Isolation of *Salmonella* Typhimuriumin from Some Psittaciformes Species and Detection of Antibiotic Resistant Genes In Egypt

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Abstract

This study was conducted to isolate and identify Salmonella as one of important zoonotic microorganisms from different spp. of captive bred psittaciformes. A total of 300 psittaciformes (were collected from private wildlife farms, pet shops and households) belonging to 15 different species were clinically examined and samples, ((219) fecal samples, (72) cloacal samples and internal organs (intestine, liver, lung, spleen and kidney) from 9 freshly dead birds) were taken for detection of salmonella infections using traditional methods of isolation and polymerase chain reaction (PCR) based on *inv* A gene as a confirmatory accurate technique on isolated strains, estimation of the antibiotic susceptibility and detection of resistance genes. The result revealed that, the incidence of the infection constituted 3.33% (10 isolates) of total number of investigated psittacine birds and the most common affected psittacines were (5) rosy-faced lovebirds, (4) budgerigars and (1) green rosella which were bought from illegal wildlife trafficking. All 10 isolates were *Salmonella* Typhimurium confirmed by PCR based on invA gene. The antibiotic sensitivity tests revealed that, all 10 isolates were highly susceptible (100%) in vitro to amoxicillin / clavulanic acid, ciprofloxacin and gentamycin, while 100% of the isolates exhibited complete resistance to doxycycline and sul./ trimethprim. Detection of resistance genes was tested by PCR targeting (*tet*1, *tet* 2, *sul* 1and *sul*2) antimicrobial genes. Resistant genes were detected of *Salmonella* Typhimuruim isolates. (6) Against tetracycline A *tet* A gene, (7) tetracycline gene B *tet* B and (7) sulphonamide gene 1 *sul* 1, meanwhile all strains were negative for *sul* 2 resistant gene.

Introduction

Previous studies on microbiota of psittacine birds indicated that, normal microbiota of them are composed only of gram positive bacteria and yeasts ***(Lopes et al.,2014).***The presence of enterobacteria is not considered normal components of unstressed parrots microflora ***(Ritchie et al., 1994).***

Salmonella infections occur in wild birds where they can cause disease and death, or even spread from their avian hosts to domestic mammals and man. In spite of being recognized as an avian disease for over a hundred years, Salmonellosis is an emerging disease as a result of increased artificial feeding by human. *Salmonella* may be present in feces for a short time, as a result of environmental contamination ***(Tizard, 2004)***. Multiple serovars of *Salmonella* enterica originating from mammalian, reptilian and avian hosts have been reported to cause infections in human, wildlife and exotic pets harboring *Salmonella* are potential sources for human infections ***(Hoelzer et al., 2011).*** Transmission of *Salmonella* from wildlife and exotic animals to humans occurs through multiple pathways. Evidences increasingly suggest that, there could be a bidirectional transmission of *Salmonella* between domesticated and wild animals. Farm animals acquiring *Salmonella* from wildlife, could increase the risk of human infection. *Salmonella* infections in humans have also been reported through direct contact with exotic pets and wildlife, especially those in captivity ***(Krueger et al., 2014)***.

Salmonellosis is well- known cause of disease and intermittently reported disease in psittacine birds ***(Oros et al., 1998).***The risk of disease dissemination must be considered, given that captivity allows greater contact between species, favoring the transmission of infectious agents ***(Alves et al. , 2013).*** On the other hand, *Salmonella* spp. serotype most frequently isolated from psittacines is *Salmonella* Typhimurium ***(Hidasi et al., 2013)***.

So this study aimed to detect the prevalence of *Salmonella* infection in some species of captive bred psittaciformes in Egypt from different sources, as well as using of PCR based on *inv* A gene as a sensitive and a specific accurate tool for confirmation and detection of Salmonella. Beside, antibiotic sensitivity and antibiotic resistant genes were performed on *Salmonella* Typhimurium strains

Material and Method

Birds: 300 psittaciformes belonging to 15 different species (257 apparently healthy, 34 diseased and 9 freshly dead birds) were collected from different private farms of psittacines (234), pet shops (25) and households (41) in Egypt.

Samples: a total number of 300 samples were collected on aseptic condition from all investigated psittacine birds. All samples, (219) fecal samples, (72) cloacal samples and internal organs (intestine, liver, lung, spleen and kidney) from 9 freshly dead birds were labeled with code number, type of sample, bird species and date of collection and were submitted to bacteriological and serological examination.

Bacteriological examination of collected samples:

1- Cultivation in liquid media:

The swabs from sample (fecal, cloacal and tissue) were collected aseptically and inoculated into selenite F broth incubated at 37°C for 18-24 hours.

2- Plating out onto solid media:

A loopfull from the incubated broth was placed and streaked onto Salmonella Sigella agar "S S Agar", MacConkey agar, Xylose lysine deoxychoclate agar "XLD agar" and Brilliant green agar plate **(Oxoid)**, then incubated at 37°C for 24 hours. Semisolid nutrient agar "0.4 %"**(Oxoid)** was used for detection of motility as well as preservation of the isolated strains as those were carried out by ***Wilson*** and ***Miles (1975)***. Suspected colonies were subjected to morphological and biochemical identification according to ***Cruickshank et al., (1975)***.

