Molecular studies on antimicrobial resistance genes in *salmonella* isolated from poultry flocks in Egypt

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A B S T R A C T

This study was aimed to investigate the wide spread resistance to different antimicrobial groups in between different Salmonellae isolated from poultry flocks in Egypt. A total of 63 Salmonella isolates (19 from chickens, 3 from ducks, 2 from turkeys, 2 from quails and 2 from pigeons) were examined in this study for their wide spread resistance against different antimicrobial agents. The antimicrobial susceptibility was applied on all isolates then PCR was applied for all resistant isolates to detect the resistance genes of different antimicrobial agents (*qep*A, *qnr*S, *aac (6') - ib-cr*) for quinolone resistant isolates, *bla* Tem for β lactam resistant isolates, *sul*1 for Sulfonamide resistant isolates, *flo*R for Florphenicol resistant isolates, *aad*A2 for Streptomycin resistant isolates and *tet*A (A) for Oxytetracycline resistant isolates, in addition to gyrase enzyme gene (*gyr*A). DNA sequencing was done for three selected isolates to detect possible mutations in the quinolone-resistance determining regions of the *gyr*A gene.

Keywords: Salmonella, resistance genes, poultry

(http://www.bvmj.bu.edu.eg) **(BVMJ-28(2): 176-187, 2015)**

1. INTRODUCTION

Avian salmonellosis is a problem of economic concern to all phases of poultry industry from production to marketing. 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 (Cruchaga *et al*., 2001). Antimicrobial resistance is a global public health problem. Although all countries are affected, the extent of the problem in the developing nations is unknown (Ang *et al.*, 2004). From the beginning of the 1990s, strains of Salmonella which are resistant to a range of antimicrobials, including first-choice agents or the treatment of human and animals, have emerged and are threatening to become a serious public health problem. This resistance results from the use of antimicrobials both in human and animal husbandry (WHO, 2006). This study aimed to detect the different antimicrobial resistance genes in between different types of Salmonellae.

2. MATERIAL AND METHODS

2.1. Source of Salmonella strains used in this study

A total of 63 isolates were used in this study; 19 strains isolated from chickens (*S.* Typhimurium, *S.* Apeyeme, *S.* Kentucky, *S.* Daula, *S.* Newport, *S.* Tamale, *S.* Molade, *S.* Colindale, *S.* Lexington, S. Bargny, *S.*

Enteritidis, *S*. Papuana, *S*. Labadi, *S*. Santiago, *S*. Magherafelt, *S*. Rechovot, *S*. Takoradi, *S*. Angers, *S*. Shubra and untyped Salmonella), 3 strains isolated from ducks (*S*. Inganda, *S*. Infantis, *S*. Larochelle and untyped Salmonella), 2 strains isolated from turkeys (*S*. Virchow and *S*. Vejle), 2 strains isolated from quails (*S*. Shangani and *S*. Jedburgh) and 2 strains isolated from pigeons (*S*. Alfort and *S*. Wingrove). The used isolates were collected from different poultry species in which they were isolated in Reference Laboratory for Veterinary Quality Control in Dokki.

2.2. Antimicrobial susceptibility testing

The antimicrobial susceptibility testing was done according to Finegold and Martin (1982) using the agar disc diffusion method on Mueller Hinton agar (Oxoid) plates. The used antimicrobial agents were Florphenicol (30 µg), Nalidixic acid (30µg), Flumequine (30µg), Ciprofloxacin (5µg), Enrofloxacin (5µg), Norfloxacin (10µg), Levofloxacin (5µg), Amoxicillin (10 µg), Ampicillin – sulbactam (20µg), Cefotaxime (30µg), Ceftriaxone (30µg), Ceftazidime (30µg), Neomycin(30µg), Amikacin (30 µg), Gentamycin (10µg) , Streptomycin (10µg), Oxytetracyclin (30µg) and Sulfamrthoxazole-Trimethoprim (25µg). The zones of inhibition that formed were measured to assess resistance or susceptibility according to the interpretation criteria established by (CLSI, 2011).

