

Original Research

## Class I Integron and $\beta$ -lactamase encoding genes of multidrug resistance *Salmonella* isolated from pigeons and their environments

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**Abstract:** Seroprevalence of *Salmonella* spp. was investigated in pigeon and its surrounding environment of Sharkia province, Egypt. Samples were randomly collected from fifty freshly dead squabs, forty freshly dead adult pigeons, sixty diseased adult pigeons and 100 apparently healthy adult pigeons. Bacterial isolates were tested for their susceptibility to 17 different antimicrobial discs, by using the disc diffusion method. The bacterial isolates were tested for Class I and  $\beta$ -lactamase encoding genes by using PCR. In vitro sensitivity of all *Salmonella* isolates were completely resistant to Streptomycin, Amoxicillin, clavulanic acid, Amoxicillin, Ampicillin and Ceftazidime (100%). Class I integron were characterized in 70% *Salmonella* isolates from squabs, 42.9% in adult pigeons and 14.3% in pigeon environment which confer their resistance to streptomycin and ampicillin. Meanwhile TEM-1  $\beta$ -lactamase was characterized in 20% of tested *Salmonella* isolates from squabs including *S. Enteritidis*, 42.9% of tested *Salmonella* isolates from adult pigeons including *S. Enteritidis* which confer their resistance to cephalosporin and not detected in all isolates from pigeons environments. In conclusion TEM-1  $\beta$ -lactamase was characterized in 20% of *Salmonella* isolates from squabs while Class I integron was characterized in 70% *Salmonella* isolates from squabs.

**Key words:** *Salmonella*, seroprevalence, antimicrobial, sensitivity, Class I integron,  $\beta$ -lactamase.

### Introduction

Salmonellosis in pigeon caused by *S. Typhimurium* and *S. Enteritidis* (1). All *Salmonella* strains which isolated from pigeon were identified as *S. Typhimurium*. In vitro antibiotic sensitivity of the isolates revealed 100% sensitivity towards ciprofloxacin followed by gentamicin, norfloxacin and chloramphenicol, co-trimoxazole, cephalexin and ceftiofur. None of the *Salmonella* isolates were sensitive to ampicillin (2). Among the *S. Typhimurium* isolates from squabs, seven resistance profiles were identified: penicillins, aminoglycosides, fluoroquinolones, lincosamides, phenicols, tetracyclines and sulphonamides; four resistance profiles were identified in the isolates of *S. Braenderup* and *S. Lomita*: aminoglycosides, fluoroquinolones, lincosamides and polymyxin. The distribution of resistance to antibiotics, was dependent on serotype identity (3). *S. Typhimurium* strains show higher resistance to ampicillin, tetracycline and nitrofurantoin (2). Multidrug resistance was found in *Salmonella Typhimurium* strains, with resistance ranging from 3 to 8 antimicrobial drug. All strains were susceptible to trimethoprim-sulfamethoxazole and chloramphenicol and resistant to ceftriaxone and ceftiofur (4). By using of PCR and DNA sequencing, 59.5% *S. Enteritidis* isolates were found to carry class I integrons. Class I integron well established and documented in Gram-negative microorganisms, with its role in the distribution of antimicrobial resistance. Class I integrons are associated with a variety of resistance gene cassettes, but most integrons contain *aadA* resistance gene determinant and encoding streptomycin-spectinomycin gene resistance (5). Class I integrons were further sequenced and the *dfzA25* (750 bp) and *bla*<sub>PSE1</sub> (1250 bp) gene cassette were identified (6). Class 2 integron is

commonly found to be associated with Tn7 transposon family (Tn7, Tn1825, Tn1826 and Tn4132), carrying both of its recombination site *attI2* and promoter Pc found within such transposons (7). Its 3' conserved segment (3'-CS) contains 5 genes (*tnsA*, *tnsB*, *tnsC*, *tnsD* and *tnsE*) functioning in the movements of transposon, which mediates mobility of class 2 integron via a preferential insertion into a unique site within bacterial chromosomes (8). The presence of ESBL genes and other resistance gene in the avian *S. Enteritidis* was recovered and harbored the *bla*<sub>CTX-M</sub> gene that associated with genes that confer resistance to trimethoprim, sulfamethoxazole or streptomycin. It was also *bla*<sub>TEM</sub> positive that associated with the genes responsible for resistance against penicillins, ceftiofur and ceftazidime (9). In Egypt, *Salmonella* infection represents a serious problem in pigeon, especially in the absence of hygiene conditions and vaccination programs. In light of the above and as a consequence of scarce data on macrolide resistance of *Salmonella* in Egypt, the current study was conducted to assess the seroprevalence of *Salmonella* in Pigeons and their environment in Sharkia province, Egypt, and to evaluate their antimicrobial resistant profile, further characterize the Class I integron and  $\beta$ -lactamase genes of multidrug resistance *Salmonella* species.