Serological identification of the isolated Salmonella:

Serotyping of isolated Salmonella was carried out in serological unit, Animal Health Research Institute, Dokki, Giza according to ***Edwards and Ewing (1972)***.

Antimicrobial susceptibility testing by disc diffusion method:

The test was performed according to the procedures of ***(NCCLS, 2007)*** using disc diffusion technique **(Table 1)**.

1-Preparation of *Salmonella* isolates for DNA extraction:

Pure colony of *Salmonella* from selective medium was transferred to nutrient agar medium and incubated for 24 hr at 37°C **(Shanmugasamy *et al.*, 2011)** then, 3 ml phosphate buffered saline was added on the medium and harvesting the growth by pipetting them and collecting in 15 ml falcons tube (pelleting).

2-Bacterial genomic DNA extraction:(Freschi *et al*., 2005)

1- 100µl from pellet was transferred into eppendorff tube after vortex.

2- Eppendorff tube was put into heat block at 95°C for 10 minutes, then to freezer overnight, centrifugation at 13,000 xg for 3 minutes.

3- The supernatant (extracted DNA) was transferred into another clean eppendorff.

3-DNA amplification (polymerase chain reaction):

DNA samples were tested (in 25ml. reaction volume in a 0.2 PCR tube. The reaction mixture consisted of 12.5 ml. master mix (Thermo

Scientific), 3 ml. Bacterial DNA, 0,25 ml. of each primer (Table 2) (conc. 25 pmol) and nuclease free water up to 25 ml., then thermal cycling in a programmable heating block (Coyvorporation, Grasslake, Michan, USA) was done.

4-Molecular identification of Salmonella spp. gene:

a) PCR protocol of *inv*A gene:

***** Initial denaturation at 94° C/ 5 min.

* Denaturation at 94° C / 0.5 min.

* Annealing at 64° C / 0.5 min.

* Extension at 72° C / 45 sec.

* Cycles repeated for 35 times with final extention at 72°C/ 7 min.

b) PCR protocol of *tet* A and *tet* B genes:

***** Initial denaturation at 94° C / 5 min.

* Denaturation 94°C / 30 sec.

* Annealing 55° C / 30 esc.

* Extention 72° C / 45 sec.

* Cycles repeated for 35 times with final extention at 72 °C/ 7 min.

c) PCR protocol of *sul*1 and *sul*2 genes:

* Initial denaturationat 94°C/ 3 min.

* Denaturation 94°C/1 min.

* Annealing for *sul*1 gene 51°C/ 1 min.,and for *sul*2 gene 57°C/1 min. *Extention at 72° C / 1 min.

* Cycles repeated for 35 times with final extention at 72°C / 10 min.

5-Identification of the PCR products:

Following amplification, 10 of each reaction products taken for electrophoresis on 1.5% (W/ V) agarose gel containing 1 x TAE buffer (0.01 m Tris acetate 0.002 M EDTA) and ethidium bromide (0.5 mg/ ml) The electrophoresis at 100 volts for 35 minutes in an electrophoresis unit. The presence of specific amplified DNA bands was detected by visualization with UV light at wave length 421 run and compared with molecular size marker (Ladder) with MW 100 bp and measure MW100-1000 bp.

Table (1): *Antimicrobial susceptibility testing by disc diffusion method:*

Antibiotic	Code	Conc.	Standard zone of inhibition (mm)		
			sensitive	intermediate	resistant
Ampicillin	AMP	10µg	≥ 17	14–16	≤ 13
Amoxicillin	AML	10µg	≥ 17	14–16	≤ 13
Amoxicillin / clavulanic acid	AMC	30µg	≥ 18	14–17	≤ 13
Ciprofloxacin	CIP	5 µg	≥ 21	16–20	≤ 15
Gentamicin	GN	10 µg	≥ 15	13–14	≤ 12
Neomycin	N	30µg	≥ 17	13-16	≤ 12
Streptomycin	S	10 µg	≥ 15	12–14	≤ 11
Tetracycline	TE	30 µg	≥ 15	12–14	≤ 11
Doxycycline	DO	30 µg	≥ 14	11–13	≤ 10
Spiramycin	SP	100 µg	≥ 24		≤ 19
Sulfa./ trimethoprim	SXT	25 µg	≥ 16	11–15	≤ 10

Molecular detection of Salmonella isolates:

Table (2): *Oligonucleotide primers encoding for inv A gene and antibiotic resistant genes:, tetracycline and Sulfonamides:*

