Molecular Detection of some Antimicrobial Resistance Genes in *Salmonella* Species Isolated from Commercial Layers in Egypt

Mohammed A. Lebda¹, Waffa M. Mohammed², Samah Eid² and Rehab I. Hamed³

¹Avian and Rabbit Medicine Department, Faculty of Veterinary Medicine, Zagazig University, 44511, Egypt

²Reference Laboratory for quality control on poultry production (RLQP). Animal Health Research Institute, Dokki Lab., Giza, Egypt

³Reference Laboratory for quality control on poultry production (RLQP). Animal Health Research Institute, Sharkia Branch, Egypt

Article History: Received: 7/1/2017 Received in revised form: 25/2/2017 Accepted: 2/3/2017

Abstract

The present study was done to investigate the wide spread resistance to some antimicrobial groups among *Salmonellae* isolated from replacement and layer flocks in Egypt. A total of 24 *salmonellae* were isolated from 200 birds (apparently healthy or diseased suffered from diarrhea, dehydration, respiratory distress and decrease of egg production) and serotyped into *S. Enteritidis*, *S. Typhimurium*, *S. Kentucky* and *S. Newport*. Twenty-one *Salmonella* isolates were examined for resistance genes against different antimicrobials. The resistance pattern of all *Salmonella* isolates was done using antibiogram, the resistant isolates were examined for the presence of resistance coding genes using PCR technique. The investigated resistance genes were (*qnrS*, *aac (6')-ib-cr*) for quinolone resistant isolates, *blaTEM* for β -lactam resistant isolates, *aadA1* and *aadA2* for aminoglycosides resistant isolates and *tetA(A)* and *tetA(B)* for tetracycline resistant isolates. Resistant genes percentages for *tetA(A)*, *tetA(B)*, *blaTEM*, *aadA1*, *aadA2*, *aac (6')-ib-cr* and *qnrS* in the examined isolates were 70%, 20%, 93.3%, 30%, 80%, 10% and 15%, respectively. In conclusion, at the study area, antimicrobial resistance genes are widely spread in *Salmonella* isolates. Thus, minimizing the influence of antibiotics in treatment and prevention.

Keywords: *Salmonella*, Resistance Genes, Replacement Layer, Layers, Egypt

Introduction

Paratyphoid infection is a problem of economic concern to all phases of poultry industry from production to marketing. *Salmonella* spp. is gram negative, motile rods by peritrichous flagella. The genus *Salmonella* is divided into two species *Salmonella enterica* and *S. bongori*, *Salmonella enterica* itself is comprised of six subspecies, namely *S. enterica* subspecies *enteric*, *S. enterica* subspecies *salamae*, *S. enterica* subspecies *Arizonae*, *S. enterica* subspecies *diarizonae*, *S. enterica* subspecies *indica* and *S. enterica* subspecies *houtenae* [1]. The number of serotypes in each species and subspecies of *Salmonella* was reported to be around 2522 [2-5]. As a result of extensive use of antibiotics in human and veterinary medicine, serious increase in the spreading of multiple antibiotic resistant *Salmonella* has occurred [6]. Prolonged use, misusing, and overusing

antibiotics led to in efficiency and enhanced the severity of the disease [7,8]. These resistant *Salmonellae* can be transmitted to humans through food such as poultry meat, therefore, they constitute a major public health problem [9]. Thus, this study was planned to detect antibiotic resistance genes in *Salmonella* infected replacement and layer flocks to select the highly effective antimicrobial agents for treatment and prevention.

Material and Methods

Samples collection

A total of 200 (apparently healthy (60), diseased (75), freshly dead (65) replacement and layers were collected from Sharkia Governorate and subjected to postmortem examination. The internal organs (liver, caecum, ovary, heart and lungs) were

aseptically collected and subjected to bacterial isolation and identification.

Bacteriological examination

Isolation of *Salmonella* spp. was done according to ISO 6579 [10]. Briefly, samples were collected in buffered peptone water and incubated at 37°C for 24 h, after pre-enrichment 0.1 mL of the broth culture was transferred into a 10 mL Rappaport Vassiliadis Soya (RVS) broth and incubated at 42°C for 24-48 h. Another 1 mL of pre-enrichment broth was transferred into a tube containing 10 mL of Muller Kauffman Tetrathionate Novobiocin (MKTTN) broth and incubated at 37°C for 24-48 h. A loop-full of material from the RVS broth and MKTTn was transferred and streaked separately onto the surface of Xylose Lysine Deoxycholate agar (XLD agar), Hektoen Enteric (HE agar), MacConkey's agar and S-S agar separately. Presumptive colonies were selected and purified on nutrient agar plates for further identification.

Biochemical identification

Identification of the isolates using oxidase reaction, hydrolysis of urea, H₂S production, Lysine decarboxylation, Indole test, Methyl red (MR) test, Voges Proskauer (VP) test and Simmon's Citrate agar were done according to Cruickshank *et al.* [11].