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**Table 1.** Partial sequences alignment of integron and B-lactam primers.

Primer	Target	Sequence (5' to 3')	Amplicon size (bp)
<b>Integron</b>			
5'-CS	Class 1	GGCATCCAAGCAGCAAG	Variable
3'-CS	integron	AAGCAGACTTGACCTGA	
hep74	Class 2	CGGGATCCCCGACGGCATGCACGATTGTA	Variable
hep51	integron	GATGCCATCGCAAGTACGAG	
<b>B-Lactam</b>			
TEM-F	<i>blaTEM</i>	ATAAAATTCTTGAAGACGAAA	1080
TEM-R		GACAGTTACCAATGCTTAATC	
CMY-F	<i>blaCMY</i>	GACAGCCTCTTTCTCCACA	1007
CMY -R		TGGAACGAAGGCTACGTA	
OXA-F	<i>blaOXA</i>	TCAACTTTCAAGATCGCA	591
OXA-R		GTGTGTTTAGAATGGTGA	
SHV-F	<i>blaSHV</i>	TTATCTCCCTGTTAGCCACC	795
SHV-R		GATTGCTGATTTCGCTCGG	

## Materials and Methods

### Clinical specimens

A total number of 400 birds were collected from different areas in Sharkia province, Egypt, over a three-year period, from March 2011 to May 2014, to be examined. Ninety five diseased squabs "showing gastrointestinal manifestations", 50 freshly dead squabs and 55 apparently healthy squabs. Forty freshly dead adult pigeons, 60 diseased adult pigeons and 100 apparently healthy adult pigeons were randomly collected. Specimens from Liver, intestine, lymph nodes and cloacal swabs were collected. Environmental specimens for *Salmonella* isolation were isolated from 90 samples of feedstuffs, water, land filter paper and swabs from workers' hands. Meanwhile sixty samples were collected from wash water after slaughtered pigeon, swabs from trays and swabs from workers' hands. Specimens were placed into separate sterile containers and then transported to the laboratory in an icebox within 24 hours for *Salmonella* isolation.

### Isolation and identification of *Salmonella* species.

Twenty five gram of each sample were minced and homogenized in a separate sterile blender, then placed in a sterile flask containing 225 ml of 1% pepton water and incubated at 37°C for 18 hrs. The prepared samples were pre-enriched in incubator at 37°C for 24 hrs. One ml of the pre-enrichment culture was inoculated into tube containing 10 ml of Rappaport-Vassiliadis soy (RVS) broth, at 41.5°C for 24hrs. A loop full from the inoculated and incubated RVS broth was streaked on XLD, MacConkey and S.S agar plates and incubate at 37°C for 24 hrs. Suspected colonies were picked up and streaked onto slope agar and incubated at 37°C for 24 hrs., and used as a stock culture for further identification. The purified bacterial isolates were subjected to cultural, morphological and biochemical identification According to (10).

### Antimicrobial sensitivity of the isolated *Salmonella* species.

Bacterial isolates were tested for their susceptibility to Amoxicillin/Clavulanic acid (AMC 30 µg), Amoxicillin (AML 10µg), Ampicillin (AMP 10µg), Cefazidime (CAZ 30µg), Ceftriaxone (CRO30µg), Cefotaxime (Ctx30µg), Erythromycin (E15µg), Oxytetracycline

(OT30µg), Sulbactam / Cefoperazone (Scf15µg), Sulphamethoxazole /Trimethoprim (SXT25µg), Sulbactam / Ampicillin (SAM20µg), Tetracycline (TE30µg), Chloramphenicol (C 30µg), Ciprofloxacin (CIP 5µg), Enrofloxacin (ENR 5µg), Norfloxacin (NOR 2µg) and Streptomycin (S 10µg). The disc diffusion method was carried out according to the standards and interpretive criteria described by CLSI (11). A smooth single colony was inoculated in 5ml trypticase soya broth and incubated at 37°C for 18 hrs, then turbidity was adjusted to 0.5 McFarland standard contain (<300x10<sup>6</sup> colony forming unit/ml), then few drops of the inoculated broth were flooded on to the surface of Muller-Hinton agar plates. Then the inoculated plates were over laid with antibiotic discs using a sterile forceps, The inoculated plates were incubated at 37°C for 24 hours and inhibition zones were measured.

