitted:*** *23 July 2019* ***Accepted:*** *12 Aug. 2019*

ABSTRACT

Salmonella is still the major threat to the poultry industry and humans especially that of zoonotic importance. In the present study, a total of 300 samples (liver, intestine, yolk sac and spleen) collected from 100 broiler chickens were examined bacteriologically for the presence of *Salmonella*. The isolated salmonellae were then screened for virulence encoding genes using multiplex PCR and the antimicrobial susceptibility to antibiotics using disc diffusion method. Results showed that *Salmonella* was recovered from 5.33% of the examined samples. Sixteen *Salmonella* serovars were recovered [*Salmonella* Sinchem (*n=3*) *Salmonella* Typhimurium (*n=2*), *Salmonella* Gallinarum (*n=2*), *Salmonella* Enteritidis (n=2), *Salmonella* enterica subsp. Salamae (*n=1*), *Salmonella* Virchow (n=1), *Salmonella* Kentucky (*n=2*), *Salmonella* Heidelberg (*n=1*), *Salmonella* Farsta (*n=1*) and *Salmonella* Hydra (*n=1*)]. Results also showed that all the tested salmonellae (100%) were found harbor the virulence encoding gene specific amplicon of *pagC, msgA, spiA, invA, prgH, orgA, sipB, tolC, iroN, lpfC, pefA, sitC, sifA,* and *sopB*. While, only 30% and 70% of the examined salmonellae were harbor *cdt*B and *spvB,* respectively. The antimicrobial susceptibility testing of the isolates revealed that most of the isolated *Salmonella* serovars were expressed multiple antibiotic resistance indexes (MAR) to amoxicillin, doxycycline, chloramphenicol, ampicillin, gentamicin, trimethoprim/sulphamethoxazole. In conclusion, the results of the current study demonstrated that *Salmonella* isolated from broilers chicken were found to harbor many virulence encoding genes and expressed a high degree of MDR to antibiotics commonly used in human medicine.

Keywords*: antimicrobial resistance, broilers, multiple PCR, Salmonella, virulence genes*

INTRODUCTION

Salmonella infection remains one of the most serious problems affecting the poultry industry causing high economic losses not manifested in high mortality in young birds and the high costs of treatment and prevention programs. In addition, it causes lower hatchability, fertility and decreased egg production. The genus *Salmonella* is a Gram-negative, flagellated, facultative anaerobic short bacilli, 0.7-1.5 x 2.5 µm (Forshell and Wierup, 2006). There are more than 2500 *Salmonella* serovars have been identified based on the Kauffman-White classification (Grimont and Weill, 2007, Gallegos *et al*., 2008). Some *Salmonella* serovars such as *S*. Enteritidis, *S*. Infantis, *S*. Kentucky, and *S*. Heidelberg appear to be more prevalent in poultry than other animals (Foley *et al*., 2011). Salmonellae are widespread in nature and are commonly found in the intestinal tract of mammals, birds, and reptiles. Poultry is considered the primary reservoirs of salmonellae. Some species of Salmonella are host restricted and usually

colonized the intestine of poultry and don't contaminate the carcass surface so they did not cause human food poisonings such as *S*. Pullorum and *S*. Gallinarium (Chao *et al*. 2007). Salmonellosis is a zoonotic bacterial disease of national and international importance. The worldwide distribution of salmonellosis often parallels the patterns of trade of animal products and food, and the migration patterns of humans and animals (Gilbert *et al*., 2010). In particular, two *Salmonella* serotypes, *S*. Enteritidis and *S*. Typhimurium became major causes of human illness in the 1980s and 1990s,(Bailey and Maurer, 2001; Gray and Fedorka, 2002; Mølbak *et al.,* 2006). In Egypt *S*. Enteritidis isolated from broiler chicken and chicken has been implicated in many cases of food poisoning. The human clinical signs of salmonellosis include Fever, nausea and diarrhea, vomiting and abdominal pain after an Incubation period of 12 to 72 hrs (Ammar *et al,* 2010). The severity of infection of salmonellosis was governed by the production of many virulence encoding genes. These virulence determinant genes of *Salmonella* spp. is associated either with a combination of chromosomal or plasmid factors (Oliveira *et al*. 2003). These genes have a role in adhesion, invasion, and enterotoxin production (Chuanchuen *et al*. 2010, Das *et al*. 2012, Oliveira *et al*. 2003).

This work was aimed to elucidate the most common *Salmonella* species affecting poultry. The confirmed isolates were examined for their sensitivity to the common antimicrobials used in poultry farms in Egypt. In addition, the antimicrobial susceptibility and the molecular characterization of virulence genes associated with theses Salmonellae was assessed.

Materials and Methods

Sampling

A total of 300 organ samples (liver, spleen, cecum, and yolk sac) were collected from 100 poultry carcasses suspected to be infected with *Salmonella.* The samples were collected from different poultry farms located in El-Minufyia and El-Gharbia governorates between February 2017 to December 2017.

Isolation and identification of *Salmonella*

Isolation and identification of *Salmonella* was carried according to **ISO 6579 (2002)**. Briefly, 25 gram of each sample was aseptically chopped into fine pieces and pre-enriched in buffered peptone water for 18-20 hours at 37°C. From each pre-enrichment culture, 1ml was added to 9 ml amount of selenite F broth (Oxoid) and kept at 37°C for 24 hours. Then a loopful was taken and streaked on xylose lysine deoxycholate agar (XLD; Oxoid), and kept for 24 hours at 37°C. The suspected typical colonies were picked up and examined microscopically by Gram´s stain. The biochemical identification of the obtained isolates was performed according to **ISO 6579 (2002)**. The isolates were further serotyped using "O" and "H"antisera (**Denka Seiken co., LTD)** & (**Pro–lab diagnostic, U.K).**

Genotypic characterization of virulence genes

The whole genomic DNA was extracted using QIAamp DNA mini kit following the manufacture instructions. The isolates of Salmonella were screened for the presence of 17 virulence genes using Multiplex PCR in the following 3 sets: [set 1(amplified *spvB* 717 bp, *pagC* 454bp, , *msgA* 189 bp, *cdtB* 268 bp and *spiA* 550 bp); set 2 (amplified *invA* 1070 bp , *prgH* 756 bp*, orgA* 255 bp *,sipB* 875 bp *spaN* 504 bp*, ,and tolC* 161 bp); and set 3 (amplified *iroN* 1205 bp*, lpfC* 641 bp *, pefA* 157 bp*, sitC* 768 bp*, sifA* 449 bp*,and sopB* 220 bp). Thermal conditions and reaction mixtures were used as previously described by Skyberg *et al*. (2006), Tarabees *et al*. 2017, Shehata *et al*. 2019).