Serotyping

Biochemically suspected *Salmonella* isolates were subjected to serological identification according to Kauffman-White Scheme (Kauffman, 1974) for determination of somatic (O) and flagellar (H) antigens [10]. The antisera were kindly supplied by Prof. Dr. Mohamed Ahmed, Faculty of Veterinary Medicine, Benha University, Department of Food Control.

Antibiogram

The antimicrobial susceptibility testing was performed according to Finegold *et al.* [12] using the agar disc diffusion method on Mueller Hinton agar (Oxoid) plates. Few colonies of *Salmonella* were suspended in Muller broth and incubated for 4-5 hours until the turbidity was seen. Using sterile Pasteur pipette, 1 mL of the suspension was inoculated into the surface of the plate to wet the whole of its surface. Excessive fluid was discarded by

its pipetting then plates were dried for up to 30 min then the chosen antibiotic discs were distributed to the surface of the plate.

The used antimicrobial agents were Florfenicol (30 µg), Nalidixic acid (30µg), Ciprofloxacin (5µg), Norfloxacin (10 µg), Amoxicillin (10 µg), Cefotaxime (30 µg), Amikacin (30 µg), Gentamycin (10 µg) Erthromycine (15 µg), Streptomycin (10 µg), Doxycyclin (30 µg) and Sulfamethaxazole-Trimethoprim (25 µg). The inhibition zone was measured to assess resistance or susceptibility according to the interpretation criteria established by Clinical Laboratory Standards Institute (CLSI) standard [13].

Molecular detection of resistance genes

The DNA extraction was done using QIAamp DNA Mini Kit DNA extraction kit (Catalogue no. 51304) according to manufactures' guidelines. The Oligonucleotide primers synthesized by Metabion (Germany) targeting several antibiotic resistance genes were used. Primers specific for tetracycline resistance gene *tetA*(A) and *tetA*(B) are F (5'-GGT TCA CTC GAA CGA CGT CA-3'); R (5'-CTG TCC GAC AAG TTG CAT GA-3') and F (CCT CAG CTT CTC AAC GCG TG-3'); R (5'-GCA CCT TGC TCA TGA CTC TT-3'), respectively [14]. Aminoglycosides resistance genes (*aadA1* and *aadA2*) were detected using primers F (5'-TAT CAG AGG TAG TTG GCG TCAT-3'); R (5'-GTT CCA TAG CGT TAA GGT TTC ATT -3') and F (5'-TGT TGG TTA CTG TGG CCG TA-3') R (5'-GAT CTC GCC TTT CAC AAA GC -3'), respectively [14,15]. β-lactams resistance gene (*blaTEM*) was detected using primers F (5'-ATC AGC AAT AAA CCA GC -3') R (5'-CCC CGA AGA ACG TTT TC -3') [16]. Quinolones resistance genes (*aac(6')-ib-cr* and *qnrS*) were detected using primers F (5'-CCC GCT TTC TCG TAG CA-3'), R (5'-TTA GGC ATC ACT GCG TCT TC-3') and F(5'-ACG ACA TTC GTC AAC TGC AA -3'),R (5'-TAA ATT GGC ACC CTG TAG GC-3'), respectively [17]. Reaction volume of 30 µL contained 12.5 µL of EmeraldAmp Max PCR Master Mix (Takara, Japan), 1 µL of each primer of 20 pmol concentrations, 4.5 µL of nucleus free water and 6 µL of template DNA. Amplified DNA was separated by electrophoresis on 1.5% agarose gel (ABgene)

in 1x TBE buffer at room temperature. For gel analysis, 15 µl of the products was loaded in each gel slot. A 100 bp DNA Ladder (Qiagen, USA) was used to determine the fragment sizes. The gel was photographed by a gel documentation system and the data was analyzed through computer software. The positive control was kindly provided by the Reference Laboratory for the Control of Poultry Production, Dokki, Giza.

Results

Cultural and biochemical characters

The cultural characters of the isolated salmonellae appeared on XLD agar are smooth

pink colonies with black center (H_2S production) while, H-E agar they appeared as deep blue colored colonies. On MacConkey's agar, the colonies were pale, colorless smooth, transparent (non lactose fermenter) and on S-S agar they appeared as pale colored colonies with or without black centers.

Application of different biochemical tests revealed the following results; negative oxidase reaction, negative urea hydrolysis, positive reaction on TSI agar, positive reaction on LI agar, negative Indole reaction, positive MR reaction, negative VP reaction and positive on Simmon's Citrate agar.

Table 1: Isolation percentages of Salmonellae in organs of infected layer chickens

Samples	Positive Number	Percent
Liver	9	37.5%
Caecum	8	33.3%
Ovary	5	20.8%
Heart	2	8.3%
Lungs	0	0%

Occurrence of Salmonella spp. in different samples

Out of 200 examined birds, 24 (12%) were positive for *Salmonella* isolation. The isolation percentages from liver, caecum, ovaries, heart and lungs were 37.5%, 33.3%, 20.8% and 8.3%, respectively, while none of the lung samples were positive (Table1).