Primer	DNA sequences (5' to 3')	Target gene	Amplicon size	Annealing Temp.		Ref.
***Inv* A gene F**	5'GTGAAATTATCGCCACGTTCGGGCAA-3'	*Inv A*	284 pb.	64 ºC		**Rahn *et al.* (1992)**
***Inv* A gene R**	5'- TCATCGCACCGTCAAAGGAACC-3'					
***tet* (A) F*tet* (A) R**	GTGAAACCCAACATACCCC GAAGGCAAGCAGGATGTAG	*tet (A)*	741 bp.	55 ºC		**Ma *et al.* (2007)**
***tet* (B) F*tet* (B) R**	CAGTGCTGTTGTGTCATTAA GCTTGGAATACTGAGTGTAA	*tet (B)*	571 bp.	55 ºC		
Sul1 F Sul1 R	TTCGGCATTCTGAATCTCAC ATGATCTAACCCTCGGTCTC	*Sul1*	547 bp.	51 ºC		**Harel *et al.* (1991)**
Sul2 F Sul2R	CGGCATCGTCAACATAACC GTGTGCGGATGAAGTCAG	*Sul2*	543 bp.	57 ºC		**Ma *et al.* (2007)**

Result and Discussion

Psittacine birds are frequently commercialized in illegal wildlife trade and when apprehended by the responsible public departments are often found in poor sanitary conditions. In these cases, these birds become susceptible to several pathogens, such as enterobacteria and can cause intestinal and extraintestinal opportunistic infections. ***(Lopes et al., 2015).***

Source of positive *Salmonella* psittacines in the present study was illegal trafficking during period of import ban which demonstrate, that psittacines from illegal trafficking can be an infected with *Salmonella* and this result agreed with

Goncalves et al. (2010); Hidasi, et al. (2013) and Matias et al. (2016). These birds were under stress condition that, may be enhance the presence of *Salmonella* that is an opportunistic microorganism, this agreed with ***Dorrestein et al. (1997)*** who stated that, *Salmonella* are ubiquitous microorganisms and, under suitable conditions can survive and multiply in environment for a long time. Many animals can be subclinical carriers and birds may become infected by ingestion of contaminated food and / or drinking water or contact with carriers (e.g., rodents, wild birds, or domesticated species).

In addition the presence of enterobacteria not considered normal, but actually categorized as a contamination. ***(Marietto et al., 2010)***.

There have been many reports on isolation of *Salmonella* from caged birds such as psittacines birds ***(Sawa et al. 1981)***. Asymptomatic *Salmonella* carriage in wild birds is thought to be high, as many species acquire the organisms and become intestinal carriers without showing any visible signs and can be considered apparently healthy birds ***(Gamal- Eldein et al., 2008).***

The present study was conducted upon captive bred, species of order psittaciformes from different sources, private wildlife farms, pet shops and households .This study has been carried out on a total number of 300 psittacine birds belonging to 15 different species (257 apparently healthy, 34 diseased and 9 died during period of sampling).

At necropsy of (9) dead psittacines birds, {(1) budgerigar, (2) lovebird} from farm (A), {(3) Amazon and (3) lovebird} from farm (C), (Table 3), that died during period of sampling, no focal necrosis were seen in the post mortum samples, However no *Salmonella* was isolated in these birds, this result disagreed with each of ***Oros, et al., (1998)*** who isolated *Salmonella* Airozonea from the liver with multifocal necrotic hepatitis in Sulpher crested cockatoo (Cacatus galerita) with no previous clinical signs, ***Vigo, et al. (2009)*** who isolated *Salmonella* Typhimurium from liver, spleen, heart, lung, kidney and intestine of Blue and gold macaw (Ara ararauna) chicks died of fatal *Salmonella* and ***Piccirillo et al. (2010)*** who isolated *Salmonella* Typhimurium from 2 Molucan cockatoos (Cacatus moluccensis) and at post mortum reported necrotic foci surrounded by a hyperemic halo were observed in lungs, heart, liver, spleen, kidneys and intestine. In addition contrast to each of ***Cardona et al. (2016)*** who reported a case of unusual salmonellosis in female African grey parrot and at necropsy , revealed sever fibrinous pericarditis, moderate hydrocoelom, diffusely reddened lungs, and a green discoloration of the liver and

Siqueira et al. (2017) who isolated *Salmonella* Typhimurium from dead pet pittacine birds with multifocal necrotic hepatitis.

Salmonella spp. are not regular members of the intestinal microbiota of psittacine and, therefore isolating these bacteria from asymptomatic or immunosupressed individuals indicates a possibility of disease ***(Goncalves et al., 2010)***.

As shown in Table (3), the prevalence of *Salmonella* was in apparently healthy psittacine birds in farm (A), (10/80), 12.5% and 10/257, (3.89%) to the total investigated apparently healthy birds. This result go in hand with ***Lopes et al., (2015) and Suphoronski et al. (2015),*** they declared that, gram negative bacteria may be isolated from healthy psittacines. Also, ***Evance (2011)*** stated that, *Salmonella* spp. were isolated from several captive psittacines birds, whether they are asymptomatic carriers or clinically diseased. Previous studies proved that the normal microbiotas of these birds are Gram positive bacteria and yeast and this was disagreeing with our result. In addition ***Lopes et al. (2014)*** stated that, in most birds presented negative in the study of *Salmonella* spp. in captive psittacines may not imply the absence of this pathogen in these birds, since the intermittent excretion is a well- known characteristic of this microorganism.

