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ANTIBIOGRAM AND CHARACTERIZATION OF SOME PLASMID MEDIATED GENES OF *SALMONELLA* SPECIES ISOLATED FROM PIGEON.

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ABSTRACT

To determine sensitivity of isolated Salmonella species to various antimicrobial agents and molecular characterization of some common genes responsible for antibiotic resistance phenotypes.

In this study, bacteriological isolation of Salmonella species isolated from 400 samples which were collected from squabs and adult pigeon and their surroundings. Bacterial isolates were tested for their susceptibility to 17 different antimicrobial discs as mentioned before by the disc diffusion method. Using PCR for screening of antimicrobial resistance genes, 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.

Prevalence of Salmonella in slaughtered squabs liver ,intestine and intestinal lymph nodes was 1.8%, 5.5% and 3.6% respectively ;and in

adult pigeons 1%, 3% and 2%, respectively. Sensitivity of all Salmonella isolates were completely resistant to Streptomycin, Amoxicillin/clavulanic acid, Amoxicillin, Ampicillin and Ceftazidime. Class1 integron was characterized in 70% Salmonella isolates from squabs, 42.9 % in adult pigeons and 14.3% in pigeon enviroments which confer their resistance to streptomycin and ampicillin. TEM-1 β-lactamase was characterized in 20% of tested Salmonella isolates from squabs including Salmonella enterica serovar Entertidis, 42.9% of tested Salmonella isolates from adult pigeons including S. Entertidis which confer their resistance to cephalosporin and not detected at all isolates from pigeons environments.

In this study, many multidrug-resistant Salmonella species were isolated and various types of antimicrobial resistant genes were identified from pigeons and their environments. Strikingly, many of these resistance genes are recorded in clinical bacterial isolates from humans.

Key words: Salmonellosis, antimicrobial, sensitivity, Plasmid, Class1 integron, β-lactamase

INTRODUCTION

Salmonellosis has one of the highest mortality rates of infectious bacterial diseases in pigeons ***(Ruben Lanckriet, 2010)***. *Salmonella* were shown to survive and multiply in the dropping for up to one month after their deposition by pigeons. Salmonellosis in pigeon caused by *Salmonella enterica* serovar Typhimurium and *S.*Enteritidis ***(Dumitrache,2013).***

Antibiotics are widely used to control bacterial infections, also used as a feed additive to promote growth and prevent livestock disease ***(Sarmah et al. ,2006). Smith et al. (2010)*** investigated the antimicrobial

susceptibility of some isolates of *Salmonella enterica*, of 15 isolates belonging to 11 different serovars analyzed, one isolate of *Salmonella* Typhimurium was resistant to multiple drugs namely ampicillin, amoxicillin/clavulanic acid, chloramphenicol and tetracycline. ***Farghaly and Heba (2011)*** collected 150 samples from adult pigeons died suddenly from different pigeon's farms at different localities in Egypt. *Salmonella* Typhimurium was isolated in ratio of 50%. Studying sensitivity of the isolated bacterial strains revealed fluoroquinolone antibiotics (ciprofloxacin, enrofloxacin and danofloxacin) were found to be effective against most of tested bacterial strains followed by gentamycin, colistin sulphate and sulphamethoxazole.

Pankaj et al. (2013) recovered 12 *Salmonella* Typhimurium isolates from 150 samples from pigeons. Antibiotic sensitivity of the isolates revealed 100 % sensitivity towards ciprofloxacin followed by gentamicin, norfloxacin and chloramphenicol (91.67 % each), co-trimaxazole (75.0 %), cephalexin (66.67 %) and cephotaxime (58.33 %). The strains showed lower sensitivity to tetracycline and nitrofurantoin (8.33 % each) followed by oxytetracycline, streptomycin, furazolidone (16.67 %each) and colistin (33.33 %). None of the *Salmonella* isolates were sensitive to ampicillin.

Integrons play a major role in the spread of antibiotic resistance genes in Gram-negative bacteria ***(Rowe-Magnus et al. ,2001).***

Integrons are genetic elements able to capture individual antibiotic resistance genes including those encoding various β-lactamase and in the process promote their transcription and expression ***(Collis et al.,1998, Martinez et al.,1999 and Hanau et al.,2002). Farzaneh et al.(2011)***

characterized 84 *Salmonella* Entertidis isolates for antimicrobial resistance patterns and class I integrons. By PCR and DNA sequencing, 59.5% *S.*Enteritidis isolates were found to carry class I integrons. The integrons were further sequenced and the *dfrA25* (750 bp) and *bla* $_{PSE1}$ (1250 bp) gene cassette were identified.

Lin et al. (2006) reported that resistance to β- lactam antibiotics of many Gram negative bacteria was as a result of β- lactamases. The first plasmid mediated β- lactamases in Gram-negative bacteria, *bla*TEM, was described in the early 1960s. Another common plasmid-mediated β-lactamase is *bla*SHV.