2.3. 2.3. Detection of resistance genes in isolated Salmonellae using PCR

DNA was extracted from the isolates that showed resistance in antimicrobial susceptibility tests. The isolates that showed resistance to quinolones were examined for presence of [*qep*A, *qnr*S and *aac*(6')-ib-cr] genes while the isolates that showed resistance to Sulfamethoxazol/ Trimethoprim were examined for presence of *sul* 1 gene but the isolates that showed resistance to B- lactams were examined for the presence of *bla* TEM gene. The isolates that showed resistance to Florfenicol were examined for the presence of *flo*R gene while the isolates that showed resistance to Streptomycin were examined for presence of *aad*A2 gene but the isolates that showed resistance to Oxytetracyclin were examined for *tet*A (A) gene. The DNA extraction for the selected isolates was performed using ABIOpure Genomic DNA extraction kit. The Oligonucleotide Primers which provided from Metabion (Germany) are listed in table (1). The primers were utilized in a 25 µl reaction containing 12.5 µl of EmeraldAmp Max PCR Master Mix (Takara, Japan), 1 µl of each primer of 20 pmol concentrations, 4.5 µl of water, and 6 µl of template. The reaction was performed in a Biometra thermal cycler. The products of PCR were separated by electrophoresis on 1-1.5% 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.

2.4. Sequencing of the gyrA gene

DNA sequencing for *gyr*A gene was done for 3 representative strains (*S*. Infantis from ducks, *S*. Enteritidis from chickens and *S*. Vejle from turkeys). PCR products were purified using QIA quick PCR Product extraction kit (QIAGEN Inc, Valencia, CA, USA). Big dye Terminator V3.1 cycle sequencing kit. (Perkin-Elmer/Applied Biosystems, Foster City, CA) was used for the sequence reaction and then it was purified using Centrisep spin column. DNA sequences were obtained by Applied Biosystems 3130 genetic analyzer (HITACHI, Japan) and the phylogenetic tree was created by the MegAlign module of Lasergene DNA Star software.

3. RESULTS

3.1. Results of the antimicrobial susceptibility tests for the isolated Salmonellae

Fifty five isolates from a total of 63 Salmonella isolates showed the highest percentage of resistance (87.3%) to Nalidixic acid and Flumequin. Five isolates from these 63 Salmonella isolates showed the lowest percentage of resistance (7.9%) to Levofloxacin. Amikacin showed highest sensitivity (100 %) in all isolates from all examined species but 8 isolates showed the lowest sensitivity (12.7%) to Nalidixic acid and Flumequin (Table 2) and (Table 3).

3.2. Results of Polymerase Chain Reaction technique for different resistance genes in the examined isolates.

All Salmonella isolates that showed resistance against different antimicrobial agents were examined by PCR to detect the incidence of resistance genes in the examined isolates which was (87%) for *sul* 1, (83.7%) for *tet*A (A), (77.8%) for *flo*R, (69.4%) for *bla* TEM, (53.1%) for *aad*A2, (19.6%) for *aac* (6')-ib-cr, (10.7%) for *qnr*S and (3.5%) for *qep*A (Table, 4).

3.3. DNA sequencing

The 3 *gyr*A sequenced strains (*S*. Infantis, *S*. Enteritidis and *S*. Vejle; isolates number 14, 40, 45 respectively in this study) showed two sits of mutation in the amino acids of *S*. Enteritidis (isolate NO. 40) (KP290113) at the position 87 which changed from Aspartic acid (D) (standard protein) to Glycine (G) (mutated protein) and at the position 176 mutated from Valine (V) (standard protein) to Alanine (A) (mutated protein). No mutations in *gyr*A gene of *S*. Infantis (KP290114) or *S*. Vejle (KP290112) were recorded in this study (Table, 5).

Table 1. Oligonucleotide primers for PCR.