Meanwhile, in this study as shown in Table (3) there was no isolation of *Salmonella* from pet shops nor households and this result disagree with ***Seeperadsingh and Adesiyn (2003)*** who reported 6 spp. of *Salmonella* isolated from pet birds were obtained from pet shops and households which may pose a health risk to their owners and contacts.

As shown in **Table (4**) the bacteriological examination of 300 samples revealed that, 10 *Salmonella* isolates were positive from apparently healthy birds demonstrated a (3.33%*) Salmonella* prevalence rate in the total investigated psittacine birds and (10.33 %) among the examined birds in a private wildlife farm group, farm A, 92 psittacines while no Salmonella was isolated from 34 diseased psittacines nor 9 psittacine birds died during period of study.

This low prevalence Salmonellosis in the present study, (3.33%) almost agreed with the study of ***Matias et al. (2016)*** and ***Siqueira et al. (2017)*** who isolated a single *Salmonella* Typhimurium (1/75), 1.33% prevalence rate, while disagreed with ***(Akhter, et al. 2010)*** who isolates 21 *Salmonella* species. out of 45 samples with a prevalence rate (46.6%).

Among the investigated (Table 5), 15 Psittaciformes spp. *Salmonella* was isolated from 3 spp., Lovebirds

{5/66,(7.57% of total lovebird}, Green rosella {1Green rosella 1/18, 5.55% of total rosella} and budgerigar {4/85, 4.7% of total budgerigar }and this result similar to ***Oros et al. (1998)*** who isolated *Salmonella* Arizonae from captive sulpher crested cockatoo ***(Cactus galerita*** *and* ***Tizard, 2004)*** stated that, the wild and exotic birds, such as budgerigars, may harbor *Salmonella* spp. in their intestine**.** Moreover, ***Abd-El –Latif*** *and* ***El-Said, (2003)*** isolated *Salmonella* spp. from 50 psittacines**,** ***Seepersadsingh and Adesiyun, (2003)*** isolated *Salmonella* from pet birds**.** Also**,** ***Allgayer et al. (2008)*** investigated 13 captive psittacines birds for *Salmonella* and the most commonly infected were orange-winged parrot (*Amazona amazonica*), (28%) and red-spectacled parrot (*Amazona pretrei*), (20%) and ***Enas (2008)*** isolated *Salmonella* from budgerigar**.** Also**,** ***Vigo et al. (2009)*** isolated *Salmonella* strains from 2 blue and gold macaw (*Ara ararauna*), ***Akhter et al. (2010)*** isolated 21 *Salmonella* spp. from a total of 45 samples were collected from 5 types of caged parrots (Gray cockatiels, Rose ringed parakeet, Alexandrine parakeet, Red breast parakeet and Blossom head parakeet) of Dhaka Zoo and ***Evance (2011)*** isolated *Salmonella* spp. from caged parrots. ***Cardona et al. (2016)*** isolated *Salmonella* spp. from an adult female African grey parrot and ***Siqueira et al. (2017)*** isolated one single *Salmonella* in Amazon parrot (*Amazona aestiva*).

Ten positive *Salmonella* were isolated from cloacal swabs,(10/34), 29.4% to the total no. of cloacal swabs in farm (A) and 13.88% (10/72) to total no. of cloacal samples, (Table 6), this similar to ***Akhter et al. (2010),*** who reported that, irrespective to the types of parrots, the higher percentage of different bacteria was isolated from cloacal swabs and also similar with ***Bezerra et al. (2013), Lopes et al. (2014)*** and ***Lopes et al. (2015).***

While contrast to ***Sareyyüpoglu et al. (2008)*** reported that, 5 (2.7%) fecal samples were found to harbor *Salmonella* spp. out of 108 fecal samples collected from pet birds in Ankaraand and ***Hidasi et al. (2013)*** isolated one *Salmonella* spp. from fecal samples.

Salmonella Typhimurium was detected by serological identification in all 10 *Salmonella* isolates. This go in hand with ***Piccirillo et al. (2010)*** who said that, *Salmonella* spp. serotype most frequently isolated from psittacines is *S.* Typhimurium.