Serotyping of Salmonella isolates

The isolated 24 salmonellae were classified under four different serogroups including *S. Enteritidis*, *S. Typhimurum*, *S. Kentucky* and *S. New port*. The most prevalent serogroups were *S. Enteritidis* and *S. Typhimurium* with the percentage of 54.2% and 20.8%, respectively. Other serotypes including *S. Kentucky* and *S. Newport* were also recorded with the percentages of 8.3% and 4.2%,

respectively. While, 3 isolates (12.5%) were untypeable

Antimicrobial susceptibility

The results in Table 2 show that *Salmonella* isolates revealed high resistance to nalidixic acid (95.2%) and Amoxycillin (71.4%). Moderate resistance to erythromycin (47.6%), streptomycin (47.6%), doxycycline (52.4%), gentamycin (47.6%), florophenicol (52.4%) and sulfamethaxazole/ trimethoprim (38.1%) was observed. Moreover, the isolates were sensitive to amikacin (80.9%); ciprofloxacin (76.2%), norofloxacin (71.2%) and cefotaxime (52.4%). Table (3) shows that multiple drug resistance to more than two antimicrobial drugs was observed in all the examined isolates.

Table 2: Antibigram of different *Salmonellae* isolates

Antibiotic	Percentage of samples (n)		
	Sensitive	Intermediate	Resistant
Nalidixic acid	0	4.8% (1)	95.2% (20)
Amikacine	80.9% (17)	19.1% (4)	0
Ciprofloxacin	76.2% (16)	23.8% (5)	0
Norofloxacin	71.4% (15)	28.6% (6)	0
Cefotaxime	52.4% (11)	33,3 % (7)	14.3% (3)
Doxycycline	38.1% (8)	14,28% (3)	47.6% (10)
Sulfamethoxazol / trimethoprim	33.3% (7)	38.1% (8)	28.6% (6)
Streptomycine	23.8% (5)	28,58% (6)	47.6% (10)
Florophenicol	19.1% (4)	57.14% (12)	23.8% (5)
Gentamycin	19.1% (4)	33.33% (7)	47.6% (10)
Erthromycine	14.3% (3)	38,1% (8)	47.6% (10)
Amoxycillin	9.3% (2)	19.05% (4)	71.4 % (15)

The remained 3 isolates out of 24 obtained isolates were untypable.

Table 3: Antibiotic resistance pattern of *Salmonella* serotypes

Isolate No	Serotype	Resistance profile
1	<i>S. Enteritidis</i>	NA-AML-DO-E
2	<i>S. Typhimurium</i>	NA-AML-SXT
3	<i>S. Kentucky</i>	NA-AML-S-E- DO
4	<i>S. Newport</i>	NA-CN-E-SXT
5	<i>S. Enteritidis</i>	NA-CN-S-E-SXT
6	<i>S. Enteritidis</i>	NA-AML-DO-F
7	<i>S. Typhimurium</i>	NA-AML-CN-
8	<i>S. Kentucky</i>	NA-AML-CN-S-SXT
9	<i>S. Enteritidis</i>	NA-CN-DO-S-F
10	<i>S. Enteritidis</i>	NA-DO-E-F-SXT
11	<i>S. Typhimurium</i>	CN-S-E-SXT
12	<i>S. Enteritidis</i>	NA-AML-CN-S
13	<i>S. Enteritidis</i>	NA-AML-DO-SXT
14	<i>S. Typhimurium</i>	NA-DO-S-E
15	<i>S. Typhimurium</i>	NA-AML-S-E-CTX
16	<i>S. Enteritidis</i>	NA-AML-CN-DO
17	<i>S. Enteritidis</i>	NA-AML-E-F-CTX
18	<i>S. Enteritidis</i>	NA-AML-DO-S-E
19	<i>S. Enteritidis</i>	NA-AML-CN-DO
20	<i>S. Enteritidis</i>	NA-AML-CN
21	<i>S. Enteritidis</i>	NA-AML-S--F-CTX

NA=Nalidixic acid AK=Amikacine CIP=Ciprofloxacin NOR=Norfloxacin AML=Amoxycillin CN=Gentamycin
DO=Doxycyclin S=Streptomycine E=Erthromycine F=Florophenicol CTX=Cefotaxime
SXT=Sulfamethozol\trimethoprim

The remained 3 isolates out of 24 obtained isolates were un typable.

Molecular detection of resistance associated genes

Salmonella isolates that showed phenotypic resistance against different antimicrobial agents using antibiotic susceptibility test were examined by PCR to identify some resistance coding genes. The results revealed that *tetA(A)*, *tetA(B)*, *blaTEM*, *aadA1*, *aadA2*, *aac*

(6')-*ib-cr* and *qnrS* were identified in 70%, 20%, 93.3%, 30% ,80 %, 10% and 15%, respectively (Table 4).