Primers	Nucleotide Sequence	References
*tet*A (A) (Tetracycline)	F(5'-GGTTCACTCGAACGACGTCA -3') R(5'-CTGTCCGACAAGTTGCATGA -3')	Randall *et al*., 2004
*aad*A2 (Streptomycin)	F (5'-TGTTGGTTACTGTGGCCGTA -3') R(5'-GATCTCGCCTTTCACAAAGC -3')	Walker *et al*., 2001
*bla*TEM (B-lactamas)	F(5′-ATCAGCAATAAACCAGC -3′) R(5′-CCCCGAAGAACGTTTTC -3′)	Colom *et al*., 2003
sul 1 (Sulfonamides)	F(5'- CGGCGTGGGCTACCTGAACG-3') R(5'- GCCGATCGCGTGAAGTTCCG-3')	Sabarinath *et al*., 2011
*qep*A (Quinolones)	F(5'-CGTGTTGCTGGAGTTCTTC -3') R(5'-CTGCAGGTACTGCGTCATG -3')	Cattoir *et al*., 2008
aac(6')-ib-cr (Quinolones)	F(5'-CCCGCTTTCTCGTAGCA -3') R(5'-TTAGGCATCACTGCGTCTTC -3')	Lunn *et al*., 2010
*qnr*S (Quinolones)	F(5'-ACGACATTCGTCAACTGCAA -3') R(5'-TAAATTGGCACCCTGTAGGC -3')	Robicsek *et al*., 2006
*flo*R (Florphenicol)	F(5'-TTTGGWCCGCTMTCRGAC -3') R(5'-SGAGAARAAGACGAAGAAG -3')	Doublet *et al*., 2003
*gyr*A (Quinolones mutation gene)	F (5'-AAATCTGCCCGTGTCGTTGGT-3') R(5'-GCCATACCTACTGCGATACC-3')	Fàbrega *et al*., 2009

Table 2. Numbers and percentages of Salmonella isolates from poultry exhibiting resistance to various antimicrobials.

Antimicrobial agent	resistant chicken isolates		resistant duck isolates		resistant turkey isolates		resistant quail isolates		resistant pigeon isolates		Total	
	No	%*	No	%	No	%	No	%	No	%	No	%**
Florphenicol	19	44.2	4	40	1	33.3	3	60	0	0	27	42.9
Nalidixic acid	43	100	9	90	1	33.3	1	20	1	50	55	87.3
Flumequin	43	100	9	90	1	33.3	1	20	1	50	55	87.3
Ciprofloxacin	15	34.9	0	0	0	0	0	0	0	0	15	23.8
Enerofloxacin	23	53.2	2	20	0	0	0	0	0	0	25	39.7
Norfloxacin	9	20.9	1	10	0	0	0	0	0	0	10	15.9
Levofloxacin	5	11.6	0	0	0	0	0	0	0	0	5	7.9
Amoxicillin	39	90.7	5	50	1	33.3	2	40	2	100	49	77.8
Ampicilliln/sulbctam	33	76.7	6	60	0	0	2	40	2	100	43	68.3
Cefotaxime	7	16.3	0	0	0	0	0	0	0	0	7	11.1
Ceftriaxone	10	23.3	1	10	0	0	0	0	0	0	11	17.5
Ceftazidime	11	25.6	1	10	0	0	0	0	0	0	12	19.1
Neomycin	18	41.9	4	40	1	33.3	0	0	0	0	23	36.5
Amikacin	0	0	0	0	0	0	0	0	0	0	0	0
Gentamycin	13	30.2	1	10	0	0	0	0	0	0	14	22.2
Streptomycin	23	53.5	6	60	1	33.3	2	40	0	0	32	50.8
Oxytetracyclin	34	79.1	6	60	1	33.3	1	20	1	50	43	68.3
Sulfa+trimethoprim	25	58.1	4	40	1	33.3	1	20	0	0	31	49.2

* The percentage calculated by dividing the number of isolates that showed resistance to each antimicrobial agent individually on the number of Salmonella isolates from each species (19/43x100= 44.2%). ** The percentage calculated by dividing the total number of isolates that showed resistance from all species to each antimicrobial agent individually on the total number of Salmonella isolates from all species (27/63x100= 42.9%).