Loana et al.(2006) investigated by PCR and DNA sequencing the presence of ESBL genes and other resistance gene in the avian *S.*Enteritidis recovered during monitoring program in Spain and found that it harbored the *bla*CTX-M gene that associated with genes that confer resistance to trimethoprim, sulfamethoxazole or streptomycin.

This study was aimed to, isolation and identification of the *Salmonella* species isolated from pigeons and its environments, testing the sensitivity of different *Salmonella* isolates to various antimicrobial agents. Molecular characterization of some resistance genes responsible for antibiotic resistance phenotypes.

MATERIAL AND METHODS

Samples:

Samples were collected from diseased pigeon, apparently healthy slaughtered and freshly dead pigeon and their environment, as shown in Table (1 and 2).

Preparation of the samples:

The obtained samples were collected under aseptic condition. Twenty five gm of each sample were minced and homogenized in a separate sterile blender, then placed in a sterile flask containing 225 ml of 1% peptone water and incubated at 37℃ for 18 hrs according to ***(Koneman et al.,1997).*** The prepared samples were pre-enriched in incubator at 37℃ for 24 hrs. Selective enrichment was done as following, 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 incubated 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.

Identification of bacterial isolates:

The purified bacterial isolates were subjected to cultural, morphological and biochemical identification According to ***(Koneman et al.,1997).***

Antibiotic sensitivity tests:

A total of 17 antibiotic discs were obtained from Oxoid company and used for studying the antibiotic sensitivity patterns of isolated *Salmonella* serotypes. They included Amoxicillin/Clavulanicacid (AMC 30 µg), Amoxicillin (AML 10µg), Ampicillin (AMP 10µg), Ceftazidime (CAZ 30µg),Ceftriaxone(CRO30µg), Cefotaxime (Ctx30µg), Erythromycin (E15µg),Oxytetracycline(OT30µg),Sulbactam/ Cefoperazone (Scf15µg), Sulphamethoxazole/ Trimethoprim (SXT25µg), Sulbactam/ Ampicillin

(SAM20μg),Tetracycline(TE30μg),Chloramphenicol(C30μg),Ciprofloxacin (CIP 5μg), Enrofloxacin (ENR 5μg), Norfloxacin (NOR 2μg) and Streptomycin (S 10μg).

The susceptibility to different antimicrobial discs was done by the disc diffusion method according to the standards and interpretive criteria described by CLSI **(Clinical and Laboratory Standards Institute, 2002).**

PCR:

Bacterial DNA preparation:

A smooth single colony was inoculated in 5ml nutrient broth and incubated at 37°C for 18 hours, then 200 μl from bacterial culture was mixed with 800 μl of distilled water then made vortex for good mixing then heating at 96°C for 5 minutes in heat block. The resulting solution was centrifuged at 10,000 rpm for 5 minutes and the 200 μl from supernatant was used as the DNA template.

PCR amplification:

Amplification reactions were carried out with 10 μl of boiled bacterial suspensions, 250 mM deoxynucleoside triphosphate, 2.5 mM MgCl2, 50 pmol of primers (as shown in Table 3) and 1 U of Ampli*Taq* Gold DNA Polymerase (Applied Biosystems, Foster City, CA, USA). Distilled water was added to bring the final volume to 50 μl. The PCR products were subjected to electrophoresis in a 1.0% agarose gel, stained with ethidium bromide and visualized under UV light.

Screening for antimicrobial resistance genes: as described previously (Table 4) ***(Ahmed et al.,2007b).***

RESULTS

Prevalence of *Salmonella* in pigeon:

■ In squabs:

Out of 200 squabs samples examined, the prevalence rate of *Salmonella* species was (5%) as shown in table (1). The incidence rate of *Salmonella* differ according to health status of examined squabs, as it was high in diseased squabs (2%) followed by apparent healthy slaughtered and freshly dead squabs (1.5%).

■ In adults:

Out of 200 adult pigeons samples examined, the prevalence rate of *Salmonella* species was (3.5%) as shown in table (1). The incidence rate of *Salmonella* differ according to health status as it was high in apparent healthy slaughtered adults (1.5%) followed by diseased and freshly dead adults (1%).

Prevalence of *Salmonella* in environments:

Out of 150 samples examined, 7 *Salmonella* species were isolated. The highest prevalence occurred in land filterpaper from different private pigeon farmer houses (8%) as shown in table (2).

Identification of the isolated *Salmonella*:

Morphological identification:

On MacConkey agar, *Salmonella* colonies appeared colourless or pale (non-lactose fermenter).On XLD agar, *Salmonella* colonies appeared as red colonies with a black center. On S.S agar, *Salmonella* colonies appeared as white colonies with a black center. Gram's stain smears from suspected colonies showed Gram-negative rod-shaped bacilli ***(Koneman et al.,1997)***.