Meanwhile, in previous studies are agreed to our findings that isolated *Salmonella* Typhimurium from psittacines **as** ***Ward et al. (2003)*** isolated 4/45, 8.88% *Salmonella* Typhimurium in a population of 45 lorikeets and lories, ***Piccirillo et al. (2010)*** reported 2 fatal cases of

Salmonella Typhimurium in Moluccan cockatoos and ***Krawiec et al. (2015).***

However, disagreed with others who isolated other serotypes, ***Oros et al. (1998)*** isolated *Salmonella* Arizonae from captive sulpher crested cockatoo (*Cactusgalerita*)**,** ***Seepersadsingh and Adesiyun (2003)*** isolated 6 isolates of *Salmonella* species with 2 isolates of serotype Aberdeen and one isolate each of Thompson, Rubislaw, Panama and Newport**,** ***Allgayer et al. (2008)*** isolated *Salmonella* spp. from 13 different spp. psittacines birds but specific tests for *Salmonella* Typhimurium were negative, one *Salmonella Lexington* from (31) Blue – fronted Amazon (*Amazona aestiva*), one *Salmonella* Saintpaul from (16) Red- and – green Macaw (*Ara macao*) and one *Salmonella Newport*from (06) Budgerigar and ***Akhter et al. (2010)*** isolated (5) *Salmonella* Pullorum from caged parakeets. In addition, ***Goncalaves et al. (2010)*** isolated *Salmonella* Enteriditis in 3 captive specimens of Amazona aestiva out of 103 investigated birds, 2.9% and ***Enas (2015)*** isolated *Salmonella* Paratyphoid , *S.* Chester, *S.* Infantis, and untypable S. strains Moreover, ***Lopes et al. (2014)*** and ***Matias et al. (2016)*** isolated 2 *Salmonella* Panama strains from 2 chestnut capped black birds (Chrysomurufi capillus).

There is scarce information about antimicrobial resistance and diseases in pet birds, however there are reports involving free-living birds as potential disseminators of *E. coli* and *Salmonella* spp. resistant to cefalosporins, ampicillin, streptomycin, sulfoxazole and tetracycline isolated from passerines ***(Andres et al., 2013).***

Antimicrobial resistance in non-typhoidal Salmonella is common, and in some places, it has been increasing in recent years ***(Centers for disease control and prevention, 2013)***.

While a growing body of research has found evidence of AMR in *Salmonella* spp. isolates derived from free-living wildlife, including birds. Wildlife species possess antimicrobial resistance determinants and the prevalence rate of AMR genes in these isolates could be as high as 100% ***(Botti et al., 2013).***

In this study the most effective antibiotics (100%) sensitivity were amoxicillin /clavulanic acid, ciprofloxacin and gentamycin. Sixty%, 40%, 30%, 30%, 30% and 20% susceptible to neomycin, streptomycin, ampicilin, amoxicillin, spiramycinand tetracycline respectively while 100% of the isolates exhibited complete resistance to doxycycline and Sulph./ Trimethobrim, 80% to tetracycline, 50% to spiramycin, 30% ampicillin, 20% amoxicillin,

20% streptomycin and 10% neomycin, Table (7) and Fig. (1) These results agreed with ***Meakins et al. (2008)*** who mentioned that, despite wide use of fluoroquinolones such as ciprofloxacin, the levels of resistance to these antimicrobials remain low. Moreover, agree with ***Rahmani et al. (2011) and Abd- El latif and El Said, (2003)*** whom reported that, most of isolated Salmonella strains were resistant to amoxicillin, fluoquine, streptomycin and penicillin**.** ***El Sharkawy et al. (2017)*** reported tetracycline resistance in the *Salmonella* Typhimurium isolates 58 (86.6%) in a total 615 broiler flocks. The study observed 20% resistance rate to amoxicillin, also, disagree with ***Leonard et al. (2012)*** who recorded a sensitivity rate of (86.7%) against amoxicillin and disagree with ***Enas (2008)*** who reported that, amikacin, chloramphenicol and tetracycline were the most effective drugs against *Salmonella* and the isolated *Salmonella* was resistant to amoxclavulanic acid, erythromycin and penicillin. Moreover disagreed with ***Vigo et al. (2009)*** who reported that, all *Salmonella* strains isolated from 2 blue and gold macaw (*Ara ararauna*) was sensitive to trimethobrim-sulfamethoxazole while in our study resistance to sulph./ Trimethobrim was 100%. In addition, disagreed with ***Matias et al. (2016)*** who reported that resistance of one strain of *Salmonella* Typhimurium and 2 strains of *Salmonella* Panama (isolated from wild birds) to multiple antimicrobial drugs, like ampicilin, ceftriaxone, ceftifur, tetracycline, gentamycin, enrofloxacin and ciprofloxacin.

It is assumed that, the multidrug resistance in this result might be due to their frequent application of these antibiotics which suggest paying more attention when using these antibiotics.

The excessive use of a specific antimicrobial agent may explain the difference between the sensitivity profiles observed among the surveys. Since it is known that, continuous exposure of the bacteria to an antimicrobial agent tends to select this microorganism to resistance ***(Arias and Carrilho, 2012)***.

The presence of multidrug resistant strains, if not controlled, can be considered a condition of sanitary risk to the birds, as well as to free-living animals that may be exposed to the introduced birds. Birds carrying resistant strains may spread these bacteria and, consequently, affect other wild animals through direct or indirect contact with contaminated feces ***(Hebla et al., 2011)***.

The antimicrobial susceptibility tests on psittacines from illegal trade revealed that, the enterobacteria found in the intestinal microbiota of the studied birds presented high multidrug resistance rates, which the most frequent resistance was to

azithromycin among the various isolated strains and this may be a consequence inadequate use of this antibiotic at some part of the life of these birds ***(Lopes et al., 2015)***.