### PCR amplification and sequence analysis of class I Integron and B-Lactam genes

Amplification reactions were carried out with 10 µl of boiled bacterial suspensions, 250 mM deoxynucleoside triphosphate, 2.5 mM MgCl<sub>2</sub>, 50 pmol of primers and 1 U of AmpliTaq Gold DNA Polymerase (Applied Biosystems, Foster City, CA, USA). Distilled water was added to bring the final volume to 50 µl. The class 1 integron primers, 5'-CS and 3'-CS, which amplify the region between the 5'-conserved segment (5'-CS) and 3'-conserved segment (3'-CS) of class I integron, were used as previously described (Table 1). For screening of class 2 integrons, PCR was performed with the primer pair, hep74 and hep51, specific to the conserved regions of class II integron (12). PCR products were subjected to electrophoresis in a 1.0% agarose gel, stained with ethidium bromide and visualized under UV light. The bacterial isolates were tested for OXA, SHV, TEM, CTX-M and CMY β-lactamase-encoding genes by PCR using universal primers for the OXA, SHV, TEM, CTX-M and CMY families (12).

## Results

### Prevalence of *Salmonella* infection in squabs

Out of 200 squabs samples examined, the prevalence rate of *Salmonella* infection in squabs was (5%) as shown in table (2).

**Table 2.** Prevalence of *Salmonella* in squabs (number=200).

Health status of squabs	No. of examined squabs	<i>Salmonella</i> positive samples in squabs	
		No.	%
Diseased	95	4	2
Freshly dead	50	3	1.5
Apparent healthy Slaughtered	55	3	1.5
Total	200	10	5

**Table 3.** Prevalence of *Salmonella* in adults (number=200).

Health status of adult pigeons	No. of examined adult pigeons	<i>Salmonella</i> positive	
		No.	%
Diseased	60	2	1
Freshly dead	40	2	1
Apparent healthy Slaughtered	100	3	1.5
Total	200	7	3.5

**Table 4.** Prevalence of *Salmonella* isolates in the environment.

Environment	Type of examined sample	No. of examined sample	<i>Salmonella</i> positive	
			No.	%
Environment of diseased and freshly dead pigeons	Feed stuffs	25	1	4
	Water	25	0	0
	Land filter Paper	25	2	8
	Swabs from worker's hand	15	1	6.6
Environment of apparent healthy pigeons	Wash water after washing	30	1	3.3
	Swabs from trays	15	1	6.6
	Swabs from worker's hands	15	1	6.6
Total		150	7	4.66

**Prevalence of *Salmonella* in adults**

Out of 200 adult pigeons samples examined, the prevalence rate of *Salmonella* species was (3.5%) as shown in Table (3).

**Prevalence of *Salmonella* in environments**

Seven *Salmonella* species out of 150 examined samples, were isolated. The highest prevalence occurred in land filter paper from different private pigeon farmer houses (8%) as shown in Table (4).

**Resistance of isolated *Salmonella* from squabs to various antimicrobial agents**

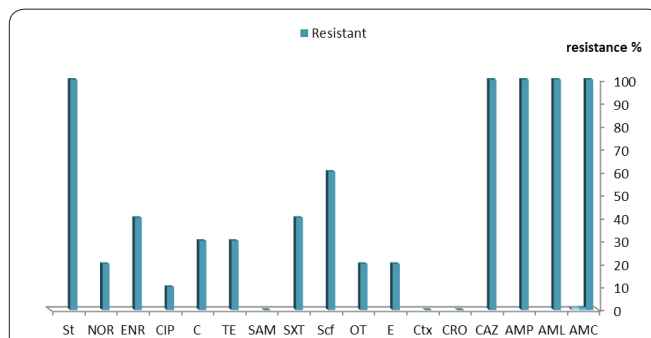
The in vitro sensitivity of 10 *Salmonella* isolates from squabs shown high resistance to Streptomycin, Ceftazidime, Ampiciline and Amoxicillin, while shown low resistance to Ciprofloxacin, as presented in Figure (1).