Sensitivity of the isolated *Salmonella* to various antimicrobial agents.:

■ Sensitivity of the isolated *Salmonella* in squabs:

The in vitro sensitivity % of 10 Salmonella isolates from squabs are presented in table (5).

■ Sensitivity of the isolated *Salmonella* in adult pigeons:

The in vitro sensitivity % of 7 *Salmonella* isolates from adult pigeons are presented in table (5).

■ Sensitivity of the isolated *Salmonella* in environments:

The in vitro sensitivity % of 7 *Salmonella* isolates from pigeons environments are presented in table (5).

Incidence of class I integron and resistance gene cassettes:

■ In squabs:

PCR-screening results detected class I integron in 7 (70%) bacterial isolates. As shown in table(6) and figure (1) *S.*Typhimurium (2 isolates), *S.* Entertidis (2 isolates) , *S.* Montevideo (2 isolates) and *S.* Agona (1 isolate).

■ In adults:

PCR-screening results detected class I integron in 3(42.9%) bacterial isolates. As shown in table (7) and figure (2_ lower part) *S.* Entertidis (3 isolates).

■ In pigeons environments:

PCR-screening results detected class I integron in 1(14.3%) bacterial isolate. As shown in table (8) and figure (2_upper part) *S.* Agona (1 isolates). DNA-sequencing results for the inserted gene cassettes identified 6 types of class 1 integrons. The identified antimicrobial resistance genes were dihydrofolate reductase types : *dfrA1*

and *dfrA25* which confer resistance to sulphamethoxazole/Trimethoprim; aminoglycoside adenyltransferase (*aadB)* which confer resistance to streptomycin, gentamycin and spectinomycin; chloramphenicol acetyltransferase (*catB3*) which confers resistance to chloramphenicol, sulphonamide resistance gene (*sul1*) which confer resistance to sulphonamide and β-lactamase gene (bla_{Pse1}) which confers resistance to ampicillin.

N.B. : All isolates were negative for class 2 integron.

Incidence of β-lactamase –encoding genes:

■ **In squabs:**

As shown in table(6) and figure (3) *blaTEM* was identified by PCR in 2 (20%) bacterial isolates of *S.* Entertidis. All isolates were negative for *blaCTX-M, blaSHV, blaOXA* and *blaCMY* resistance genes .

■ **In adults pigeons:**

As shown in table(7) and figure (4_lower part) *blaTEM* by PCR in 3 (42.9%) bacterial isolates of *S.* Entertidis. All isolates were negative for *blaCTX-M blaSHV, blaOXA* and *blaCMY* resistance genes .

■ **In pigeons environments:**

As shown in table(8) and figure (4_lower part) by PCR and DNA-sequencing all isolates were negative for *blaCTX-M, blaTEM, blaSHV, blaOXA* and *blaCMY* resistance genes.

The *blaTEM* a narrow-spectrum β-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.

Table (1): Prevalence of *Salmonella* in squabs and adults pigeons.

Health status	No. of examined		*Salmonella* positive samples			
	squabs	adult	squabs		adult	
			No.	%	No.	%
Diseased	95	60	4	2	2	1
Freshly dead	50	40	3	1.5	2	1
Apparent healthy Slaughtered	55	100	3	1.5	3	1.5
Total	**200**	**200**	**10**	**5**	**7**	**3.5**

Table (2): prevalence of *Salmonella* isolates in the environment.

	Type of examined sample	No. of examined sample	*Salmonella* positive	
			No.	%
Environment of diseased and freshly dead pigeons	Feed stuffs	25	1	4
	Water	25	0	0
	Land filterpaper	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

Table (3): Primers used in this study:

Primer	Target	Sequence (5' to 3')	Amplicon size (bp)	Reference or GenBank accession no.
Integron				
5'-CS 3'-CS	Class 1 integron	GGCATCCAAGCAGCAAG AAGCAGACTTGACCTGA	variable	*Levesque et al. (1995)*
hep74 hep51	Class 2 integron	CGGGATCCCGGACGGCATGCACGA TTGTAGATGCCATCGCAAGTACGAG	variable	*Ahmed et al. (2007b)*
B-Lactam				
TEM-F TEM-R	*blaTEM*	ATAAAATTCTTGAAGACGAAA GACAGTTACCAATGCTTAATC	1080	*Ahmed et al.(2007b)*
CMY-F CMY -R	*blaCMY*	GACAGCCTCTTTCTCCACA TGGAACGAAGGCTACGTA	1007	*Ahmed et al.(2007b)*
OXA-F OXA-R	*blaOXA*	TCAACTTTCAAGATCGCA GTGTGTTTAGAATGGTGA	591	*Ahmed et al.(2007b)*
SHV-F SHV-R	*blaSHV*	TTATCTCCCTGTTAGCCACC GATTTGCTGATTTCGCTCGG	795	*Ahmed et al.(2007b)*
CTX-M-F CTX-M-R	*blaCTX-M*	CGCTTTGCGATGTGCAG ACCGCGATATCGTTGGT	550	*Ahmad et al.(2007b)*

Table (4): PCR conditions and amplicon size.