One of the earliest steps in the pathogenic cycle of the facultative intracellular pathogen Salmonella species was the invasion of the intestinal epithelium, *inv* A was a member of this locus, and it was the first gene of an operon consisted of at least two additional invasion genes ***Galan et al. (1992) and Lamb et al. (2014)*** recommended the use of invA primer due accuracy, sensitivity and uniform distribution among *Salmonella*.

To assess potential virulence of *Salmonella* isolates by the presence or absence of genes, Polymerase chain reaction (PCR) was used to detect *Salmonella* virulence genes. All samples tested positive using PCR, amplifying the invasion gene *inv*A gene, at 284 bp. Fig. (2), these results were in agreement with ***Krawiec et al. (2015)*** while nearly similar with ***Hudson et al. (2000)*** who detected 15 positive *inv* A gene in a total of 22 *Salmonella* isolates.

Detection of resistant genes was tested by PCR targeting *tet*A, *tet*B, *sul* 1 and *sul* 2. PCR detected *tet*A gene, (740 bp) with an incidence rate of 60%, Fig (3). , *tet B* gene, (571bp) with an incidence rate 70%, Fig. (4) and *sul*1 gene, (574 bp) with an incidence rate 70%, Fig (7) while there was no detection at all of *sul*2, Fig. (6) Which refers that in this study the incidence rate of *tet A* gene is higher than that of *tet B* and so it is disagreed in percentage of detection with ***Eid and Shalaby (2013)***, who reported the incidence rates detected in their study for *tet* A and *tet* B genes by PCR was 90% and 40%, respectively and ***(Hamada et al. (2003), Asai et al. (2006) and Shahada et al., (2006)*** who stated that, the most common tetracycline resistance determinant in chickens belonged to *tetA* gene. Moreover, agreed with ***El Sharkawy et al. (2017)*** reported tetracycline resistance in the *S.* Typhimurium isolates 58 (86.6%) in a total 615 broiler flocks, correlated with the presence of *tet* C (96.6%), and *tet*A gene (84.5%), (*Sul*1 and *Sul*3). All tested strains were negative for *tet*B codon. *tet* A codon was also found in all of the non-typable *Salmonella* strains.

Table (3): *Prevalence of Salmonella in different Sources of collection:*

Source of sample	Apparently healthy			Diseased			Dead	
	No.	positive *Sal.*		No	positive *Sal.*		No	% positive *Sal.*
		No.	%		No	%		
Farm (A)	80	10/80	12.5%	9	0/9	0%	3	0%
Farm (B)	49	0/49	0%	49	0/49	0%	0	0%
Farm (C)	62	0/62	0%	25	0/25	0%	6	0%
Pet shops	41	0/41	0%	41	0/41	0%	0	0%
House holds	25	0/25	0%	25	0/25	0%	0	0%
Total No.	257	10/257	3.89%	34	0/34	0%	9	(0%)

Table (4*): Source of examined birds and positive samples***.**

Source of examined psittaciformes	No. of examined birds	No. of positive samples	Percentage (%)
Farm (A)	92	10/92	10,9%
Farm (B)	49	0/49	0%
Farm (C)	93	0/93	0%
Pet shops group	41	0/41	0%
House hold group	25	0/25	0%
Total No.	**300**	**10/300**	**3,33%**

Table

(5) : *Prevalence of Salmonella infection in examined psittacines*

Scientific Name	Common Name	Examined (No.)	Positive salmonella	
			(No.)	(%)
Ara macao	Macaw	2	0/2	0%
Platycercus caledonicus	Green rosella	18	1/18	5,55%
Pesphotus varius	Mulga parrot	5	0/5	0%
Psephotus haematonotus	Red – rumpedparrakeet	25	0/25	0%
Neophema splendida	Splendid or Scarlet-chested parrots (Splendida)	1	0/1	0%
Neopsephotus bourkii	Bourke's parrot	4	0/4	0%
Neophema pulchella	Turquoisine parrot	2	0/2	0%
Psittacus erithacus	African grey parrot	17	0/17	0%
Melopsittacus undulatus	Budgerigar	85	4/85	4,7%
Nymphicus hollandicus	Cockateil	16	0/16	0%
Polytelis alexandrae	Princess of Wales (paralceet)	3	0/3	0%
Psittaculakrameri	Indian ring head parakeet	9	0/9	0%
Amazona amazonica	Orange-winged amazon	28	0/28	0%
Agaponis roseicollis	Rosy-faced love bird	60	5/60	8.33%
Agaponis taranta	Black-winged lovebird	6	0	0%
Agaponis fischeri	Fischer's lovebird	6	0/6	0%
Total lovebird		72	5/72	6.94%
Pionus senilis	White-caped parrot	9	0/9	0%
Cacatus goffiana	Goffin's cockatoo	4	0/4	
Total No.		**300**	**10/300**	**3,33%**