**Incidence of resistance in *Salmonella* isolated from adult pigeons**

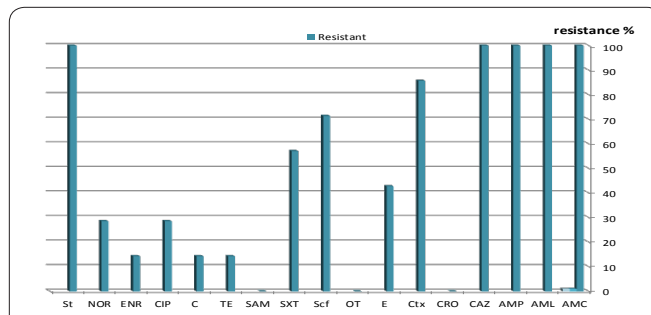
Isolated *Salmonella* species from adult pigeon shown high resistance to streptomycine, Ceftazidime, Ampiciline and Amoxicillin, while low resistance shown to Enrofloxacin, Chloramphenicol and Tetracycline , as shown in figure 2.

**Resistance of isolated *Salmonella* species from surrounding environments to various antimicrobial agents**

*Salmonella* species which isolated from surrounding environment shown high resistance to Streptomycine,



**Figure 1.** Incidence of resistance in *Salmonella* isolated from squabs (number=10).

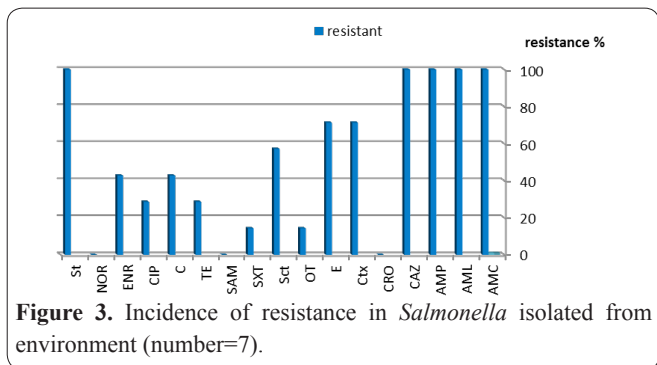


**Figure 2.** Incidence of resistance in *Salmonella* isolated from adult pigeons (number=7).

Ceftazidime and Ampicillin while shown low resistance to Oxytetracycline and Sulphamethoxazole, as presented in figure 3.

**Incidence of class I integron and resistance gene cassettes in squabs**

PCR-screening results detected class I integron in



**Figure 3.** Incidence of resistance in *Salmonella* isolated from environment (number=7).

7 (70%) bacterial isolates. As shown in table (5) and figure (4) *S. Typhimurium* (2 isolates), *S. Enteritidis* (2 isolates), *S. Montevideo* (2 isolates) and *S. Agona* (1 isolates).

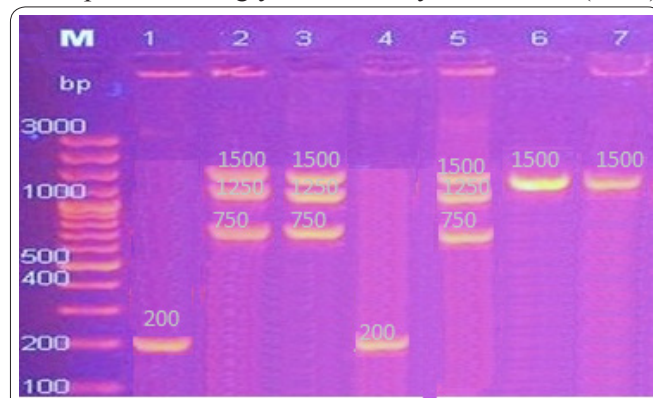
**Incidence of class I integron and resistance gene in adults**

PCR-screening results detected class I integron in 3(42.9%) bacterial isolates. As shown in table (6) and figure (5) *S. Enteritidis* (3 isolates).

**Incidence of class I integron and resistance gene in pigeons environments**

PCR-screening results detected class I integron in 1(14.3%) bacterial isolates. As shown in table (7) and fi-

gure (6) *S. Agona* (1 isolates). DNA-sequencing results for the inserted gene cassettes identified 6 types of class I integrons. The identified antimicrobial resistance genes were dihydrofolate reductase types : *dfrA1* and *dfrA25* which confer resistance to sulphamethoxazole/Tri-methoprim; aminoglycoside adenytransferase (*aadB*)



**Figure 4.** Agarose gel electrophoresis (1%) for the PCR products of class I integron in squabs. **M:** DNA digested with *HindIII* used as size marker. Lanes 1,4: *S. Typhimurium* integron gene cassette carrying 200 bp gene (*sull*). Lanes 2,5: *S. Enteritidis* integron gene cassette carrying 750bp, 1250bp and 1500bp genes (*dfrA25*, *blaPse*, *dfrA1-orf*, *aadB* and *catB3*). Lane 3: *S. Agona* integron gene cassette carrying 750 bp, 1250bp and 1500bp genes (*dfrA25*, *blaPse*, *dfrA1-orf*, *aadB* and *catB3*). Lanes 6,7 : *S. Montevideo* integron gene cassette carrying 1500bp gene (*aadB* and *catB3*).