Gene	Hot start	Denat.	Anneal.	Prim. ext.	Cy.	Final ext.	Target
Integrons							
Class 1 integron	94°C/10 min	94°C /1 min	55°C /1 min	72°C /3min	30	72°C /10min	Variable
Class 2 integron	94°C/10 min	94°C /1 min	55°C /1 min	72°C /3min	30	72°C /10min	Variable
β-lactamases							
CMY	94°C/10 min	94°C /1 min	55°C /1 min	72°C /1min	35	72°C /7 min	1007 bp
OXA	94°C/10 min	94°C /1 min	56°C /1 min	72°C /1min	35	72°C /10min	591 bp
SHV	94°C/10 min	94°C /30 sec	50°C /30 sec	72°C /1min	30	72°C /10min	795 bp
TEM	94°C/10 min	94°C /30 sec	50°C /30 sec	72°C /1min	30	72°C /10min	1080 bp
CTX-M	95°C/10 min	95C/30 sec	55°C /30 sec	72°C/30sec	30	72°C / 5min	550 bp

Table (5): Sensitivity % of *Salmonella* isolated from squabs, adult pigeon and environments

Antimicrobial agents	squabs			adults			environments		
	S*	M**	R***	S	M	R	S	M	R
Amoxicillin/clavulanic acid	0	0	100	0	0	100	0	0	100
Amoxicillin	0	0	100	0	0	100	0	0	100
Ampicillin	0	0	100	0	0	100	0	0	100
Ceftazidime	0	0	100	0	0	100	0	0	100
Ceftriaxone	0	100	0	0	100	0	0	100	0
Cefotaxime	80	20	0	0	14.3	85.7	0	28.6	71.4
Erythromycin	80	0	20	57.1	0	42.8	28.6	0	71.4
Oxytetracycline	20	60	20	28.6	71.4	0	42.8	42.8	14.3
Sulbactam cefoperazone	20	20	60	28.6	0	71.4	0	42.8	57.2
Sulfamethoxazole/ Trimethoprim	60	0	40	42.8	0	57.1	71.4	14.3	14.3
Sulbactam ampicillin	100	0	0	100	0	0	100	0	0
Tetracycline	50	20	30	85.7	0	14.3	71.4	0	28.6
Chloramphenicol	40	30	30	57.1	28.6	14.3	42.8	14.3	42.8
Ciprofloxacin	10	80	10	28.6	42.8	28.6	28.6	42.8	28.6
Enrofloxacin	40	20	40	42.8	42.8	14.3	42.8	14.3	42.8
Norfloxacin	30	50	20	28.6	42.8	28.6	57.2	42.8	0
Streptomycin	0	0	100	0	0	100	0	0	100

*S: Sensitivity% ** M: Moderate % *** R: Resistance %

Table (6): Incidence of class 1 integron and antimicrobial resistance genes in multidrug resistance *Salmonella* isolated from squabs.

No.	Bacteria	Resistance phenotype	Class1 integron	Resistance gene
1	*S.* Typhimurium from freshly dead squabs	AMC, AML, AMP, CAZ, Ctx, E, OT, S	+ve (200bp)	*sul1*
2	*S.* Typhimurium from apparent healthy slaughtered squabs.	AMC, AML, AMP, CAZ, Scf, CIP, S	_ve	_ve
3	*S.* Typhimurium from apparent healthy slaughtered squabs	AMC, AML, AMP, CAZ, Scf, S	_ve	_ve
4	*S.* Typhimurium from diseased squabs	AMC, AML, AMP, CAZ, Ctx, E, OT, S	+ve (200bp)	*sul1*
5	*S.* Enteritidis from freshly dead squabs	AMC, AML, AMP, CAZ, Ctx, E, SXT,ENR, NOR , S	+ve (750bp, , 1250bp and1500bp)	*dfrA25, blaPse, dfrA1-orf aadB, catB3.* ***and*** *blaTEM*
6	*S.* Enteritidis from diseased squabs	AMC, AML, AMP, CAZ, Ctx, E, SXT, C. ENR, NOR , S	+ve (750bp, ,1250bp and1500bp)	*dfrA25, blaPse, dfrA1-orf, aadB, catB3,* ***and*** *blaTEM*
7	*S.* Agona from freshly dead squabs	AMC, AML, AMP, CAZ, Ctx, E, Scf, SXT, C, S	+ve (750bp,1250bp and1500bp)	*dfrA25, blaPse, dfrA1-orf, aadB* and *catB3*
8	*S.* Agona from apparent healthy slaughtered squabs	AMC, AML, AMP, CAZ, Ctx, E, Scf, SXT, TE, C, S	_ve	_ve
9	*S.* Montevideo from diseased squabs	AMC, AML, AMP, CAZ, Ctx, E, OT, Scf, TE, ENR, S	+ve (1500bp)	*aadB* and *catB3*
10	*S.* Montevideo from diseased squabs	AMC, AML, AMP, CAZ, Ctx, E, OT, Scf, TE, ENR, S	+ve (1500bp)	*aadB* and *catB3*