Antimicrobial susceptibility testing:

The susceptibility of the serotyped *Salmonella* was tested against the following antimicrobials; amoxicillin (30µg), ampicillin (10µg), chloramphenicol (30µg), doxycycline (30µg), sulphamethoxazole/trimethoprim (25µg) and Gentamycin (10µg), using the disc diffusion method according to the procedures established by CLSI, (2015). The media and antimicrobial discs were supplied by (Oxoid). Inhibition zones were measured to assess resistance or susceptibility.

Results and Discussion

Salmonella infections in poultry are the most important source of *Salmonella*-associated food poisoning in humans (Hedican *et al*., 2010). In the present study, the obtained data showed that among the examined 300 samples, 16 samples (5.33%) were positive for

Salmonella. This result is in agreement with that obtained by (Abd-El- Atif, 2014) who isolated 64 (5.33%) *Salmonella* from the examined 1200 samples. In addition, this outcome is nearly similar to that obtained by several researchers. (Abd El-Ghany *et al*., 2012) showed that *Salmonella* was isolated from 3.84% to 5.06% of the examined samples collected from four chicken flocks located at El-Kalubia governorate, Egypt. (Issa *et al*., 2017) demonstrated that only seven samples (11.5%) among the examined 61 samples were found positive for *Salmonella*. In contrast, a lower incidence rate was reported by (Menghistu *et al*., 2011) who showed that only seven samples (2.7%) of the examined 220 poultry tissue samples and 40 egg samples were positive for *Salmonella*. (Mahmoud, 2016) showed that 43 samples among the examined 348 chicken samples (12.4%) collected from Dakahlia and Damietta Governorates, Egypt were positive for *Salmonella*. (Andoh *et al*., 2016) who successfully isolated *Salmonella* from 94/200 (47%) of the examined samples. In addition, (El-Sharkawy *et al*., 2017) revealed that *Salmonella* was recovered from 41% of the examined samples collected from broilers farms located in Kafr El- Sheikh. (Nidaullah *et al*., 2017) isolated *Salmonella* serotypes from 161 of the examined 182 samples (88.46%). While (EL-Sheikh, 2018) found Sixteen (16%) out of 100 balady chickens positive for *Salmonella* isolation. Furthermore, a higher incidence was reported by many studies including (Uddin *et al*., 2018) and (Tarabees *et al*. 2019) and (Mohsen, 2019). These discrepancies in results could be attributed to the geographical distribution of the samples, the management and housing conditions, the breed of birds and other factors not investigated under the conditions of the current study.

The data also revealed that 10 serovars were successfully serotyped from chickens 3 *S*. Sinchem (18.75%), 2 *S*. Gallinarum (12.5%), 2 *S*. Kentucky (12.5%), 2 *S*. Typhimurium (12.5%), 2 *S*. Enteritidis (12.5%), 1 *S*. Salamae (6.25%), 1 *S*. Heidelberg (6.25%), 1 *S*. Hydra (6.25%), 1 *S*. Virchow (6.25%) and 1 *S*. Farsta (6.25%). These outcomes are inconsistent with that previously obtained by Dogru *et al*. (2010) who recovered 32 *Salmonella* serovars from 400 of the examined chicken carcasses as follows; 22 *S*. Enteritidis (68.7%), 5 *S*. Virchow (15.6%), 3 *S*. Typhimurium (9.3%) and 2 *S*. Hadar (6.2%). In contrast, Barua *et al*, (2013) revealed that that *S*. Virchow and *S*. Kentucky were the two predominant serovars isolated from the broiler farms. While (Osman *et al*. 2014) demonstrated that *S*. Enteritidis was the most frequent isolate 2/150 (1.3%), followed by *S*. Typhimurium, *S*. Virchow, *S*. Larochelle. While (Abd-El-Atif, 2014) isolated 7 *S*. Enteritidis, 21 *S*. Typhimurium, 7 *S*. Kentucky, 5 *S*. *arizonae*, 2 *S*. Hydra, 1 *S*. Anatum, 1 *S*. Paratyphi A, 4 *S*. Agona, 1 *S*. Bloomsbury, 2 *S*. Derby, 3 *S*. Rubislow, 1 *S*. Senftenberg, 4 *S*. Virchow, 5 *S*. Cerro with a percentage 10.93%, 32.81%, 10.93%, 7.81%, 3.12%, 1.56%, 6.25%, 1.56%, 3.12%, 4.68%, 1.56%. 6.25%, 7.81%, respectively. Nabil (2015) isolated 8 *S*. Typhimurium (18.6%), 1 *S*. Apeyeme (2.3%), 4 *S*. Kentucky (9.3%), 1 *S*. Daula (2.3%), 6 *S*. Newport (14 %), 3 *S*. Tamale (7%), 3 *S*. Molade (7%), 1 *S*. Colindale (2.3%), 1 *S*. Lexington (2.3%), 2 *S*. Bargny (4.7%), 2 *S*. Enteritidis (4.7%), 1 *S*. Papuana (2.3%), 1 *S*. Labadi (2.3%), 2 *S*. Santiago (4.7%), 2 *S*. Magherafelt (4.7%), 1 *S*. Rechovot (2.3%), and 1 untyped *Salmonella* (2.3%) and 3 serovars were isolated from chickens farms located at Damietta Governorate including 1 *S*. Takoradi (2.3%), 1 *S*. Angers (2.3%) and 1 *S*. Shubra (2.3%). Andoh *et al*. (2016) revealed that sixteen different serovars were identified mainly *S*. Kentucky, *S*. Nima, *S*. Muenster, *S*. Enteritidis, and *S*. Virchow were the most prevalent types. (Aslam *et al*., 2017) stated that *S*. Hadar was the most common serovar isolated from chicken and *S*. Heidelberg was the most prevalent serovar isolated from turkey meat. In contrast, (El-Sheikh, 2018) showed that only 7 *Salmonella* serovars were isolated from chickens including *S*. Enteritidis, *S*. Infantis, *S*. Newlands, *S*. Kentucky, *S*. Wey bridge, *S*. Naestved, and *S*. Ferruch. (Sharma *et al*., 2019) demonstrated that *S*. Kentucky, *S*. Virchow, and *S*. Typhimurium were the predominant identified serovars. These differences in results could be attributed to the geographical distribution of *Salmonella*. In addition, this study provides further evidence for the emergence of new *Salmonella* serovars

may have zoonotic importance require further investigations in future studies.