Discussion

In this study, 200 replacement and layers from different poultry farms at Sharkia Governorate were examined for the presence of *Salmonella* species. Twenty-four (12%) of the

examined birds were found positive. This was in agreement with Rehan [18] and Mohamed [19] who isolated *Salmonella* species from Dakahlia and Damietta Governorates in Egypt with an isolation rates of 12% and 12.4%, respectively. Meanwhile, these results were higher than 9.2% and 3.4% reported by AL-

Abadi and Al-Mayah [20] and AL-Hakeem [21], respectively. These differences in the overall prevalence of *Salmonella* may be related to several factors such as environment, hygienic conditions of the farm and health status of the examined chicken.

Table 4: Occurrence of resistance genes among examined bacterial strains isolated from layer chickens

Resistance gene	Number of examined resistant isolates	Number of positive (%)
<i>qnrS</i>	20	3 (15%)
<i>acc(6)-ib-cr</i>	20	2(10%)
<i>blaTEM</i>	15	14(93.33)
<i>tetA(A)</i>	10	7(70%)
<i>tetA(B)</i>	10	2(20%)
<i>aadA1</i>	10	3(30%)
<i>aadA2</i>	10	8 (80%)

The prevalence of the isolated bacteria from different internal organs of the examined layers in this study showed that the highest percentage of *Salmonella* was recorded in liver (37.5%) followed by cecum (33.3%), ovary (20.8%) and heart (8.3%). These results are in agreement with Dhahar *et al.* [22] and AL-Iedani [23]. AL-Abadi and Al-Mayah [20] recorded that the highest percentage of *Salmonella* isolation was from cecum which is the primary sites of colonization of *Salmonella* due to the anatomical and structural location that allows the cecum to act as a blind sac with low content flow rate.

Salmonella strains which are resistant to a range of antimicrobials have emerged and are threatening to become a serious public health problem [8]. This resistance results from the misuse of antimicrobials both in human and poultry husbandry. In this study, 21 typable *Salmonella* isolates from replacement and layers were examined against 12 different antimicrobial agents belonged to different groups such as quinolone, phenicol, β -lactams, aminoglycosides, tetracyclines, cephalosporins and sulfonamide groups. Antibioqram of *Salmonella* isolates showed high resistance to nalidixic acid (95.2 %) and amoxicillin (71.4%). The obtained results differ from Habrun *et al.* [24] who reported that 100% *Salmonella* isolates were sensitive to

florophenicol and streptomycin, while, 92 of the isolates (58%) were sensitive to nalidixic acid. In addition, Okamoto *et al.* [25] reported that florophenicol is the most effective antimicrobial for treating *Salmonella* infection as a result of lack of use of this antimicrobial in animals feed since 2003. In contrary to the obtained results, Cardoso *et al.* [26] reported that 100% of *Salmonella* isolates showed sensitivity to doxycycline, while, Shivhare *et al.* [27] reported high sensitivity of *Salmonella* spp. isolated from poultry to norfloxacin, while, all the isolates were resistant to sulfonamides trimethoprim. Moreover, our results agreed with Snow *et al.* [28] who reported that all *Salmonella* isolates from commercial layer flocks in UK were sensitive to amikacin. The results in this study differ from Zdragas *et al.* [29] who reported 5% resistance to streptomycin and 2% to nalidixic acid. Khan *et al.* [30] reported that 87.9% of salmonellae were sensitive to ciprofloxacin and amikacin. Moreover, Donado-Godoy *et al.* [31] reported that the resistance pattern of *Salmonella* isolated from chickens was 15% to ciprofloxacin, trimethoprim-sulfamethoxazole, streptomycin, and nalidixic acid. On the other hand, 8 *S. Enteritidis* isolates showed 100% sensitivity to ciprofloxacin, norfloxacin and gentamycin [26]. Also, Yah and Eghafona [32] reported that 183 *Salmonella* isolates from

chickens were highly resistant to chloramphenicol, gentamycin, trimethoprim and sulfamethoxazole and to lesser extent resistant to ciprofloxacin. Our results agreed with Threlfall [33] and Hendriksen [34] who recorded that *S. Typhimurum* isolates were

resistant to amoxicillin, chloramphenicol, streptomycin and sulfonamides. In contrary to the obtained results in the current study, Asway *et al.* [35] mentioned that the isolated *Salmonella* strains were resistant to ciprofloxacin.

Figure 1: Exemplar of gel pictures showing amplified products of resistance associated genes; In each photo “P” stands for positive control, “neg”: Negative control and numbers indicates lanes with positive and negative samples; A: *qnrS* gene (417bp); B: *acc(6)-ib-cr* gene (113bp); C: *aadA1* gene(484bp); D: *blaTEM* gene(516 bp); E: *aadA2* gene(622bp); F: *tetA(A)* gene(576 bp); G: *tetA(B)* gene(633 bp).