**Table 5.** Incidence of class 1 integron and antimicrobial resistance genes in multidrug resistance *Salmonella* isolated from squabs.

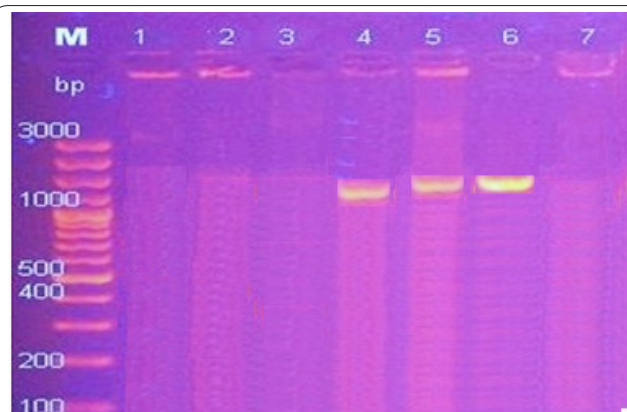
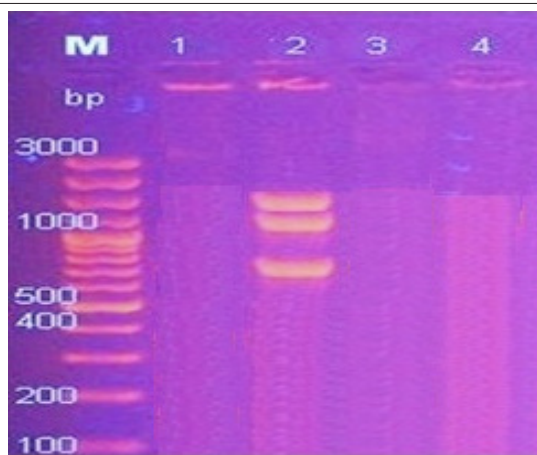
No.	Bacteria	Resistance phenotype	Class I integron	Resistance gene
1	<i>S. Typhimurium</i> from freshly dead squabs	AMC, AML, AMP, CAZ, Ctx, E, OT, S	+ve (200bp)	<i>sull</i>
2	<i>S. Typhimurium</i> from apparent healthy slaughtered squabs.	AMC, AML, AMP, CAZ, Scf, CIP, S	_ve	_ve
3	<i>S. Typhimurium</i> from apparent healthy slaughtered squabs	AMC, AML, AMP, CAZ, Scf, S	_ve	_ve
4	<i>S. Typhimurium</i> from diseased squabs	AMC, AML, AMP, CAZ, Ctx, E, OT, S	+ve (200bp)	<i>sull</i>
5	<i>S. Enteritidis</i> from freshly dead squabs	AMC, AML, AMP, CAZ, Ctx, E, SXT, ENR, NOR, S	+ve (750bp, 1250bp 1500bp)	<i>dfrA25</i> , <i>blaPse</i> , <i>dfrA1-orf</i> , <i>aadB</i> , <i>catB3</i> , <i>blaTEM</i>
6	<i>S. Enteritidis</i> from diseased squabs	AMC, AML, AMP, CAZ, Ctx, E, SXT, C. ENR, NOR, S	+ve (750bp, 1250bp 1500bp)	<i>dfrA25</i> , <i>blaPse</i> , <i>dfrA1-orf</i> , <i>aadB</i> , <i>catB3</i> , <i>blaTEM</i>
7	<i>S. Agona</i> from freshly dead squabs	AMC, AML, AMP, CAZ, Ctx, E, Scf, SXT, C, S	+ve (750bp, 1250bp 1500bp)	<i>dfrA25</i> , <i>blaPse</i> , <i>dfrA1-orf</i> , <i>aadB</i> , <i>catB3</i>
8	<i>S. Agona</i> from apparent healthy slaughtered squabs	AMC, AML, AMP, CAZ, Ctx, E, Scf, SXT, TE, C, S	_ve	_ve
9	<i>S. Montevideo</i> from diseased squabs	AMC, AML, AMP, CAZ, Ctx, E, OT, Scf, TE, ENR, S	+ve (1500bp)	<i>aadB</i> <i>catB3</i>
10	<i>S. Montevideo</i> from diseased squabs	AMC, AML, AMP, CAZ, Ctx, E, OT, Scf, TE, ENR, S	+ve (1500bp)	<i>aadB</i> <i>catB3</i>