4. DISCUSSION

According to the results concerning antimicrobial susceptibility tests in table (2)& (3), 55 isolates showed the highest percentage of resistance (87.3%) to Nalidixic acid and Flumequin. Five isolates showed the lowest percentage of resistance (7.9%) to Levofloxacin. All Salmonella isolates from all examined species were sensitive to Amikacin (100 %) but 8 isolates from all examined isolates showed the lowest sensitivity (12.7%) to Nalidixic acid and Flumequin. The obtained results were differed from Boris *et al*., (2012) who reported that all Salmonella isolates were sensitive to Chloramphenicol and Streptomycin (100%) while 92 isolates (58%) were sensitive to Nalidixic acid and of 66 (41.7%) isolates were sensitive to all antibiotics. Contrary to these results Cardoso *et al*., (2006) who reported that Salmonella showed sensitivity to doxycycline hydrochloride with 100%. However Khan *et al.*, (2010) stated that all Salmonella isolates exhibit (100%) resistant to Cephalexin and Rifampicin while about 90% and 88% of the isolates were resistant to Ampicillin and tetracycline. In this study forty three chicken isolates showed resistance with 100% against Nalidixic acid and Flumequin but five isolates showed the lowest resistance (11.6%) to levofloxacin while 43 chicken isolates were sensitive to Amikacin (100%) but 38 isolates were sensitive to Levofloxacin with a percentage

Table 3. Numbers and percentages of sensitive Salmonella isolates from poultry.

Antimicrobial agent	sensitive chicken isolates		sensitive ducks isolates		sensitive turkeys isolates		sensitive quails isolates		sensitive pigeons isolates		Total	
	No	%*	No	%	No	%	No	%	No	%	No	%**
Florphenicol	24	55.8	6	60	2	66.7	2	40	2	100	36	57.1
Nalidixic acid	0	0	1	10	2	66.7	4	80	1	50	8	12.7
Flumequin	0	0	1	10	2	66.7	4	80	1	50	8	12.7
Ciprofloxacin	28	65.1	10	100	3	100	5	100	2	100	48	76.2
Enerofloxacin	20	46.5	8	80	3	100	5	100	2	100	38	60.3
Norfloxacin	34	79.1	9	90	3	100	5	100	2	100	53	84.1
Levofloxacin	38	88.4	10	100	3	100	5	100	2	100	58	92.1
Amoxicillin	4	9.3	5	50	2	66.7	3	60	0	0	14	22.2
Ampicilliln/sulbctam	10	23.3	4	40	3	100	3	60	0	0	20	31.7
Cefotaxime	36	83.7	10	100	3	100	5	100	2	100	56	88.9
Ceftriaxone	33	52.4	9	90	3	100	5	100	2	100	52	82.5
Ceftazidime	32	74.4	9	90	3	100	5	100	2	100	51	80.9
Neomycin	25	58.1	6	60	2	66.7	5	100	2	100	40	63.5
Amikacin	43	100	10	100	3	100	5	100	2	100	63	100
Gentamycin	30	69.8	9	90	3	100	5	100	2	100	49	77.8
Streptomycin	20	46.5	4	40	2	66.7	3	60	2	100	31	49.2
Oxytetracyclin	9	20.9	4	40	2	66.7	4	80	1	50	20	31.7
Sulfa+trimethoprim	18	41.9	6	60	2	66.7	4	80	2	100	32	50.8

* The percentage calculated by dividing the number of isolates that were sensitive to each antimicrobial agent individually on the number of Salmonella isolates from each species (24/43x100= 55.8%). ** The percentage calculated by dividing the total number of isolates that were sensitive to each antimicrobial agent individually (from all species) on the total number of Salmonella isolates from all species (36/63x100= 57.1 %).