All *Salmonella* isolates from poultry origin were resistant to at more than one antimicrobial, indicating multiple drug resistance. These findings confirm that poultry is a major reservoir of multi-resistant *Salmonella* and this had no correlation with the presence of antimicrobial resistance gene indicating that other mechanisms of acquiring resistance could be present [25,36]. Molecular identification of *Salmonella* resistance genes showed that *qnrS* and *aac(6')-ib-cr* resistance genes for quinolones were reported with the percentages of 15% and 10 %, respectively. This with inconstant with Kim *et al.* [37] who investigated the prevalence and characteristics of plasmid mediated quinolone resistance

genes (*qnr*, *aac(6')-ib-cr*) and found that 3.2% of the isolates contained *qnr* genes but none carried the *aac(6')-ib-cr*. While, Hao *et al.* [38] reported *aac(6')-ib-cr* and *qnrS* resistance genes in *Salmonella* isolates with the percentages of 20.2% and 1.6%, respectively.

The *blaTEM* gene, a gene encoded for β -lactamases resistance was reported in the present study with the percentage of 93.3%. This was similar to a study performed by Hur *et al.* [39] who reported that 90.5% of penicillin resistant *S. Enteritidis* carried the *blaTEM* gene. In another study, the percentage of *blaTEM* was 10% which was identified in 10 *Salmonella* isolates from retail chicken meat [40]. However, *blaTEM* gene was

detected in 51.6% resistant *Salmonella* isolates [41]. Aslam *et al.* [42] reported that the percentage of *bla*TEM gene in *Salmonella* isolated from retail meats in Canada was 17% and this gene was the most common resistance genes found. A total of 108 *S. Indiana* possessed *bla*TEM gene with a percentage of 81.2% [43]. The *aadA2* gene, a gene encoded for streptomycin resistance was reported in the present study with the percentage of 80%. These results agree with a study performed by Shahada *et al.* [44] who reported that all streptomycin resistant *S. Infantis* from poultry in Japan carried *aadA1* gene, while, Mohamed [19] recorded that 17 isolates possessed *aadA1* gene with the percentage 53.1%. Sheng *et al.* [45] reported lower percentage of *aadA2* gene in only three *Salmonella* isolates out of seventy-three isolates.

The *tetA* gene, a gene encoded for tetracycline resistance was reported in the present study with the percentage of 70%. Higher percentage was obtained by Lu *et al.* [46] who reported that 108 *Salmonella* isolates possessed *tetA* gene with the percentage of 81.2%. Moreover, Shahada *et al.* [44] reported that 89% of oxytetracycline-resistant *Salmonella* from poultry carried the *tet(A)* gene. Percentages ranging from 66.7%-100% of *Salmonella* carriage of tetracycline resistance gene *tetA* were reported by several studies [39,47-50]. Another study by Aslam *et al.* [42] reported that the percentage of *tet(A)* gene in *Salmonella* isolates was 28%. Bacci *et al.* [51] recorded that *tet* class genes are considered the most common types in gram negative bacteria, also *tetA* and *tetB* genes are located inside non-conjugative transposons which are important way for the horizontal transfer of antibiotic resistance.

Conclusion

It could be concluded that the antimicrobial resistance genes of *Salmonella* infected chickens were extensively spread in the study area. Thus, leading to minimizing the influence of antibiotics in both treatment and prevention and increase public health significance. Therefore, other tools of prevention and treatment are important to avoid this problem.

Conflict of interest

The authors declare no conflict of interest.

References

- [1] Selvaraj, R.; Das, D.; Ganguly, S.; Ganguli, M.; Dhanalakshmi, S. and Mukhoadhayay, K. (2010): Characterization and antibiogram of *Salmonella* spp from poultry specimens. *J Microbiol Antimic*, 2(9) :123-126.
- [2] Brenner, F. W.; Villar, R. G.; Angulo, F. J.; Tauxe, R. and Swaminathan, B. (2000): *Salmonella* nomenclature. *J Clin Microbial*, 38(7):2465-2467.
- [3] Su, L. and Chiu, C. (2007): *Salmonella*: clinical importance and evolution of nomenclature. *Chang Gung Med J*, 30(3):210-219.
- [4] Bertrand, S. (2010): Rapport Annuel 2009-Centre National de Référence des *Salmonella* et *Shigella* –Souches de *Salmonella* et *Shigella* isolées en Belgique en 2009.
- [5] Oludairo, O.O.; Kwaga, J.K.P.; Dzikwi, A.A. and Kabir, J. (2013): The genus *Salmonella*, isolation and occurrence in wildlife. *Int J Microbiol Immunol Res*, 1(5):47-52.
- [6] Cruchaga, S.; Echieta, A.; Aladuena, A.; Garcia-Pena, J.; Frias, N. and Usera, M.A. (2001): Antimicrobial resistance in *Salmonella* from human food and animals in Spain. *J. Antimicrob Chemother*, 47: 315-321.
- [7] WHO (2002): World Health organization. Department of communicable diseases surveillance and response. Geneva, Fact sheet N194.
- [8] WHO (2005): World Health organization. Drug-resistant *Salmonella*. Food safety Geneva, Fact sheet N139.
- [9] Khallaf, M.; Ameer, N.; Terta, M.; Lakranbi, M.; Senouci, S. and Ennaji, M.M. (2014): Prevalence and antibiotic resistance of *Salmonella* isolated from chicken meat marketed in Rabat, Morocco. *International Journal of Innovation and Applied Studies*, 6(4):1123-1128.