The severity of *Salmonella* infection is controlled by the expression of m the current study, *Salmonella* serovars were screened for the presence of 17 virulence encoding genes using multiplex PCR. These genes were involved in invasion, tissue damage and survival in the macrophages (Skeyberg, 2006). The data here in showed that *pagC*, *msgA, spiA*, *invA*, *prgH*, *orgA*, *sipB*, *tolC*, *iroN*, *lpfC*, *pefA*, *sitC*, *sifA*, *sopB* were reported with a percentage of 100% in 10 *Salmonella* isolates, While *spvB* and *cdtB* were found in 70% and 30% of the examined *Salmonella* serovars. These results are nearly similar to that obtained by Ammar *et al*. (2016) who revealed that *invA* gene was the most prevalent one (100%), followed by *hilA* (88.24%), *stn* (58.82%), and *fliC* genes (52.94%), while *sopB* and *pefA* genes were found in 41.18% of the examined *Salmonella* serovars. While, *sefC* and *spvC* encoding genes were found in 11.76 and 5.88%, of the examined 17 *Salmonella* serovars. While (Skyberg *et al.,* 2006) found that 11 encoding genes (*invA, orgA, prgH, tolC, spaN*, *sipB, sitC, pagC, msgA, spiA,* and *iroN*) out of the examined 17 genes were successfully amplified in the examined *Salmonella* serovars. The remaining genes (*lpfC, cdtB, sifA, pefA,* and *spvB*) were successfully amplified in 10%–90% of the examined Salmonellae isolated from sick birds and in 3.75%–90% of the healthy birds. In contrast, (Tarabees *et al*., 2017) found that *sitC, sopB, sifA, lpfC, spaN, sipB, invA, spiA*, and *msgA* genes were detected in *S.* Enteritidis. While, the *sitC, iroN*, *sopB, sifA, lpfC, spaN, sipB, invA*, and *tolC* genes were successfully amplified in S. Typhimurium. (Susmita, 2017) showed that *invA* and *spv*C encoding genes specific amplicons were detected in S. Gallinarium. (Ammar *et al.,* 2018) stated that the *inv*A gene was present in 100% of examined *Salmonella* serovars. (Shehata *et al*., 2019) demonstrated that the most predominant virulence genes in the examined *Salmonella* serovars isolates were *iroN, cdtB, spaN, invA,* and *orgA*, which were found in 17 (94.4%), 15 (83.3%), 14 (77.7%), 13 (72.2%), and 12 (66.7%) of the examined *Salmonella* serovars, respectively. While, *sipV, IpfC*, *sopB, prgH*, and *sitC* virulence genes were successfully amplified in 7 (38.8%), 7 (38.8%), 7 (38.8%), 5 (27.7%) and 3 (16.6%) of the examined salmonellae, respectively. In addition, *spiA, pagC, msgA, tolC, sifA* and *pefA* genes were not successfully amplified in all the examined serovars (Shehata et al, 2019). Sever and Akan (2019) demonstrated that the presence of the virulence encoding genes was varied greatly among the examined *Salmonella* serovars. The data of the present study highlighted the importance of multiplex PCR as a rapid and effective technique that can be used for the assessing of the presence of virulence encoding determinants among *Salmonella* serovars. In addition, the data draw the attention toward vigilant monitoring programs for the presence of different of *Salmonella* and especially that of zoonotic importance.

The confirmed *salmonella* serovars were further examined for their antimicrobial susceptibility to some antibiotics that commonly used in poultry farms. The collected data showed that the examined *Salmonella* serovars were highly sensitive to Doxycycline, Chloramphenicol, followed by Amoxicillin, Ampicillin, Gentamicin and Sulphamethoxazole +Trimethoprim, correspondingly. (Boris *et al*., 2012) reported that almost examined salmonellae were sensitive to gentamicin, chloramphenicol, ampicillin and tetracycline. (Taddele *et al*., 2012) found sensitivity to amoxicillin 93.3% of isolated *Salmonella* strains. This is nearly with (Putturu *et al*., 2013) who stated that *S.* Enteritidis was highly sensitive to ciprofloxacin followed by chloramphenicol, amikacin, gentamicin, amoxicillin, streptomycin, tetracycline, nalidixic acid, ampicillin and sulfonamide. this result agree with the out of Ahmed (2014) who investigated that all strains were sensitive to gentamycin, ciprofloxacin, colistin sulphate, doxycycline hydrochloride, neomycin, chloramphenicol, ampicillin and amoxicillin. Hasan *et al*. (2017) reported that on the basis of antibiotic sensitivity tests *Salmonella* spp. isolates were highly sensitive to gentamicin followed by doxycycline. The obtained result was different from the out of (Yah and Eghafona, 2007) reported that 183 *Salmonella* isolates showed variable resistance patterns to the antibiotics. (Sodagari *et al*., 2015) showed