Amoxicillin/ Clavulanicacid **(AMC)**,Amoxicillin **(AML)**, Ampicillin **(AMP)**, Ceftazidime **(CAZ)**, Ceftriaxone **(CRO)** ,Cefotaxime **(Ctx)**, Erythromycin (E), Oxytetracycline **(OT)**, Sulbactam/ Cefoperazone **(Sct)**, Sulphamethoxazole/ Trimethoprim **(SXT)**, Sulbactam/Ampicillin **(SAM)**, Tetracycline **(TE)**, Chloramphenicol **(C)**, Ciprofloxacin **(CIP)**, Enrofloxacin **(ENR)**, Norfloxacin **(NOR)** and Streptomycin **(St)**.

Table (7): Incidence of class 1 integron and antimicrobial resistance genes in multidrug resistance *Salmonella* isolated from adult pigeons.

No.	Bacteria	Resistance phenotype	Class1 integron	Resistance gene
1	*S.* Typhimurium from freshly dead adults	AMC, AML, AMP, CAZ, Ctx, E, NOR , S	_ve	_ve
2	*S.* Typhimurium from diseased adults.	AMC, AML, AMP, CAZ, Scf, CIP, S	_ve	_ve
3	*S.* Typhimurium from diseased adults.	AMC, AML, AMP, CAZ, Ctx, E, CIP , S	_ve	_ve
4	*S.* Enteritidis from freshly dead adults	AMC, AML, AMP, CAZ, Ctx, SXT ,Scf, NOR , S	+ve (1500bp)	*aadB, catB3 and blaTEM*
5	*S.* Enteritidis from apparent healthy slaughtered adults	AMC, AML, AMP, CAZ, Ctx, SXT ,Scf, ENR , S	+ve (1500bp)	*aadB, catB3, and blaTEM*
6	*S.* Enteritidis from apparent healthy slaughtered adults	AMC, AML, AMP, CAZ, Ctx, SXT ,Scf, S	+ve (1500bp)	*aadB, catB3, And blaTEM*
7	*S.* Agona from apparent healthy slaughtered adult	AMC, AML, AMP, CAZ, Ctx, SXT ,Scf, E, TE, C, S	_ve	_ve

Amoxicillin/Clavulanicacid(**AMC**),Amoxicillin(**AML**),Ampicillin(**AMP**),Ceftazidime(**CAZ**),Ceftriaxone(**CRO**),Cefotaxime (**Ctx**),Erythromycin(**E**),Oxytetracycline(**OT**),Sulbactam/Cefoperazone(**Sct**),Sulphamethoxazole/Trimethoprim(**SXT**),Sulbactam/Ampicillin(**SAM**),Tetracycline(**TE**),Chloramphenicol(**C**),Ciprofloxacin(**CIP**),Enrofloxacin **(ENR),** Norfloxacin **(NOR)** and Streptomycin(**St**).

Table (8): Incidence of class 1 integron and antimicrobial resistance genes in multidrug resistance *Salmonella* isolated from pigeons environments.

No.	Bacteria	Resistance phenotype	Class1 integron	Resistance gene
1	*S.* Typhimurium from feed stuff " environment of diseased and freshly dead pigeon".	AMC, AML, AMP, CAZ, Ctx, E, C, CIP, ENR, S	_ve	_ve
2	*S.* Typhimurium from land filterpaper " environment of diseased and freshly dead pigeon"..	AMC, AML, AMP, CAZ, Ctx, E, C, CIP,ENR, S	_ve	_ve
3	*S.* Typhimurium from swabs of worker's hand " environment of diseased and freshly dead pigeon".	AMC, AML, AMP, CAZ, Scf, S	_ve	_ve
4	*S.* Typhimurium from wash water after washing " environment of apparent healthy slaughtered pigeon".	AMC, AML, AMP, CAZ, Scf, S	_ve	_ve
5	*S.* Typhimurium from swabs of worker's hands. " environment of apparent healthy slaughtered pigeon".	AMC, AML, AMP, CAZ, Ctx, E, S	_ve	_ve
6	*S.* Agona from swabs of trays " environment of apparent healthy slaughtered pigeon".	AMC, AML, AMP, CAZ, Ctx, E, TE, C, S	+ve (750bp,1250bp and1500bp	*dfrA25, blaPse, dfrA1-orf, aadB and catB3*
7	*S.* Virginia from land filterpaper " environment of apparent healthy slaughtered pigeon".	AMC, AML, AMP, CAZ, Ctx, E, OT, Scf ,TE ,ENR, S	_ve	_ve

Amoxicillin/Clavulanicacid(**AMC**),Amoxicillin(**AM L**), Ampicillin **(AMP)**, Ceftazidime **(CAZ)**, Ceftriaxone **(CRO)**, Cefotaxime **(Ctx)**, Erythromycin **(E)**, Oxytetracycline **(OT)**, Sulbactam/ Cefoperazone **(Sct)**, Sulphamethoxazole/ Trimethoprim **(SXT)**, Sulbactam/ Ampicillin **(SAM)**, Tetracycline **(TE)**, Chloramphenicol **(C)**, Ciprofloxacin **(CIP)**, Enrofloxacin **(ENR)**, Norfloxacin **(NOR)** and Streptomycin(**St**).