**Table 6.** Incidence of class I integron and antimicrobial resistance genes in multidrug resistance *Salmonella* isolated from adult pigeons.

No.	Bacteria	Resistance phenotype	Class I integron	Resistance gene
1	<i>S. Typhimurium</i> freshly dead adults	AMC, AML, AMP, CAZ, Ctx, E, NOR, S	_ve	_ve
2	<i>S. Typhimurium</i> from diseased adults.	AMC, AML, AMP, CAZ, Scf, CIP, S	_ve	_ve
3	<i>S. Typhimurium</i> from diseased adults.	AMC, AML, AMP, CAZ, Ctx, E, CIP, S	_ve	_ve
4	<i>S. Enteritidis</i> from freshly dead adults	AMC, AML, AMP, CAZ, Ctx, SXT, Scf, NOR, S	+ve (1500bp)	<i>aadB</i> , <i>catB3</i> and <i>blaTEM</i>
5	<i>S. Enteritidis</i> from apparent healthy slaughtered adults	AMC, AML, AMP, CAZ, Ctx, SXT, Scf, ENR, S	+ve (1500bp)	<i>aadB</i> , <i>catB3</i> , and <i>blaTEM</i>
6	<i>S. Enteritidis</i> from apparent healthy slaughtered adults	AMC, AML, AMP, CAZ, Ctx, SXT, Scf, S	+ve (1500bp)	<i>aadB</i> , <i>catB3</i> , and <i>blaTEM</i>
7	<i>S. Agona</i> from apparent healthy slaughtered adult	AMC, AML, AMP, CAZ, Ctx, SXT, Scf, E, TE, C, S	_ve	_ve

**Table 7.** Incidence of class I integron and antimicrobial resistance genes in multidrug resistance *Salmonella* isolated from pigeons environments.

Bacteria	Resistance phenotype	Class I integron	Resistance gene
<i>S. Typhimurium</i> from feed stuff " environment of diseased and freshly dead pigeon".	AMC, AML, AMP, CAZ, Ctx, E, C, CIP, ENR, S	_ve	_ve
<i>S. Typhimurium</i> from land filterpaper " environment of diseased and freshly dead pigeon".	AMC, AML, AMP, CAZ, Ctx, E, C, CIP, ENR, S	_ve	_ve
<i>S. Typhimurium</i> from swabs of worker's hand " environment of diseased and freshly dead pigeon".	AMC, AML, AMP, CAZ, Scf, S	_ve	_ve
<i>S. Typhimurium</i> from wash water after washing " environment of apparent healthy slaughtered pigeon".	AMC, AML, AMP, CAZ, Scf, S	_ve	_ve
<i>S. Typhimurium</i> from swabs of worker's hands. " environment of apparent healthy slaughtered pigeon".	AMC, AML, AMP, CAZ, Ctx, E, S	_ve	_ve
<i>S. Agona</i> from swabs of trays " environment of apparent healthy slaughtered pigeon".	AMC, AML, AMP, CAZ, Ctx, E, TE, C, S	+ve (750bp, 1250bp and 1500bp)	<i>dfrA25</i> , <i>blaPse</i> , <i>dfrA1-orf</i> , <i>aadB</i> and <i>catB3</i>
<i>S. Virginia</i> from land filterpaper " environment of apparent healthy slaughtered pigeon".	AMC, AML, AMP, CAZ, Ctx, E, OT, Scf, TE, ENR, S	_ve	_ve

**Figure 5.** 1% Agarose gel electrophoresis for the PCR products of class I integrons in adult pigeons. **M** : DNA digested with *HindIII* used as size marker. **Lanes 4,5,6:** *S. Enteritidis* integron gene cassette carrying 1500bp genes (*aadB* and *catB3*).**Figure 6.** 1% Agarose gel electrophoresis for the PCR products of class I integron in pigeon environments. **M**: DNA digested with *HindIII* used as size marker. **Lanes 2:** *Agona* integron gene cassette carrying 750bp, 1250bp and 1500bp genes (*dfrA25*, *blaPse*, *dfrA1-orf*, *aadB* and *catB3*).

which confer resistance to streptomycin, gentamycin and spectinomycin; chloramphenicol acetyltransferase (*catB3*) which confers resistance to chloramphenicol, sulphonamide resistance gene (*sulI*) which confer resistance to sulphonamide and  $\beta$ -lactamase gene (*bla<sub>pse1</sub>*) which confers resistance to ampicillin. All isolates were negative for class II integron.