that high antimicrobial resistance rates were observed to nalidixic acid (92.8%), tetracycline (81%), sulfamethoxazole/ trimethoprim (61.2%), streptomycin (56.7%), and kanamycin (36.9%), chloramphenicol (3.6%), amoxicillin-clavulanic acid (5.4%), and ampicillin (11.7%). Moe *et al*. (2017) reported that *Salmonella* isolates were resistant to trimethoprim-sulfamethoxazole (70.3%), tetracycline (54.3%), streptomycin (49.3%), and ampicillin (47.1%), chloramphenicol (29.7%), amoxicillin-clavulanic acid (17.4%), ciprofloxacin (9.4%), tobramycin (8.7%), gentamicin (8%), cefazolin (7.2%), lincomycin-spectinomycin (5.8%), and norfloxacin (0.7%). The obtained results are inconsistent with that reported by Asif *et al*. (2017) who showed that *S*. Enteritidis was resistant to ampicillin (82.2%), tetracycline (80 %), augmentin (77. 14 %), and chloramphenicol (54,2%) with an overall multidrug resistance index of 0.5. (Uddin *et al*., 2018) stated that *Salmonella* isolated from different sources were resistant to tetracycline, neomycin, ampicillin, novobiocin, cephradine, piperacillin-tazobactam, and cefepime in percentages of 89%, 80%, 80%, 75%, 74%, 100%, 94%, and 90%, correspondingly. (Mohsen, 2019) demonstrated that the isolated *Salmonella* serovars were resistant to tobramycin, amikacin, ampicillin/sulbactam, amoxicillin/clavulanic acid and doxycycline in a percentage of 75%, 58.3%, 50%, 58.3%, and 50%, respectively. The present study showed that most of *Salmonella* serovars recovered from poultry were sensitive to the tested antibiotics except *S*. Enteritidis, *S*. Typhimurium, *S*. Virchow, and *S*. Hydra. These variations in results might be attributed to the intensive use of these antibiotics in poultry farms as therapeutics or prevention. Therefore, the results of the current study encourage regular testing of *Salmonella* isolated from poultry for antimicrobial susceptibility to avoid transmission of these serovars to human food chains.

In conclusion, the data of the present study showed that new serovars of *Salmonella* were recovered from poultry. These *Salmonella* serovars were found to harbor many virulence encoding genes. These serovars expressed variable degrees of resistance to antibiotics and this requires regular monitoring of the isolated *Salmonella* for their antimicrobial susceptibility especially that of zoonotic importance. Finally further investigations are warranted to elucidate the emergence of new *Salmonella* serovars in particular *S*. Farsta as a potential threat to humans.

Table (1):- Serotyping of isolated *Salmonella* species.

Type of isolated *Salmonella* strains	Antigenic analysis
S.Sinchem	3,10:L,v:Z35
S.Gallinarum	1,9,12:-:-
S.enterica subsp.Salamae	1,9,12:9,m,[s],t:[1,5,7]
S.Kentucky	8,20,I,Z6
S.Entertidis	1,9,12,g,m,-
S.Typhimurium	1,4,[5],12,I,1,2
S.Heidelberg	1,4,5,12r,1,2
S.Hydra	21,c,1,6
S.Virchow	6,7,14:r:1,2
S.Farsta	4,12:i:e,n,x

Table (2):- Incidence of *Salmonella* serovars

S. serovars	Number of serovars	Percentage
S. Sinchem	3	18.75%
S. Gallinarum	2	12.5%
S. enterica subsp.Salamae	1	6.25%
S. Kentucky	2	12.5%
S. Entertidis	2	12.5%
S. Typhimurium	2	12.5%
S. Heidelberg	1	6.25%

S.Hydra	1	6.25%
S.Virchow	1	6.25%
S.Farsta	1	6.25%

Table (3):- the result of Multiplex -PCR of virulence genes associated with *salmonella* isolates:

Salmonella serovare	virulence genes															
	spvB	***pagC***	***msgA***	***cdtB***	***spiA***	***invA***	***prgH***	***orgA***	***sipB***	***tolC***	***iroN***	***lpfC***	***pefA***	***sitC***	***sifA***	***sopB***
***S.* S**	_	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
***S.* G**	+	+	+	_	+	+	+	+	+	+	+	+	+	+	+	+
***S.*e**	+	+	+	_	+	+	+	+	+	+	+	+	+	+	+	+
***S.* K**	_	+	+	_	+	+	+	+	+	+	+	+	+	+	+	+
***S.* E**	+	+	+	_	+	+	+	+	+	+	+	+	+	+	+	+
***S.*T**	_	+	+	_	+	+	+	+	+	+	+	+	+	+	+	+
***S.* Hb**	+	+	+	_	+	+	+	+	+	+	+	+	+	+	+	+
***S.* H**	+	+	+	_	+	+	+	+	+	+	+	+	+	+	+	+
***S.*V**	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
***S.* F**	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

S.*S** = *S.* Sinchem , ***S.*G**= *S.* Gallinarum*,* ***S.*e** =*S.*enterica subsp.Salamae ,S.*K**= *S.* Kentucky*,* ***S.*E**= *S.* Entertidis *,* ***S.*T** =*S.* Typhimurium, ***S.* Hb**= *S.* Heidelberg, ***S.*H**= *S.* Hydra*,* ***S.*V** =*S.*Virchow, ***S.*F** = *S.* Farsta

Table (4) *Salmonella* species susceptibility testing to different antimicrobial agents

Isolates type	**antimicrobial agents**					
	AML	DO	C	AM	CN	SXT
S. Sinchem 1	S	S	S	S	S	S
S. Sinchem 2	I	S	I	S	I	S
S. Sinchem 3	S	S	S	S	S	S
S. Gallinarum 1	S	S	S	S	I	S
S. Gallinarum 2	S	S	S	I	S	S
S. enterica subsp.Salamae	I	S	S	S	S	S
S. Kentucky1	S	I	S	I	I	I
S. Kentucky2	I	S	S	S	S	S
S. Entertidis1	R	R	S	R	S	I
S. Entertidis2	S	S	S	S	S	S
S. Typhimurium1	S	S	S	S	S	S
S.Typhimurium2	R	R	I	R	R	R
S.Heidelberg	R	S	S	S	R	S
S.Hydra	R	R	I	R	R	R
S.Virchow	R	S	I	R	S	I
S.Farsta	R	S	S	S	S	S

S=Sensitive, **I**= Intermediate, **R**= Resistance, **AML=** Amoxicillin**, DO=** Doxycycline**, C=** Chloramphenicol**, AMP=** Ampicillin**, CN=** Gentamycin**, SXT=** Sulpha+Trimethoprim**.**