(88.4%) and 4 isolates were sensitive to Amoxicillin giving the lowest percentage (9.3%) and these results agreed with Snow *et al*., (2011) who reported that all Salmonella isolates from commercial layer flocks in UK were sensitive to Amikacin with (100%). The results in this study differ from Zdragas *et al*., (2012) who reported (5%) of 23 resistance to Streptomycin (highest resistance rates) and (2%) to Tetracycline and Nalidixic acid (lowest rate), Munawwar *et al.,* (2010) who reported (100%) resistance to Cephalexin and Rifampicin in Salmonella isolated from chicken meats in Dubai, while 87.88% of these Salmonellae were sensitive to ciprofloxacin and amikacin. Nine duck isolates in this study showed the highest resistance with (90%) to Nalidixic acid and Flumequin while Norfloxacin, Ceftriaxone, Ceftazidime and Gentamycin came in lowest percentage with (10%).Ciprofloxacin, Levofloxacin, Cefotaxime and Amikacin showed (100%) sensitivity while Nalidixic acid, Flumequin showed the lowest sensitivity with a percentage of (10%). These results agreed with Rahman *et al*., (1999) who detected that *S.* Enteritidis was susceptible to Cefotaxime and Ciprofloxacin, Pan *et al.,* (2009) who reported that Salmonella displayed a high level of resistance to Ampicillin, Streptomycin and Tetracycline. While these results differ from Hegazy (1991) who reported that 19 Salmonellae isolated from ducks in Kafr El- Sheik governorate were resistant to Ampcillin and Cephaloxin with percentage of 100% while Flumequine and Enrofloxacin were highly sensitive.

Table 4. Results of Polymerase Chain Reaction technique for different resistance genes from the examined isolates.

No	serotype	source	*qep*A	*qnr*S	*aac*(6')-ib-cr	*sul*1	bla TEM	*flo*R	*aad*A2	*tet*A (A)
1	*S*. Alfort	pigeon	-	-	-	NE	-	NE	NE	+
2	*S*.Typhmurium	chicken	-	-	-	NE	NE	NE	NE	NE
3	*S*.Typhmurium	chicken	-	-	+	NE	+	NE	-	-
4	*S*.Virchow	turkey	NE	NE	NE	NE	NE	NE	NE	NE
5	Untyped	chicken	-	-	+	NE	+	NE	NE	+
6	*S*.Typhmurium	chicken	-	-	-	NE	+	+	+	+
7	*S*. Apeyeme	chicken	-	-	-	NE	+	NE	+	+
8	*S*.Typhmurium	chicken	-	-	-	NE	+	NE	+	+
9	*S*.Typhmurium	chicken	-	-	-	+	+	NE	+	+
10	*S*.Typhmurium	chicken	-	-	-	+	NE	NE	NE	+
11	*S*. Kentucky	chicken	-	-	-	+	+	+	+	+
12	*S*. Daula	chicken	-	-	-	NE	+	NE	NE	+
13	*S*. Newport	chicken	-	-	-	+	+	NE	NE	+
14	*S*. Infantis	duck	-	+	-	+	+	+	+	+
15	*S*. Shangani	quail	-	-	-	NE	+	+	+	NE
16	*S*. Tamale	chicken	-	+	-	NE	+	NE	+	+
17	*S*. Molade	chicken	-	-	-	+	+	NE	NE	+
18	*S*.Typhmurium	chicken	-	-	-	+	+	+	+	+
19	Untyped	duck	-	+	-	+	-	+	-	+
20	*S*. Newport	chicken	-	-	-	+	+	+	+	+
21	*S*. Colindale	chicken	-	-	-	+	-	NE	-	+
22	Untyped	duck	-	-	-	NE	NE	NE	-	-
23	*S*. Shangani	quail	NE	NE	NE	NE	NE	+	+	NE
24	*S*. Newport	chicken	-	-	-	+	-	+	+	+
25	*S*.Virchow	turkey	NE	NE	NE	NE	NE	NE	NE	NE
26	*S*. lexington	chicken	-	-	-	-	-	NE	-	+
27	*S*. Bargny	chicken	-	-	+	NE	-	NE	-	+
28	*S*. Rechovot	chicken	-	+	+	+	+	+	+	NE
29	Untyped	duck	-	-	-	NE	NE	NE	NE	NE
30	*S*. Magherafelt	chicken	-	-	-	NE	+	NE	NE	-
31	*S*. Enteritidis	chicken	-	-	-	NE	-	+	NE	-
32	*S*. Inganda	duck	-	-	-	NE	-	NE	-	+
33	*S*. Magherafelt	chicken	-	+	-	+	+	NE	NE	+
34	*S*.Typhmurium	chicken	-	-	-	+	+	-	NE	NE
35	*S*. Santiago	chicken	-	-	-	NE	+	+	NE	+
36	*S*. Infantis	duck	-	-	-	NE	+	NE	NE	NE
37	*S*. Labadi	chicken	-	-	-	NE	-	-	-	+
38	*S*. Molade	chicken	-	-	+	+	+	NE	-	+
39	*S*. Tamale	chicken	-	-	+	NE	+	NE	NE	+