Table (6) *Prevalence of Salmomella in all types of samples:*

Type of sample	Source of sample	No. of samples	positive *Salmonella*	
			No.	%
Fecal samples	**Farm (A)**	55 / 92	0/55	0%
	Farm (B)	40 / 49	0/40	0%
	Farm (C)	58 / 93	0/58	0%
	Pet shops	41 / 41	0/41	0%
	House holds	25 / 25	0/25	0%
No.		219	0	0%
Cloacal swabs	**Farm (A)**	34/92	10/34	29,4%
	Farm (B)	9/49	0/9	0%
	Farm (C)	29/93	0/29	0%
	Pet shops	0/41	0	0%
	House holds	0/25	0	0%
No.		72	10	13.88%
Necropcies	**Farm (A)**	3/92	0/3	0%
	Farm (B)	0/49	0	0%
	Farm (C)	6/93	0/6	0%
	Pet shops	0/41	0	0%
	House holds	0/25	0	0%
No.		9	0	0%
Total		**300**	**10/300**	**3.33%**

Table (7): *Antibiogram of isolated Salmomella Typhimurium:*

Antimicrobial group	R		I		S	
	No.	(%)	No.	(%)	No.	(%)
Ampicillin	3	30%	4	40%	3	30%
Amoxicillin	2	20%	5	50%	3	30%
Tetracycline	8	80%	0	0%	2	20%
Doxycycline	10	100%	0	0%	0	0%
Spiramycin	5	50%	2	20%	3	30%
Amoxicilin /clavulanicacid	0	0%	0	0%	10	100%
Sulph./ Trimethobrim	10	100%	0	0%	0	0%
Ciprofloxacin	0	0%	0	0%	10	100%
Gentamicin	0	0%	0	0%	10	100%
Neomycin	1	10%	3	30%	6	60%
Streptomycin	2	20%	4	40%	4	40%

R: Resistant **I**: Intermediate **S:** Sensitive

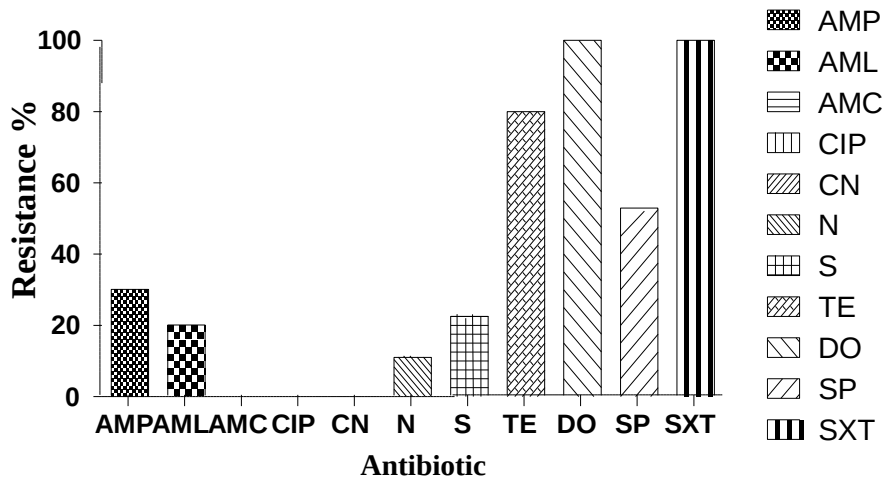

Figure (1): *Antibiotic resistance pattern of different SalmonellaTyphimurium isolated from investegated Psittacine birds*

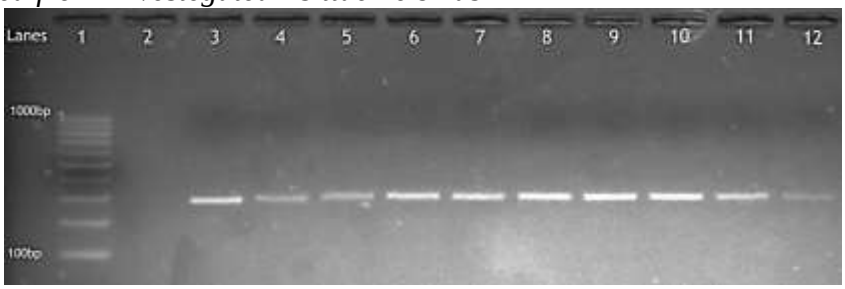

Figure (2): Electropherotic pattern of *inv* A gene PCR assay: lane 2 (negative control): *E.coli* ATCC 25922**,** lanes 3-12: positive *inv* A gene (284 bp) *Salmonella* isolates and lane 1: DNA ladder from 100-1000 (Jena Bioscience).