- [10] ISO 6579 (2002): Microbiology of food and animal feeding stuffs-horizontal method for detection of *Salmonella* spp. International standard. (4th edition).
- [11] Cruickshank, R.; Duguid, J. P.; Marmion, B.P. and Swain, R.H.A. (1975): Medical microbiology. The practice of Medical Microbiology. 12th Ed. Vol. II Churchill Living stone, Edinburgh, London and New York., 2: 170-188.
- [12] Finegold, S.M. and Martin, E.T. (1982): Diagnostic microbiology. 6th Ed., The C.V. Mosby Company, St. Louis, Toronto, London.
- [13] CLSI (2011): Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fifth Informational Supplement (M100-S25).
- [14] Randall, L.P.; Cooles, S.W.; Osborn, M.K.; Piddock, L.J.V.; Woodward, M.J. (2004): Antibiotic resistance genes, integrons and multiple antibiotic resistance in thirty- five serotypes of *Salmonella enterica* isolated from humans and animals in the UK. J Antimicrob Chemother. 53(2);208- 216.
- [15] Walker, R.A.; Lindsay, E. and Woodward, M.J. (2001): Variation in clonality and antibiotic- resistance genes among multi- resistant *Salmonella enterica* serotype Typhimurium phage-type U302 (MR U302) from humans, animals and foods. Microb Drug Resist, 7(1):13- 21.
- [16] Colom, K.; Perez, J.; Alonoso, R.; Fernandez- Aranguiz, A.; Larino, E. and Cisterna, R. (2003): Simple and reliable multiplex PCR assay for detection of *bla*TEM, *bla*SHV, *bla*OXA-1 genes in Enterobacteriaceae. FEMS Microbiol Lett, 223 (2):147-151.
- [17] Lunn, A.D.; fabrega, A.; Sanchez-Céspedes, J. and Vila, J. (2010): Prevalence of mechanisms decreasing quinolone- susceptibility among *Salmonella* spp. clinical isolates. Int Microbiol, 13(1):15-20.
- [18] Rehan, H.M.A. (2004): Application of PCR identification of *Salmonella* in chickens. M.V.Sc., Thesis, Fac. Vet. Med., Mansoura Uni., Egypt
- [19] Mohamed, N.M (2015): Molecular studies on antibiotic drug resistance *Salmonella* in poultry. PhD Thesis, Faculty of Veterinary Medicine, Benha University, Egypt.
- [20] Al-Abadi, I.K.M. and AL- Mayah, A.A.S. (2012): Isolation and identification of *Salmonella* spp. from chicken and chicken environment in Basrah province. Afr J Biol Sci, 7 (1): 33-43 .
- [21] Al-Hakeem, Z, Kh. Kh. (2003): Contamination of the parent stock environment with *Salmonella* . M.Sc Thesis. College of Veterinary Medicine. Musol Univerisity.
- [22] Dhahar, F.H.; Awni, M.D.H.; Mahmood, N.R. and Jamil, M.M. (2011): isolation and diagnosis of *Salmonella* in Animal origin food. Iraq Acad Sci J, 3(5):1-19.
- [23] AL-Iedani, A.A.; Khudor, M.H. and Ouafi, N.M. (2014): Isolation and identification of *Salmonella* spp. from poultry farms by using different techniques and evaluation of their antimicrobial susceptibilities. Bas J Vet Res, 13(1): 246-259.
- [24] Habrun, B.; Simpraga, B.; Kompes, G. and Krstulovic, F. (2012): Antimicrobial resistance and serotyping of *Salmonella enterica* subsp. *enterica* isolated from poultry in Croatia. Veterinarski Arhiv, 82(4):371-381.
- [25] Okamoto, A.S; Andreatti Filho, R.L; Rocha, T.S.; Menconi, A. and Maritto, A. (2009): Detection and transfer of antimicrobial resistance gene integron in *Salmonella* Enteritidis derived from avian material. Revista Brasileira de Ciência Avícola, 11(3):195-201.
- [26] Cardoso, M.O.; Ribeiro, A.R.; Santos, L.R.; Pilotto, F.; de Moraes, H.S.; Salle, C.P.; Rocha, S.S. and Nascimento, V.P. (2006): Antibiotic resistance of *Salmonella* Enteritidis isolated from broiler carcasses. Braz J Microbiol, 37(3): 368-371.