Table 4. (continued) Results of Polymerase Chain Reaction technique for different resistance genes from the examined isolates.

No	serotype	source	*qep*A	*qnr*S	aac(6')-ib-cr	*sul* 1	*bla* TEM	*flo*R	*aad* A2	*tet*A (A)
40	*S*.Enteritidis	chicken	-	-	+	+	NE	+	NE	+
41	*S*.Takoradi	chicken	-	-	-	+	+	-	+	-
42	*S*.Wingrove	pigeon	NE	NE	NE	NE	-	NE	NE	NE
43	*S*. Newport	chicken	-	-	-	+	+	+	-	+
44	*S*.Larochelle	duck	-	-	-	+	NE	NE	NE	NE
45	*S*. Vejle	turkey	-	+	+	+	+	+	+	+
46	*S*. Tamale	chicken	-	-	-	NE	-	NE	-	NE
47	*S*. Molade	chicken	+	-	+	NE	+	NE	-	+
48	*S*. Kentucky	chicken	+	-	-	+	+	NE	NE	+
49	*S*. Newport	chicken	-	-	-	+	+	+	NE	NE
50	*S*. Inganda	duck	-	-	-	NE	+	+	+	+
51	*S*. Kentucky	chicken	-	-	-	+	NE	NE	NE	NE
52	*S*. Newport	chicken	-	-	-	+	+	+	+	NE
53	*S*. Papuana	chicken	-	-	-	+	-	NE	-	NE
54	*S*. Kentucky	chicken	-	-	-	+	+	+	-	+
55	*S*. Santiago	chicken	-	-	+	NE	+	+	NE	NE
56	*S*. Infantis	duck	-	-	-	-	-	-	-	+
57	*S*. Angers	chicken	-	-	-	-	-	-	NE	-
58	*S*. Bargny	chicken	-	-	+	+	+	+	NE	+
59	*S*. Shubra	chicken	-	-	-	NE	NE	NE	NE	+
60	*S*. Shangani	quail	-	-	-	-	-	-	NE	-
61	*S*. Jedburgh	quail	NE	NE	NE	NE	NE	NE	NE	NE
62	*S*. Jedburgh	quail	NE	NE	NE	NE	NE	NE	NE	NE
63	Untyped	duck	NE	NE	NE	NE	NE	NE	NE	NE
Total examined Samples			56	56	56	31	49	27	32	43
No. of positive			2	6	11	27	34	21	17	36
% of positive			3.5	10.7	19.6	87	69.4	77.8	53.1	83.7

*(qep*A - *qnr*S - aac (6')-ib-cr)= resistant genes for quinolones
sul 1= resistant gene for sulfonamide & *bla* TEM= resistant genes for B-lactamases
*flo*R= resistant gene for florfenicol & *aad*A2= resistant gene for streptomycin
*tet*A (A) = resistant gene for tetracycline. &NE = not examined by PCR = sensitive samples.