Table (5). Antimicrobial susceptibility patterns

AMA	***Salmonella* serotype (No)**										**Total No. (%)**
	***S.*S(3)**	***S.*G(2)**	***S.*e(1)**	***S.*K(2)**	***S.*E(2)**	***S.*T(2)**	***S.*Hb(1)**	***S.*H(1)**	***S.*V(1)**	***S.*F(1)**	

	S	*R*	*S*	*R*	*S*	*R*	*S*	*R*	*S*	*R*	*S*	*R*	*S*	*R*	*S*	*R*	*S*	*R*	*S*	*R*	**S**	**R**
AML	2	*0*	2	0	0	0	1	0	1	1	1	1	0	1	0	1	0	1	0	1	7 (43.73 %)	6 (37.5 %)
DO	3	0	2	0	1	0	1	0	1	1	1	1	1	0	0	1	1	0	1	0	12 (75%)	3 18.75 %
C	2	0	2	0	1	0	2	0	2	0	1	0	1	0	0	0	0	0	1	0	12 (75%)	0 0%
AM	3	0	1	0	1	0	1	0	1	1	1	1	1	0	0	1	0	1	1	0	10 (62.5%)	4 25%
CN	2	0	1	0	1	0	1	0	2	0	1	1	0	1	0	1	1	0	1	0	10 (62.5%)	3 18.75 %
SXT	3	0	2	0	1	0	1	0	1	0	1	1	1	0	0	1	0	0	1	0	11 68.75%	2 (12.5 %)

AMA=Antimicrobial agent**, NO**=Number, **S**=Sensitive, **I**= Intermediate, **R**= Resistance, **AML=** Amoxicillin**, DO=** Doxycycline**, C=** Chloramphenicol**, AMP=** Ampicillin**, CN=** Gentamycin**, SXT=** Sulpha+Trimethoprim**. S. S** = *S.* Sinchem , **S. G**= *S.* Gallinarum, **S.e** = *S.*enterica subsp.Salamae ,**S. K**= *S.* Kentucky, **S. E**= *S.* Entertidis , **S.T** =*S.* Typhimurium, **S. Hb**= *S.* Heidelberg, **S. H**= *S.* Hydra, **S.V** =*S.*Virchow, **S. F** = *S.* Farsta.

Table (6) Multidrug resistance of different *Salmonella* species to antimicrobial agents

***Salmonella* serotype (No.)**	**No.of Antimicrobials to which the isolates were resistant**	**MDR (Multidrug resistance) Index**
S. Sinchem(3)	0	**0**
S. Gallinarum(2)	0	**0**
S. enterica subsp.Salamae(1)	0	**0**
S. Kentucky(2)	0	**0**
S. Entertidis(2)	3	**0.5**
S. Typhimurium(2)	5	**0.83**
S.Heidelberg(1)	2	**0.33**
S.Hydra(1)	5	**0.83**
S.Virchow(1)	2	**0.33**
S.Farsta(1)	1	**0.17**

Results of Polymerase chain reaction for detection of common virulence genes in *Salmonella* isolates

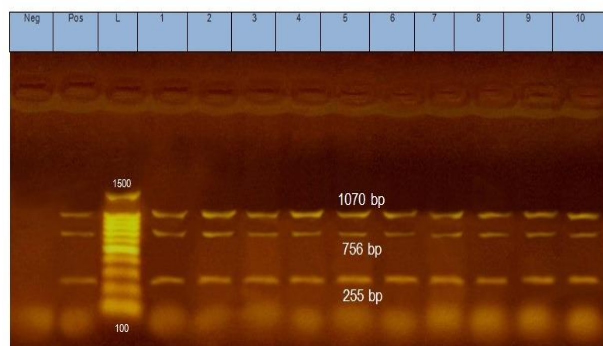

Figure (1): PCR products of *invA* (1070bp), *prgH* (756bp) ,*orgA* (255bp) genes. Lane L: 100-1500pb DNA ladder, Pos.: Positive control, Neg.: Negative control, Lane 1: *S.* Sinchem. Lane 2: *S.* Gallinarum. Lane 3: *S.*enterica subsp.Salamae, Lane 4: *S.* Kentucky, Lane 5: *S.* Entertidis, Lane 6:*S.*Typhimurium, Lane 7: *S.* Heidelberg, Lane 8: *S.*Hydra, Lane 9: *S.* Virchow, Lane 10: *S.* Farsta.

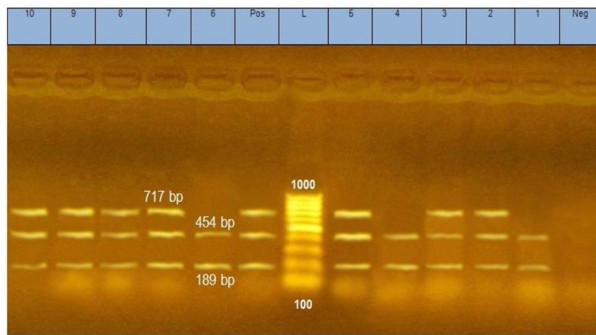

Figure (2): PCR products of the spvB (717bp), pagC (454bp), and msgA (189bp) genes. Lane L: 100-1000pb DNA ladder, Pos.: Positive control, Neg.: Negative control. Lane 1: Salmonella Sinchem. Lane 2: Salmonella Gallinarum, Lane 3: Salmonella enterica subsp.Salamae, Lane 4: Salmonella Kentucky, Lane 5: Salmonella Entertidis. Lane6: Salmonella Typhimurium, Lane 7: Salmonella Heidelberg, Lane 8: Salmonella Hydra, Lane 9: Salmonella Virchow, Lane 10: Salmonella Farsta.

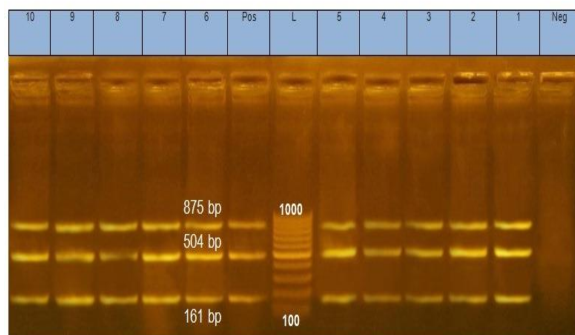

Figure (3): PCR products of the *sipB* (504 bp), *spaN* (161bp), *tolC* (875bp) genes. Lane L: 100-1000 pb DNA ladder, Pos.: Positive control.