#### Incidence of $\beta$ -lactamase –encoding genes in squabs

*BlaTEM* gene was identified by PCR in 2 (20%) bacterial isolates of *S. Enteritidis*. All isolates were negative

for *blaCTX-M*, *blaSHV*, *blaOXA* and *blaCMY* resistance genes as shown in figure (7).

#### Incidence of $\beta$ -lactamase –encoding genes in adults pigeons

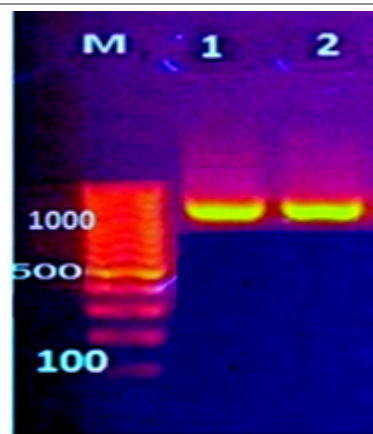
*BlaTEM* by PCR in 3 (42.9%) bacterial isolates of *S. Enteritidis*. All isolates were negative for *blaCTX-M*, *blaSHV*, *blaOXA* and *blaCMY* resistance gene.

#### Incidence of $\beta$ -lactamase –encoding genes in pigeons environments

By using of PCR and DNA-sequencing all isolates were negative for *blaCTX-M*, *blaTEM*, *blaSHV*, *blaOXA* and *blaCMY* resistance genes. The *blaTEM* a narrow-spectrum  $\beta$ -lactamase gene which confers resistance against penicillins and first generation cephalosporins. The *blaSHV* confers resistance against ampicillins and amoxicillin. While the *blaOXA* which confers resistance against ampicillins, Ceftazidime, cefotaxime. The *blaCMY* that encodes resistance to extended-spectrum cephalosporins and ampicillins.

#### Discussion

The prevalence of *Salmonella* spp. among the samples evaluated from apparently healthy pigeon was low when compared to studies from dead or dying specimens. Despite the low detection rate (2.75%), these results are a sign that the isolated serovars circulate in the pigeon population (13). In this study, seventeen

**Figure 7.** Agarose gel electrophoresis (1%) for the PCR products of *blaTEM* (1080 bp), *blaCMY* (1007bp) and *blaSHV* (795bp) in squabs. **M**: 1000bp ladder used as size marker. **Lanes 1,2** : *S. Enteritidis* (1080bp) (*blaTEM*).