Table 5. Nucleotide changes in the *gyr*A gene of Salmonella serovars.

Strain no.	Accession no.	Serovar	Species	Nucleotide change at *gyrA* Positions		
				83 TCC [Ser]	87 GAC [Asp]	176 GTA [Val]
14	KP290114	Infantis	Duck	None	None	None
40	KP290113	Enteritidis	Chicken	None	G<u>G</u>C [Gly]	G<u>C</u>A [Ala]
45	KP290112	Vejle	Turkey	None	None	None

In turkey isolates, the highest percentage of resistance (33.3%) was to Florphenicol, Nalidixic acid and Flumequin, Amoxicillin, Neomycin, Streptomycin, Oxytetracyclin, Sulfamethoxazole /Trimethoprim but other antimicrobial agents showed no resistance. These obtained result nearly in coordinated with Jodas and Hafez (2003) who reported that all of the examined Salmonella isolates from turkeys were sensitive to Enrofloxacin (98%) while these results differ from Blackburn *et al*., (1982) who reported that all Salmonella strains isolated from turkeys were (100%) resistant to Gentamicin. In addition to Pederson *et al*., (2002) who stated that 1.7 %, 1.7 %, 8.7 %, and 9 % of strains isolated from Danish turkeys between 1995 and 2000, were resistant to Gentamicin, Trimethoprim/ Sulphamethoxazole, Tetracycline and Streptomycin.

In quail isolates, 60% resistance to Florphenicol and the lowest percentage was 20% to Nalidixic acid, Flumequin, Oxytetracyclin and Sulfamethoxazole/ Trimethoprim while Ciprofloxacin, Enerofloxacin, Norfloxacin, Levofloxacin Cefotaxime, Ceftriaxone, Ceftazidime, Neomycin, Amikacin and Gentamycin showed sensitivity with 100% but Florphenicol was the lowest sensitivity with percentage of 40%. The results in this study nearly in coordinated with Rahman *et al*., (2011) and differ from Bacci *et al*., (2012) who reported that the isolated Salmonellae from quails exhibited 89.2% resistance to Sulfamethoxazole and 24.3% to Ampicillin, Gentamicin, Sulfamethoxazole and Tetracycline in addition to Helm *et al*., (1999) who reported that Salmonella typhimurium isolated from bobwhite quail was resistant to Ampicillin, Chloramphenicol, Sulfonamides, and Tetracycline.

Pigeon isolates showed resistance (100%) to Amoxicillin and Ampicilliln/ Sulbctam followed by Nalidix acid, Flumequin and Oxytetracyclin (50%) while the isolates showed sensitivity with 100% to all examined antimicrobial agents except Nalidixic acid, flumequin and oxytetracyclin that showed sensitivity with 50%. These results agreed with Jahantigh and Nill (2010) who showed a high frequency of sensitivity to Ciprofloxacin, Norfloxacin and Gentamycin with percentage of (100%) in Salmonella isolated from pigeon eggs but Banani *et al*., (2003) who found that all isolates of *S*. Enteritidis were sensitive to Ciprofloxacin, Ceftriaxone, and Florphenicol.