Neg.: Negative control, Lane 1: *Salmonella* Sinchem, Lane 2: *Salmonella* Gallinarum, Lane 3: *Salmonella* enterica subsp.Salamae,. Lane 4: *Salmonella* Kentucky. Lane 5: *Salmonella* Entertidis, Lane 6: *Salmonella* Typhimurium. Lane 7: *Salmonella* Heidelberg, Lane 8: *Salmonella* Hydra, Lane 9: *Salmonella* Virchow, Lane 10: *Salmonella* Farsta

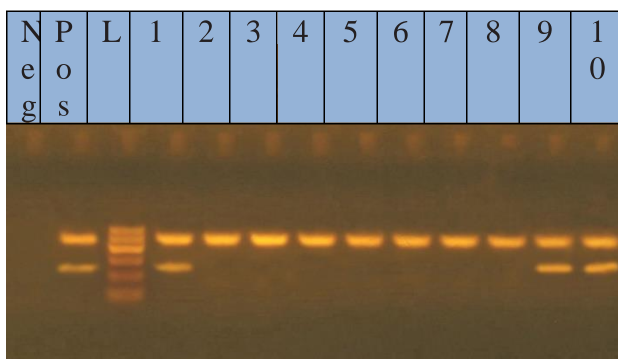

Figure (4): PCR products of the *cdtB* (268bp), *spiA* (550 bp) genes Lane L: 100-600 Pb DNA ladder, Pos.: Positive control, Neg.: Negative control, Lane 1: *Salmonella* Sinchem, Lane 2: *Salmonella* Gallinarum. Lane 3: *Salmonella* enterica subsp.Salamae, Lane 4: *Salmonella* Kentucky, Lane 5: *Salmonella* Entertidis, Lane 6: *Salmonella* Typhimurium, Lane 7: *Salmonella* Heidelberg, Lane 8: *Salmonella* Hydra, Lane 9: *Salmonella* Virchow, Lane 10: *Salmonella* Farsta.

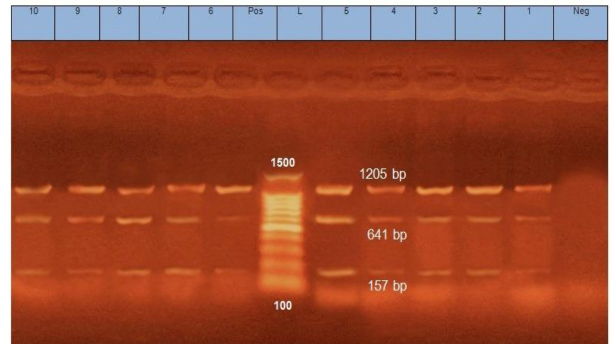

Figure (5): PCR products of the *iroN* (641, bp), *lpfC* (1205bp), *pefA* (157*bp)* genes, Lane L: 100-1500 pb DNA ladder. Pos.: Positive control. Neg.: Negative control. Lane 1: *Salmonella* Sinchem. Lane 2: *Salmonella* Gallinarum. Lane 3: *Salmonella* enterica subsp.Salamae, Lane 4: *Salmonella* Kentucky. Lane 5: *Salmonella* Entertidis. Lane 6: *Salmonella* Typhimurium. Lane 7: *Salmonella* Heidelberg, Lane 8: *Salmonella* Hydra, Lane 9: *Salmonella* Virchow, Lane 10: *Salmonella* Farsta.

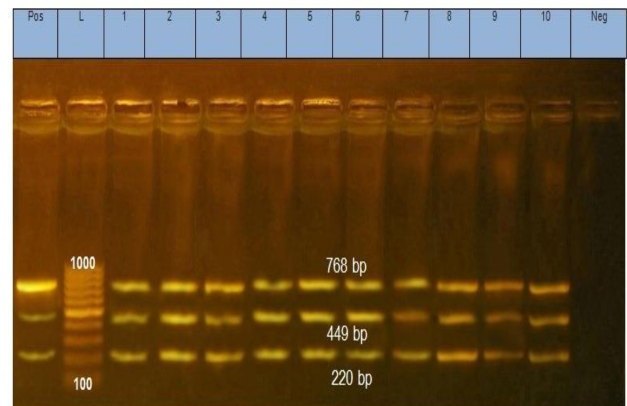

Figure (6): PCR products of the *sitC* (768bp), *sifA*(449bp), *sopB*(225bp) genes, Lane L: 100-1000kpb DNA ladder, Pos.: Positive control, Neg.: Negative control, Lane 1: *Salmonella* Sinchem, Lane 2: *Salmonella* Gallinarum, Lane 3: *Salmonella* enterica subsp.Salamae,. Lane 4: *Salmonella* Kentucky. Lane 5: *Salmonella* Entertidis. Lane 6: *Salmonella* Typhimurium, Lane 7: *Salmonella* Heidelberg, Lane 8: *Salmonella* Hydra, Lane 9: *Salmonella* Virchow, Lane 10: *Salmonella* Farsta.

References

Abd El-Ghany, A. W., El-Shafii, A. S. S. and Hatem, M. E. 2012. A Survey on *Salmonella* Species Isolated from Chicken Flocks in Egypt. Asian Journal of Animal and Veterinary Advances, 7: 489-5011.

Ammar, A., Alloui, N., Bennoune, O. and Kassah-Laouar, A. 2010. Survey of *Salmonella* serovars in broilers and laying breeding reproducers in East of Algeria. Journal Infection Dev Ctries, 4(2):103-106.

Ammar, A. M., Mohamed, A. A., Abd El-Hamid, Marwa, I. and El-Azzouny, Mona, M. 2016. Virulence genotypes of clinical *Salmonella* Serovars from broilers in Egypt. J Infect Dev Ctries, 10(4):337- 346.

Ammar, A. A., Abdeen, E. E., Abo-Shama, U. H., E. Fekry, E. and El mahallawy, E. K. 2018. Molecular characterization of virulence and antibiotic resistance genes among *Salmonella* serovars isolated from broilers in Egypt. Letters in Applied Microbiology, 68:188-195.