- [27] Shivhare, S.; Sharda, R.; Reddy, A.; Sharma, R. and Sharma, V. (2000): Antibiotic sensitivity of *Salmonella* Typhimurium isolates from domestic poultry. *Ind Vet J*, 77 (11): 998-999.
- [28] Snow, L.C.; Davies, R.H.; Christiansen, K.H.; Carrique-Mas, J.J.; Wales, A.D.; O'Connor, J.L.; Cook, A.J. and Evans, S.J. (2007): Survey of the prevalence of *Salmonella* species on commercial laying farms in the United Kingdom. *Vet Record*, 161(14):471-476.
- [29] Zdragas, A.; Mazaraki, K.; Vafeas, G.; Giantzi, V.; Papadopoulos, T. and Ekateriniadou, L. (2012): Prevalence, seasonal occurrence and antimicrobial resistance of *Salmonella* in poultry retail products in Greece. *Lett Appl Microbiol*, 55(4):308-313.
- [30] Khan, M.; Suryanarayan, P.; Ahmed, M.; Vaswani, R. and Faheem, S. (2010): Antimicrobial susceptibility of *Salmonella* isolates from chicken meat samples in Dubai, United Arab Emirates. *International Journal of Food, Nutrition and Public Health*, 3(2):149- 159.
- [31] Donado-Godoy, P.; Clavijo, V.; León, M.; Tafur, M.A.; Gonzales, S.; Hume, M.; Alali, W.; Walls, I.; Lo Fo Wong, D.M. and Doyle, M.P. (2012): Prevalence of *Salmonella* on retail broiler chicken meat carcasses in Colombia. *J Food Prot*, 75(6):1134-1138.
- [32] Yah, S.C. and Eghafona, N.O. (2007): Plasmids: A vehicle for rapid transfer of antibiotic resistance markers of *Salmonella* species in animals. *J Amer Sci*, 3 (4): 86-92.
- [33] Threlfall, E.J. (2002): Antimicrobial drug resistance in *Salmonella*: problems and perspectives in food and water-borne infection. *FEMS Microbiol Reviews*, 26(2):141-148.
- [34] Hendriksen, S.W. (2004): Animal-to-Human transmission of *Salmonella typhimurium* DT104A Variant. *Emer infect Dis J*, 10(12):2225-2227.
- [35] Asway, A.M.E. and El-latif, M.M.A. (2010): Some bacteriological and serological studies on enteritis in ducks. *Assiut Vet Med J*, 56(125):239-249.
- [36] Álvarez- Fernandez, E.; Alonso-Calleja, C.; García-Fernández, C. and Capita, R. (2012): Prevalence and antimicrobial resistance of *Salmonella* serotypes isolated from poultry in Spain: comparison between 1993 and 2006. *Int J food Microbiol*, 153(3):281-287.
- [37] Kim, J.H.; Cho, J.K. and Kim, K.S. (2013): Prevalence and characterization of plasmid-mediated quinolone resistance genes in *Salmonella* isolated from poultry in Korea isolated from poultry in Korea. *Avian Pathol*, 42(3):221-229.
- [38] Hao, H.; Yang, B.; Shi, J.; Xi, M.; Wang, X.; Cui, Y. and Meng, J. (2011): drug resistance and related genes of chicken borne *Salmonella* to quinolone and fluoroquinolones. *Wei Sheng Wu Xue Bao = Acta microbiologica Sinica*, 51(10):1413-1420.
- [39] Hur, J.; Kim, J.H.; Park, J.H.; Lee, Y.J. and Lee, J.H. (2011): Molecular and virulence characteristics of multi-drug resistant *Salmonella* Enteritidis strains isolated from poultry. *Vet J*, 189(3):306-311.
- [40] Ahmed, A.M.; Shimabukuro, H. and Shimamoto, T. (2009): Isolation and molecular characterization of multidrug-resistant strains of *Escherichia coli* and *Salmonella* from retail chicken meat in Japan. *J Food Sci*, 74(7):405-410.
- [41] Yang, B.; Qu, D.; Shen, J.; Xi, M.; Zhi, S.; Cui, S.; Ji, B. and Meng, J. (2010): Antimicrobial susceptibility and related genes of *Salmonella* serovars from retail food in Shaanxi province. *Wei Sheng Wu Xue Bao = Acta microbiologica Sinica*, 50(6):788-796.
- [42] Aslam, M.; Checkley, S.; Avery, B.; Chalmers, G.; Bohaychuk, V.; Gensler, G.; Reid-Smith, R. and Boerlin, P. (2012): Phenotypic and genetic characterization of antimicrobial resistance in *Salmonella* isolated from retail meats in Alberta, Canada. *Food Microbiol*, 32(1):110-117.