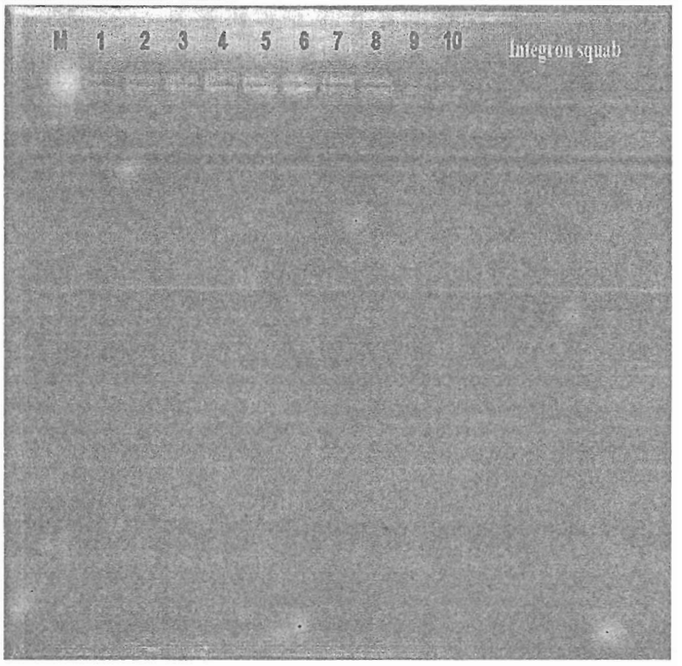

Fig (1): 1% Agarose gel electrophoresis for the PCR products of class 1 integron in squabs.

M: DNA digested with *Hind*III used as size marker

Lanes 1 , 4: *S.*Typhimurium integron gene cassette carrying 200 bp gene (*sul1*).

Lanes 5 , 6: *S.* Enteritidis integron gene cassette carrying 750bp, 1250bp and 1500bp genes (*dfrA25*, *blaPse*, *dfrA1-orf* ,*aadB* and *catB3*).

Lanes 7: *S.* Agona integron gene cassette carrying 750 bp, 1250bp and 1500bp genes (*dfrA25*, *blaPse*, *dfrA1-orf* ,*aadB* and *catB3*).

Lanes 9 , 10 : *S.* Montevideo integron gene cassette carrying 1500bp gene (*aadB and catB3).*

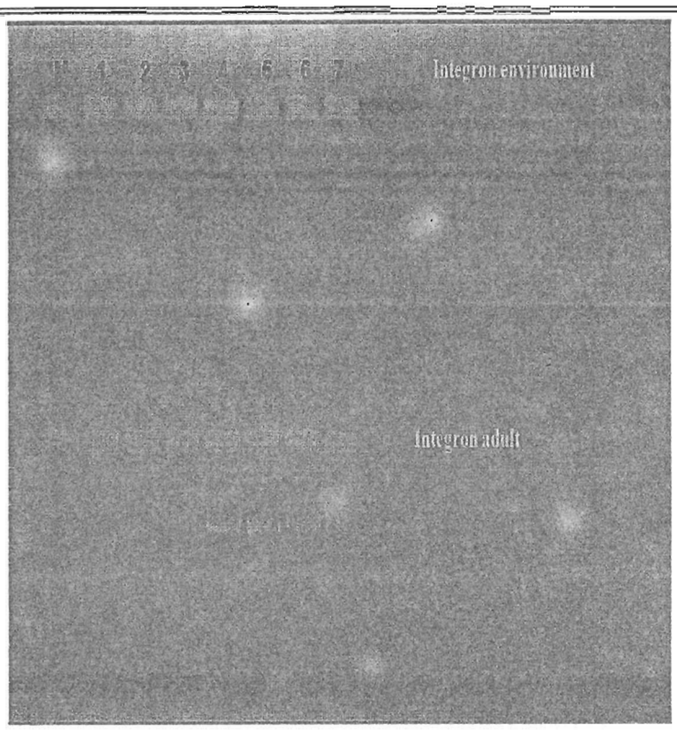

Fig (2): Upper part: 1% Agarose gel electrophoresis for the PCR products of class 1 integron in pigeon environments.