Andoh, L. A., Dalsgaard, A., Obiridanso, K. and Newman, M. J. 2016. Prevalence and antimicrobial resistance of *Salmonella* serovars Isolated from poultry in Ghana. Epidemiol Infect, 144(15):3288-3299.

Asif, M., Rahman, H., Qasim, M., Khan, T. A., Ullah, W. and Jie ,Y. 2017. Molecular detection and antimicrobial resistance profile of zoonotic *Salmonella* Enteritidis isolated from broiler chickens in Kohat, Pakistan. Journal of the Chinese Medical Association, 80(5): 303-306.

Bailey, J. S. and Maurer, J. J. 2001. *Salmonella* Species. In Food Microbiology: Fundamentals and Frontiers, 2nd en, eds Doyle, M. P. L. R. Beuchat &T. J. Montville, ASM Press, (pp.141–178). Washington D.C.

Barua, H. 1., Biswas, P. K., Olsen, K. E., Shil, S. K., Christensen, J. P. 2013. Molecular characterization of motile serovars of *Salmonella* enterica from breeder and commercial broiler poultry farms in Bangladesh. Pub. Med, 8(3):e57811.

Chao, M. R., Hsien, C. H., Yeh, C.M., Chou, S.J., Chu, C., Su, Y.C. and Yu, C.Y. 2007. Assessing the prevalence of *Salmonella* Enterica in poultry hatcheries by using hatched eggshell membranes. Poultry Science, 8(86):1651-1655.

Chuanchuen, R. K., Ajariyakhajorn, C., Koowatananukul, W., Wannaprasat, S., Khemtong, S., Samngamnim. 2010. Antimicrobial resistance and virulence genes in *Salmonella* enterica isolates from dairy cows. Foodborne Pathog Dis 7(1): 63-69.

CLSI . 2015. Clinical and Laboratory Standards Institute performance standard for antimicrobial disc susceptibility tests, approved standards, M02- A12,MO7-A10 and MO11-A8.

Dogru, A. K., Ayaz, N. D. and Gencay, Y. E. 2010. Serotype identification and antimicrobial resistance profiles of *Salmonella* species isolated from chicken carcasses. Tropical Ani. Health and Production. 42 (5): 893-897.

Das, A., S. Sree Hari., U. Shalini., A. Ganeshkumar, and M. Karthikeyan. 2012. Molecular Screening of Virulence Genes from *Salmonella* enterica isolated from Commercial Food Stuffs. Bioscience Biotechnology Research Asia, 9(1): 363-369.

El-Sharkawy, Hanem., Tahoun, A., El-Gohary, A. A., El-Abasy, Moshira., El-Khayat, F., Gillespie, T., Kitade, Y., Hafez, H.M., Neubauer, H. and El-Adawy, H. 2017. Epidemiological, molecular Characterization and antibiotic resistance of *Salmonella* enterica serovars isolated from chicken farms in Egypt. Gut Pathogens 9 (8):017-0157-1.

Foley, S.L., Nayak, R., Hanning, I.B., Johnson, T.J., Han, J. and Ricke, S.C. 2011. Population dynamics of *S\almonella enterica* serotypes in commercial egg and poultry production. Appl Environ Microbiol, 77:4273–4279.

Forshell, L.P. and Wierup, M. 2006. *Salmonella* contamination a significant challenge to the global marketing of animal foods products. Revue Scientifique Techinique Office International des Epizooties, Paris, 25. (2):541-554.

Gallegos, R., Loredo, A., Ojeda, G. and Vega, A. 2008. Identification of *Salmonella* serotypes isolated from cantaloupe and chile pepper production system in Mexico using PCR-RFLP. J. Food Protect, 71(11): 2217-2222.

Gilbert, S., Lake, R., Cressey, P., Hudson, A. and King, N. 2010. Risk Profile: *Salmonella* (Non Typoidal) in Pork and Pork Products, Institute of Environmental Science and Research Limited.

Gray, J. T. and Fedorka-Gray, P. J. 2002. *Salmonella* in Foodborne Diseases. 2nd edn, eds Cliver, D. O. & H. P. Riemann, Academic Press, (pp. 55–68).

Grimont, P.A.D and Weill, F.X. 2007. Antigenic formulae of the *Salmonella* serovars. WHO Collaborating Centre for Reference and Research on *Salmonella*, Institut Pasteur, Paris, France.

Hasan, K., Rathnamma, D., Narayanaswamy, H.D., Malathi, V., Gupta, S. and Singh, S.V. 2017. Isolation of Bacterial Pathogens associated with Broiler Mortality in Kolar. Vet. Sci, 5(7): 312-315.

Hafiz Nidaullah, Nadarajan Abirami, Ahamed Kamal Shamila-Syuhada, Li-Oon Chuah, Huda Nurul, Teik Pei Tan, Farah Wahida Zainal Abidin. and Gulam Rusul1. 2017. Prevalence of *Salmonella* in poultry processing environments in wet markets in Penang and Perlis, Malaysia. Veterinary World, 10(3): 286–292.

Hedican, E., Miller, B., Ziemer, B., Le Master, P., Jawahir, S., Leano, F. and Smith, K. 2010. Salmonellosis outbreak due to chicken contact leading to a foodborne outbreak 312 associated with infected delicatessen workers. Foodborne Pathog Dis, 7, 995- 997.

ISO 6579. 2002. Microbiology –General guidance on methods for the detection of *Salmonella*, International organize for standardization, Geneve, Switzerland.

Issa, Y., Abu-Rayyan, A., Hemidat, S. and the Environmental Health Team at Hebron. 2017. Prevalence of *Salmonella* in different poultry and meat food products in Hebron district. a prevalence study. The LANCET, 390 s(33).

Jaishree Sharma, Deepak Kumar, Sheeba Hussain, Anubha Pathak, Maani Shukla,V., Prasanna Kumar., P.N., Anisha, Richa Rautela, A.K.,Upadhyay. and S. P., Singh. 2019. Prevalence, antimicrobial resistance and virulence genes characterization of non typhoidal *Salmonella* isolated from retail chicken meat shops in Northern India.Food Control, foodcont, 01.021.