- [43] Lu, Y.; Wu, C.M.; Wu, G.J.; Zhao, H.Y.; He, T.; Cao, X.Y.; Dai, L.; Xia, L.N.; Qin, S.S. and Shen, J.Z. (2011): Prevalence of antimicrobial resistance among *Salmonella* isolates from chicken in China. *Foodborne Pathog Dis*, 8(1):45-53.
- [44] Shahada, F.; Chuma, T.; Tobata, T.; Okamoto, K.; Sueyoshi, M. and Takase, K. (2006): Molecular epidemiology of antimicrobial resistance among *Salmonella enterica* serovar Infantis from poultry in Kagoshima, Japan. *Int J Antimicrob Agents*, 28(4):302-307.
- [45] Sheng, C.; Shaohua, Z.; David, G. W.; Carl, M.; Ran L.; Hanchun, Y.; Patrick, F.; McDermott, S. and Jianghong, M. (2004): Characterization of multiple antibiotic resistant *Salmonella* serovars Isolated from Retail Meats. *Appl Environ Microbiol*, 70(1):1-7.
- [46] Lu, Y.; Zhao, H.; Liu, Y.; Zhou, X.; Beier, R.C.; Wu, G. and Hou, X. (2014): Characterization of Multidrug-Resistant *Salmonella enterica* Serovars Indiana and Enteritidis from Chickens in Eastern China. *PLoS One*, 9 (5): e96050.
- [47] Ahmed, A. M. and Shimamoto, T. (2012): Genetic analysis of multiple

antimicrobial resistance in *Salmonella* isolated from diseased broilers in Egypt. *Microbiol Immunol*, 56(4):254-261.

- [48] Nde, C.W. and Logue, C.M. (2008): Characterization of antimicrobial susceptibility and virulence genes of *Salmonella* serovars collected at a commercial turkey processing plant. *J Appl Microbiol*, 104(1):215-223.

- [49] Zhang, C.P.; Ning, Y.; Song, L.; Chen, H. and Chen, R. (2011): Investigation of resistance to tetracycline and distribution of tetracycline resistance genes of *Salmonella* isolates from poultry. *China Poult*, 33(3):18-21.

- [50] Zou, W.; Frye, J. G.; Chang, C.W.; Liu, J.; Cerniglia, C. E. and Nayak, R. (2009): Microarray analysis of antimicrobial resistance genes in *Salmonella enterica* from preharvest poultry environment. *J Appl Microbiol*, 107(3):906-914.

- [51] Bacci, C.; Boni, E.; Alpighiani, I.; Lanzoni, E.; Bonardi, S. and Brindani, F. (2012): Phenotypic and genotypic features of antibiotic resistance in *Salmonella* isolated from chicken meat and chicken and quail carcasses. *Int J Food Microbiol*, 160:16-23.

الملخص العربي

الكشف الجزيئي على بعض الجينات المقاومة لمضادات الميكروبات في السالمونيلا المعزولة من الدجاج البياض التجاري في مصر

محمد عبد العزيز لبد،^١ وفاء محمد محمد،^٢ سماح عيد،^٣ رحاب اسماعيل حامد^٣

^١ قسم طب الطيور والارانب - كلية الطب البيطري - جامعة الزقازيق

^٢ المعمل المرجعي للرقابة علي الانتاج الداجني معمل بحوث صحة الحيوان - الدقى - الجيزة

^٣ المعمل المرجعي للرقابة علي الانتاج الداجني معمل بحوث صحة الحيوان - فرع الشرقيه

تهدف هذه الدراسة الي دراسة مدي الانتشار الواسع لمقاومة عترات السالمونيلا المعزولة من قطعان الاستبدال والبيض في مصر لبعض مجموعات مضادات الميكروبات المختلفة. تم فحص ٢٤ سالمونيلا معزولة من ٢٠٠ طائر (التي تبدو سليمة واخري تعاني من اسهالات وجفاف وأعراض تنفسية وإنخفاض في معدل انتاج البيض) والتي تم تصنيفها سيرولوجيا الي *Salmonella Enteritidis*, *S. Typhimurium*, *S. Kentucky* and *S. Newport*. ٢١ معزولة من السالمونيلا تم اختبارها لاستبيان وجود جينات المقاومة للمضادات الميكروبية المختلفة. تم التعرف على النمط المقاوم للمضادات الحيوية باستخدام اختبار الحساسية. كما تم اختبار المعزولات المقاومة للتأكد من وجود جينات المقاومة للميكروبات باستخدام اختبار البلمرة المتسلسل للجينات *qnrS*, *aac(6)-ib-cr* للكينولون، *blaTEM* للبيتالاكتام، *aadA1*, *aadA2* للامينوجليكوسايدس، *tetA(A)*, *tetA(B)* للتيتراسيكلين. وقد أظهرت النتائج أن الجينات المقاومة للمضادات الحيوية *tetA(A)*, *tetA(B)*, *blaTEM*, *aadA1*, *aadA2*, *aac(6)-ib-cr* and *qnrS* جاءت بالنسب ٧٠%، ٢٠%، ٩٣،٣%، ٣٠%، ٨٠%، ١٠% و ١٥% علي التوالي. ويستخلص من هذه الدراسة مدي انتشار عزلات السالمونيلا المقاومة للمضادات الميكروبية في الدواجن بالمنطقة محل الدراسة مما يؤدي الي التقليل من تأثير المضادات الحيوية في العلاج والوقاية.