M : DNA digested with *Hin*dIII used as size marker

Lanes 6 : *Agona* integron gene cassette carrying 750bp, 1250bp and 1500bp genes (*dfrA25, blaPse, dfrA1-orf ,aadB* and *catB3*).

Lower part: 1% Agarose gel electrophoresis for the PCR products of class I integrons in adult pigeons.

M : DNA digested with *Hin*dIII used as size marker

Lanes 4 , 5 , 6: *S.*Enteritidis integron gene cassette carrying 1500bp genes (*aadB* and *catB3*).

Fig (3): Upper part: 1% Agarose gel electrophoresis for the PCR products of *blaTEM* (1080 bp), *blaCMY* (1007bp)and *blaSHV* (795bp) in pigeons environments.

M : 1000bp ladder used as size marker.

lower part: 1% Agarose gel electrophoresis for the PCR products of *blaTEM* (1080 bp), *blaCMY* (1007bp)and *blaSHV* (795bp) in adult pigeons.

M : 1000bp ladder used as size marker.

Lanes 4, 5 , 6: *S.* Enteritidis (1080bp) (*blaTEM*).

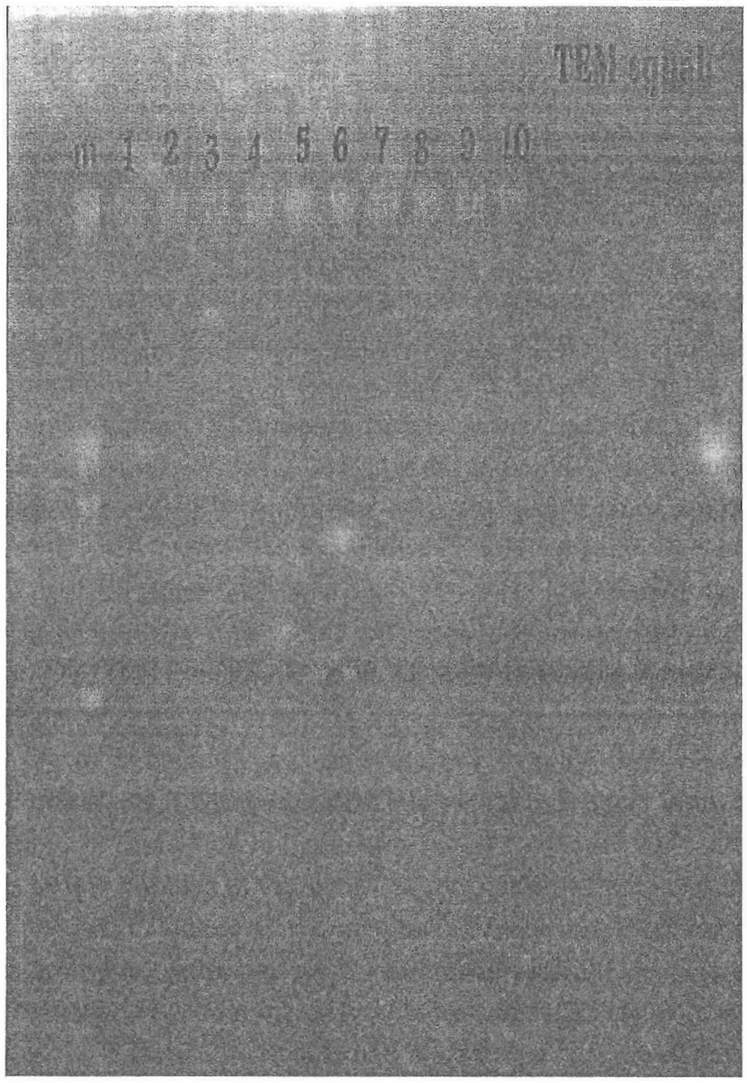

Fig (4): 1% Agarose gel electrophoresis for the PCR products of *blaTEM* (1080 bp), *blaCMY* (1007bp) and *blaSHV* (795bp) in squabs

M : 1000bp ladder used as size marker.

Lanes 5 , 6 : *S.* Enteritidis **(1080bp)** (***blaTEM***).