*Salmonella* isolates (4.75%), 10 in squabs (5%) and 7 in adult pigeons (3.5%), were isolated, prevalence of *Salmonella* in slaughtered squabs liver, intestine and intestinal lymph node was (1.8%, 5.5% and 3.6% respectively) and in adults pigeon liver, intestine and intestinal lymph node was (1%, 3% and 2% respectively). (14) Recorded only 2% *Salmonella* positive in slaughtered pigeons but (15) detected (12%) *S. Typhimurium* from pigeon liver was highly contaminated with *Salmonella* (8%) but no *S. Typhimurium* was detected in squabs carcasses. Obtained data revealed 5 strain of *Salmonella* were isolated from freshly dead squabs and adults pigeon with a percentage (6% & 5%) respectively. (16) isolate *S. Typhimurium* in ratios of 50%. In our study, 7 samples out of 150 pigeon environment samples were found to be positive to *Salmonella* species (4.7%) and serotyped as *S. Typhimurium*, *S. Agona* and *S. Virginia* (71.4%, 14.3% and 14.3%) respectively. The most effective antibiotic in squabs was Sulbactam ampicillin (100%) followed by Cefotaxime (80%), Erythromycin (80%) and Sulfamethoxazole/ Trimethoprim (60%). While in adult pigeons Sulbactam ampicillin (100%) was the most effective antibiotic followed by Tetracycline (85.7%), Erythromycin (57.1%) and Chloramphenicol (57.1%). While in pigeons environments Sulbactam ampicillin (100%) was the most effective antibiotic followed by Tetracycline (71.4%) and Norfloxacin (57.2%). This result is in accordance with (17) who reported the sensitivity of *S. Enteritidis* was (89.96%) with norfloxacin followed by chloramphenicol (73.91%). *Salmonella* isolates from squabs showed moderate sensitivity with Ceftriaxone (100%), Ciprofloxacin (80%), Oxytetracycline (60%) and Norfloxacin (50%). Also in adult pigeons showed moderate sensitivity with Ceftriaxone (100%), Ciprofloxacin (71.4%), Oxytetracycline (42.8%) and Norfloxacin (42.8%). While in pigeon environments showed moderate sensitivity to Ceftriaxone (100%), Oxytetracycline, Chloramphenicol and Enrofloxacin (42.8%). These results coincide with (17) who reported the sensitivity of *S. Enteritidis* was moderate to cefotaxime and co-trimoxazole (0.43% and 4.35% respectively). The *Salmonella* isolates from squabs, adults and pigeons environments were completely resistant to Streptomycin, Amoxicillin/clavulanic acid, Amoxicillin, Ampicillin and Cefazidime (100%). The resistance to quinolones in *Salmonella* spp. is a warning to the scientific community with knowledge about the possibility of transmission of resistance by mechanisms mutations in target genes and/or by determinants in plasmids and transposons (4). Despite the detection of phenotypic resistance to multiple antibiotics, most different serotypes isolated from clinical cases are resistant to various antimicrobials and carry the class 1 Integron gene, involved in antimicrobial multiresistance (18). In this study, class 1 integron were detected in 70% of the tested *Salmonella* isolates from squabs including *S. Typhimurium*, *S. Enteritidis*, *S. Montevideo* and *S. Agona*, 42.9% of tested *Salmonella* isolates from adult pigeons including *S. Enteritidis* and 14.3% of tested *Salmonella* isolates from pigeons environments including *S. Agona*. DNA-sequencing identified 6 types of class 1 integrons (*dfrA1*, *dfrA25*, *aadB*, *catB3*, *sul1* and *bla<sub>Pse1</sub>*) which confer resistance to sulphamethoxazole/Trimethoprim, streptomycin, genta-

mycin, spectinomycin; chloramphenicol, sulphonamide and ampicillin. These results agree with results of (19) and (6). Class II integron is situated in a decreased diversity of non-replicative transposons *Tn7* and Class II integrase genes represents an internal stop codon (TAA) (20). Most of the gene cassette arrays of Class II integron are conserved and show the lack of dynamics recombination due to integrase inactivation. The variable region of Class II integron mostly carries the three antibiotic resistance gene, namely, *dfr1*, *sat2* and *aadA*. The resistance against trimethoprim is encoded in *dfr* gene within the Class II integron and high level of resistance to trimethoprim associated with the presence of *dfrA1*, *dfrA5*, *dfrA7* and *dfrA17* (21). In this study *bla<sub>TEM</sub>* was detected in 20% of tested *Salmonella* isolates from squabs including *S. Enteritidis*, 42.9% of tested *Salmonella* isolates from adult pigeons including *S. Enteritidis* and not detected at all isolates from pigeons environments isolates. These results were agreed with (9) and (22). Resistant plasmids harboring *bla<sub>CTX-M</sub>* genes and clonal spread of strains were both responsible for dissemination of resistant *Salmonella* isolates. The presence and spread of *bla<sub>CTX-M</sub>*, especially the *bla<sub>CTX-M-27</sub>* on non-typeable plasmids in *Salmonella* isolates may pose a potential threat for public health (23).

In conclusion, prevalence of *Salmonella* is higher in squabs than adult pigeons. In vitro sensitivity of *Salmonella* isolates were completely resistant to Streptomycin, Amoxicillin/clavulanic acid, Amoxicillin, Ampicillin and Cefazidime (100%). Class I integron was characterized in 70% *Salmonella* isolates from squabs, 42.9% in adult pigeons and 14.3% in pigeon environments which confer their resistance to streptomycin and ampicillin. TEM-1  $\beta$ -lactamase was characterized in 20% of tested *Salmonella* isolates from squabs including *S. Enteritidis*, 42.9% of tested *Salmonella* isolates from adult pigeons including *S. Enteritidis* which confer their resistance to cephalosporin and not detected at all isolates from pigeons environments.

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