Marwa, M. F. Abd-Elatif. 2014. Isolation and Identification of salmonellae in Chickens Farms. M.V.Sc. Thesis, Fac.Vet. Med, Cairo Univ.

Marwa Ibrahim Abd Al-Fattah EL-Sheikh.2018. Bacteriological and molecular studies on *Salmonella* infection in chickens. M.V.Sc. Thesis, Fac. Vet. Med, Sadat City Univ.

Menghistu, H.T., Rathore, R., Dhama, K. and Agarwal, R.K. 2011. Isolation, Identification and Polymerase Chain Reaction (PCR) Detection of *Salmonella* Species from Field Materials of Poultry Origin. International Journal of Microbiological Research, 2 (2): 135-142.

Molbak, K., Olsen, J.E. and Wegener, H.C. 2006. *Salmonella* infections in Riemann HP, Cliver DO (eds.) Food infections and intoxications, Elsevier, Amsterdam, 57-136. 4.

Moe, A.Z., Paulsen, P., Pichpol, D., Fries, R., Irsigler, H., Baumann, M. P. O. and Oo., K.N. 2017. Prevalence and Antimicrobial Resistance of *Salmonella* Isolates from Chicken Carcasses in Retail Markets in Yangon, Myanmar. Journal of Food Protection, 80(6): 947-951.

Mueen Aslam., aSylvia Checkleyb., Brent Avery., cGabhan Chalmerse., Valere Bohaychukd., Gary Genslerd., Richard Reid Smith., cPatrick Boerline. 2017. Phenotypic and genetic characterization of antimicrobial resistance in *Salmonella* serovars isolated from retail meats in Alberta, Canada. Food Microbiology, 32 (1): 110117.

Nehal Mahmoud Nabil. 2015. Molecular studies on antimicrobial resistance genes in *salmonella* isolated from poultry. Doctoral dissertation, Faculty of Veterinary Medicine, Benha University.

Nurdan Karacan Sever and Mehmet Akan. 2019. Molecular analysis of virulence genes of *Salmonella* Infantis isolated from chickens and turkeys. Microbial Pathogenesis, 126:199-204.

Oliveira, S.D., C.R. Rodenbusch., Ce, M. C., S.L. Rocha. and C.W. Canal.2003. Evaluation of selective and non-selective enrichment PCR procedures for *Salmonella* detection. Lett Appl Microbiol, 36(4): 217-221.

Osman, K.M., Marouf, S.H., Erfan, A.M. and Al Atfeehy, N. 2014. *Salmonella* enterica in imported and domestic day-old turkey poults in Egypt repertoire of virulence

genes and their antimicrobial resistance profiles. Rev. sci. tech. Off. int. Epiz, 33 (3): 1017-1026.

Oxoid, M. 1998. Manual of culture media, ingredients and other laboratory service.

Putturu, R., Thirtham, M. and Eevuri, T. R. 2013. Antimicrobial sensitivity and resistance of *Salmonella* Enteritidis isolated from natural samples. Vet. World, 6(4):185-188.

Awad, A., Shehata, Shereen Basiouni, Alaa Abd Elrazek, Hesham Sultan, Reda Tarabees, Mohamed Sabry Abd Elraheam Elsayed, Shaimaa Talat, Ibrahim Moharam, Ahmed Said, Walaa Atia Mohsen. and Monika Krüger. 2019. Genotypic characterization and antimicrobial resistance of *Salmonella* enterica isolated from poultry hatcheries and commercial broiler chickens. Pakistan Veterinary Journal, 0253-8318.

Reda Tarabees, Mohamed, S. A., Elsayed, Reyad Shawish, Shereen Basiouni. and Awad, A., Shehata.2016. Isolation and characterization of *Salmonella* Enteritidis and *Salmonella* Typhimurium from chicken meat in Egypt. JIDE (The Journal of Infection in Developing Countries), 11(4):314-319.

Skyberg, J.A., Logue, C.M. and Nolan, L.K. 2006. Virulence Genotyping of *Salmonella* spp. with Multiplex PCR. Avian Diseases, 50(1):77-81.

Sodagari, H.R., Mashak, Z. and Ghadimianazar, A. 2015 Prevalence and antimicrobial resistance of *Salmonella* serotypes isolated from retail chicken meat and giblets in Iran. Journal of Infection in Developing Countries, 18. 9(5):463-469.

Susmita Pal, Samir Dey, Kunal Batabyal, Abhiroop Banerjee, Siddhartha Narayan Joardar, Indranil Samanta. and Devi Prasad Isore. 2017. Characterization of *Salmonella* Gallinarum isolates from backyard poultry by polymerase chain reaction detection of invasion (invA) and *Salmonella* plasmid virulence (*spvC*) genes. Veterinary World, 10 (7): 814-817.

Tarabees, R., Elsayed, M.S.A., Shawish, R., Basiouni, S.and Shehata, A.A. 2017. Isolation and characterization of *Salmonella* Enteritidis and *Salmonella* Typhimurium from chicken meat in Egypt. J Infect Dev Ctries, 30.11(4):314-319.

UDDIN, Muhammad Nazir, Muhammad Muhammad Farooq, Muhammad Waqas, Najeeb Ullah Khan, Waqas Ali Khan, Imran Khan, Nasiara Karim.and Muhammad Rizwan. 2018. Antibiotic assays of *Salmonella* isolate from poultry chicken of various locations in districts Swat. Pure and Applied Biology (PAB), 1 (7): 1, 78-84.

Wafaa Abd El-Ghany, A., El-Shafii, Soumaya, S.A. and Hatem, M.E. 2012. A Survey on *Salmonella* species isolated from chicken flocks in Egypt. Asian journal of animal and veterinary advances,7(6):489-501.

Walaa Atia Mohammed Mohsen. 2019. Studies on *Salmonella* microorganism in chickens. M.V.Sc. Thesis, Fac. Vet. Med, Sadat City Univ.

Yah, S.C. and Eghafona, N.O. 2007. Plasmid A vehicle for rapid transfer of antibiotic resistce markers of *Salmonella* species in animals. J.Amer. Sci, 3(4):86-92.