DISCUSSION

In this study, during period from July 2010 till July 2013, 400 pigeons, 200 squabs and 200 adult pigeons, were examined for isolation and identification of *Salmonella*. Seventeen *Salmonella* isolates (4.75%) ,10 in squabs (5%) and 7 in adult pigeons (3.5%), were isolated, these results differ from that of ***Kinjo et al. (1983)*** who isolated 9 *Salmonella* (1.3%) out of 700 feral pigeons captured in public parks and storehouses of animal feeds in three prefectures of Central Japan On the other hand ***Adesiyun et al. (1998)*** reported 8 serotypes of *S.*Typhimurium (5%) recovered from fecal and cloacal swabs of 171 racing pigeons which originated from 8 fanciers. In this study, 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 lymphnode was (1%, 3% and 2% respectively). ***Ring and Woerlen (1991)*** recorded only 2% *Salmonella* positive in slaughtered pigeons but ***Nassar and El-Ela (2000)*** detected (12%) *S.*Typhimurium from wooden pigeon carcases and liver was highly contaminated with *Salmonella* (8%) but no *S.*Typhimurium was detected in squabs carcases. On the other hand, ***Jeffrey et al. (2001)*** recorded 1.4% *Salmonella* from 18 farms (1110 squab) , 4.3% *Salmonella* from 1 farm (250 squab) and 4.1% *Salmonella* positive from 23 farms (2900 squab). ***Abd el-Aziz et al. (2002)*** revealed no positive samples for *Salmonella* from 50 squabs carcasses from different markets in Cairo and Giza governorates. In this study, we isolated 6 strain of *Salmonella* from cloacal swabs of diseased squabs and adult pigeons with a percentage (4.2% &3.3%) respectively. ***Kimpe et al. (2002)*** isolated six *Salmonella* strains from faecal samples of pigeons from lofts suffering from salmonellosis but ***Banani et al. (2003)*** isolated one hundred-eleven *Salmonella* salmples from domestic pigeons suspected to salmonellosis. *Salmonella* isolates belonged to serogroups D1(84.26%), B(8.33%) and C1(7.41%).

In this study, we isolated 5 strain of *Salmonella* from freshly dead squabs and adult pigeons with a percentage (6% &5%) respectively. ***Farghaly and Heba (2011)*** collected 150 samples from adult pigeons died suddenly from different pigeon's farms at different localities in Egypt. *Salmonella* Typhimurium were isolated 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 and this result differ from ***Veldman et al. (1995)*** who surveyed the rate of contamination with *Salmonella* species of poultry feeds and feed components. Ten percent of 360 samples were found to be contaminated. Twenty-eight serotypes of *Salmonella* were isolated, but no *Salmonella* Enteritidis was found.

In our study, 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 ***Abd El-Hamid et al.(1984)*** who reported the sensitivity of *S.* Enteritidis was (89.96%) with norfloxacin followed by chloramphenicol (73.91%).

The *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 enviroments showed moderate sensitivity to Ceftriaxone (100%), Oxytetracycline, Chloramphenicol and Enrofloxacin (42.8%). These

results coincide with ***Abd El-Hamid et al.(1984)*** who reported the sensitivity of *S.* Enteritidis was moderate to cefotaxime and co-trimaxazole (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 Ceftazidime (100%). These results agree with results of ***Ryder et al. (1980)*** who reported the resistance among strains of *S.* Typhimurium was (46.8%) for streptomycin, trimethoprim sulphamethoxazole (1.2%), chloramphenicol (0.6%), nalidixic acid (0%) and gentamycin (0%).

Class 1 integrons are the most common and well characterized class of integron. They are widely disseminated in animal and human clinical isolates of the family *Enterobacteriaceae.* ***(Goldstein et al.,2001).***

Class 1 integrons are commonly found in antibiotic-resistant clinical isolates of Gram-negative bacteria. Each class1 integron contains up to several gene cassettes encoding drug resistance, and the pool of such cassettes seems to be large. ***(Sorum et al.,2003)***.

In this study, class 1 integrons were detected in 70% of the tested *Salmonella* isolates from squabs including *S.*Typhimurium, *S.* Entertidis ,*S.* Montevideo and *S.* Agona., 42.9 % of tested *Salmonella* isolates from adult pigeons including *S.* Entertidis 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_{Psel}) which confer resistance to sulphamethoxazole/ Trimethoprim, streptomycin, gentamycin, spectinomycin; chloramphenicol, sulphonamide and ampicillin. These results agree with results of ***Farzaneh et al.(2011)***. In this study *blaTEM* was detected in 20% of tested *Salmonella* isolates from squabs including *S.* Entertidis, 42.9% of tested *Salmonella* isolates from adult pigeons including *S.* Entertidis and not detected at all isolates from pigeons environments isolates. These results was agreed with ***Loana et al.(2006).***

CONCLUSION

In conclusion, prevalence of *Salmonella* is higher in squabs than that occur in adult pigeons with a higher rate in diseased followed by freshly dead and finally by apparently healthy slaughtered birds. The most predilection site for *Salmonella* isolation were intestine followed by intestinal L.N. then liver. In vitro sensitivity of all *Salmonella* isolates were completely resistant to Streptomycin, Amoxicillin/clavulanic acid, Amoxicillin, Ampicillin and Ceftazidime(100%). Class1 integrons were characterized in 70% *Salmonella* isolates from squabs, 42.9 % in adult pigeons and 14.3% in pigeon enviroments which confer their resistance to streptomycin and ampicillin. TEM-1 β-lactamase was characterized in 20% of tested *Salmonella* isolates from squabs including *S.* Entertidis, 42.9% of tested *Salmonella* isolates from adult pigeons including *S.* Entertidis which confer their resistance to cephalosporin and not detected at all isolates from pigeons environments.

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