PCR was a perfect tool for accurate detection of Salmonella resistant genes and the results from *qep*A- *qnr*S- *aac* (6')-ib-cr, a resistant genes for quinolones were reported with a percentage of follow: (2 /56 isolates) 3.5%, (6 / 56 isolates) 10.7% and (11 / 56 isolates) 19.6% respectively. The results obtained for *qep*A gene nearly in coordinated with Lunn *et al.,* (2010) who found that one isolate (2.4%) showed the presence of *qep*A. While the results in this study differ from Hao *et al*., (2011) who reported *aac* (6')-Ib-cr and *qnr*S resistant genes in Salmonella with a percentage of 20.16% and 1.61% respectively. The *sul* 1 gene, a gene encoded for sulfonamide resistance was reported in the present study with a percentage of 87% (27 out of 31 isolates) these results differ from a study performed by Dessie *et al*., (2013) who identified *sul*2 in 33 Salmonella isolates but *sul*1 not identified in them in addition to another study performed by Zou *et al*., (2009) who detected *sul*1 in 11 of 16 isolates (68.7%).The *bla* TEM gene, a gene encoded for B- lactam resistance was reported in the present study with a percentage of 69.4% (34 out of 49 isolates) these results differ from a study performed by Hur *et al*., (2011) who reported that 19/21 penicillin resistant *S*. Enteritidis in Korea carried the *bla* (TEM) gene with a percentage of (90.5%) but in another study done by Ahmed *et al*., (2009) the percentage of *bla* (TEM-1) was 10% which was identified in between 10 Salmonella isolates from retail chicken meat in Hiroshima, Japan. The *flo*R gene, a gene encoded for florphenicol resistance

was reported in the present study with a percentage of 77.8% (21/ 27 isolates) these results nearly in accordance with Lu *et al*., (2014) who reported that 108 *S.* Indiana possessed *flo*R gene with a percentage of 81.2%. However, Cloeckaert *et al*., (2000) reported that *Salmonella enterica* serovar Agona strains isolated from poultry harbor *floR* gene, conferring cross-resistance to Chloramphenicol and Florphenicol. The *aad*A2 gene, a gene encoded for Streptomycin resistance was reported in the current study with a percentage of 53.1% (17 / 32 isolates) these results came in contrary with a study performed by Shahada *et al*., (2006) who reported that all streptomycin resistant *S.* Infantis from poultry in Japan carried *aad*A1 gene while Sheng *et al*., (2004) who reported *aad*A2 gene in three Salmonella isolates from seventy-three isolates from retail meats. The *tet* A gene, a gene encoded for tetracycline resistance was reported in the present study with a percentage of 83.7% (36 /43 isolates) these results nearly in coordinated with Lu *et al*., (2014) who reported that 108 *S.* Indiana possessed *tet* A gene with a percentage of 81.2% and Shahada *et al*., (2006) who reported that 89% of Oxytetracycline-resistant *S.* Infantis from poultry in Japan carried the *tet* (A) gene while these results differ from a study performed by Ahmed *et al*., (2012) who identified tetracycline resistance gene *tet* A in 14 out of 21 (66.7%) Salmonella isolates.

Mutations in the *gyr*A gene were known to be the main resistance mechanism to quinolones. However, there are other mechanisms as the plasmid mediated resistance and this was obvious in the current *gyr*A sequence results. *S*. Enteritidis showed double mutations in the codons Asp87 and Val176 while *S*. Infantis and *S*. Vejle showed no mutations at *gyr*A gene. The results in this study nearly in coordinated with Reyna *et al*., (1995) who stated that double and triple combinatorial mutations involving residues 83 and 87 of *gyr*A are associated with much higher resistance levels than those of single mutations and Hirose *et al*., (2002) who reported a single mutation in the *gyrA* gene, at either position 83 or 87 of *gyr*A in *Salmonella enterica* Serovar Typhi and Serovar Paratyphi A and double mutations in the *gyrA* gene at both position 83 and position 87 of *gyr*A in strains with high-level resistance to Fluoroquinolones induced by in vitro selection with ciprofloxacin. The mutations (Ser83Phe and/or Asp87Gly) were previously recorded in many reference Salmonella strains and also in strains isolated from food and domestic or wild birds as reported by (Reyna *et al*., 1995; Hirose *et al*., 2002 and De Souza *et al*., 2011).

Because of much higher resistances of Salmonella to different antimicrobials that reported in this study using sensitivity tests, from PCR and sequencing techniques, it's recommended not to apply any antimicrobial agents for Salmonella treatment without application of sensitivity tests to avoid these resistance which have adverse effect on poultry industry. Also peoples shouldn't eat under cooked poultry and poultry products to avoid infection with such resistant Salmonellae.

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