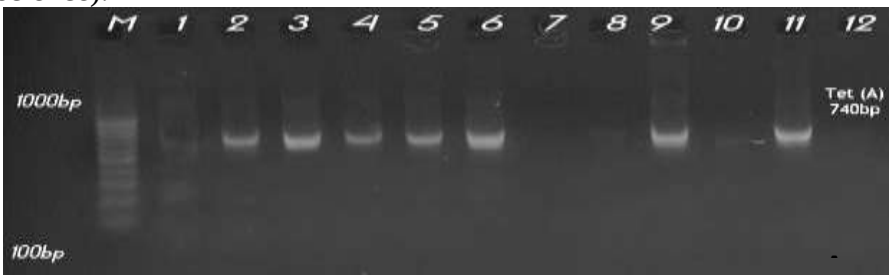

Figure (3):Electrophoretic pattern of *Salmonella* Typhimurium tetracycline resistance gene, (A) *tet*A gene 740 bp PCR assay:DNA marker GeneRuler (Thermo); 1 negative control; 2 positive control; from 3-12 examined *Salmonella* isolates.

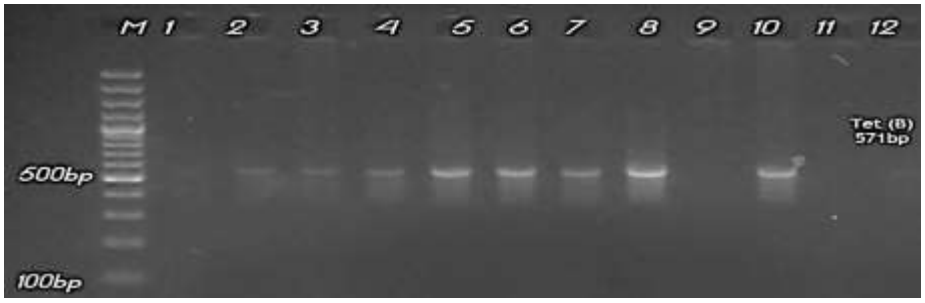

Figure (4):Electropherotic pattern of*Salmonella* Typhimurium tetracyclin resistance gene, *tet* B gene 571bp PCR assay,; DNA marker JenaBioscience (Germany) (B) M; DNA marker GeneRuler (Thermo); 1 negative control ; 2 positive control; from 3-12 examined *Salmonella* isolates.

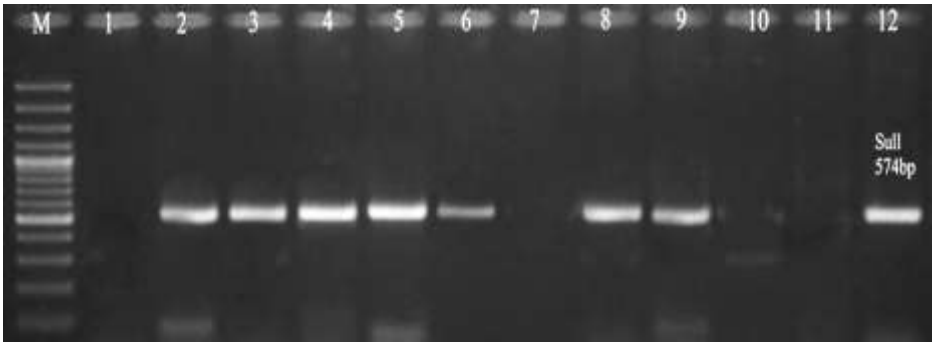

Figure (5):Electropherotic pattern of *Salmonella* Typhimurium Sulfonamide resistance gene 1, *Sul* 1 gene 574 bp PCR assy; DNA marker Gene Ruler (Thermo); 1 negative control ; 2 positive control; from 3-12 examined *Salmonella* isolates.

Figure (6):Electrophoretic pattern of *Salmonella* Typhimurium resistance Sulphonamid gene2 show negative *Salmonella* Typhimurium Sulphonamide resistant gene, *Sul* (2) gene 543 bp PCR assay ; DNA marker Gene Ruler (Thermo); 1 negative control; 2 positive control; from 3-12 examined *Salmonella* isolates.

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عزل سالمونيلا تيفميوريوم من بعض أنواع الببغاوات ورصد الجينات المقاومة للمضادات الحيوية فى مصر

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تم عمل هذه الدراسة لعزل وتصنيف السالمونيلا كواحدة من أهم الأمراض البكتيرية المشتركة على انواع مختلفة من طيور الببغاء . تم فحص 300 طائر تنتمى ل (15) نوع من طيور الببغاء من مصادر مختلفة (محلات طيور الزينة- مزارع خاصة للحياة البرية- طيور مرباة فى المنازل), كما تم تجميع (219) عينة براز, (72) مسحة من فتحة المجمع وعينات من الاعضاء الداخلية (الامعاء, الكبد, الرئه, الطحال والكلي) من 9 طيور نافقة وذلك لعزل ميكروب السالمونيلا بالطرق التقليدية وتأكيد العزل باجراء تفاعل البلمرة المتسلسل باستخدام جين (*inv* A) مع اجراء اختبارات حساسية ورصد الجينات المقاومة للمضادات الحيوية.

أظهرت النتائج أن معدل الأصابه بالسالمونيلا كان3,33 % لعدد (10) عينات من اجمالى الطيورمحل الدراسة وذلك من طيور سليمة ظاهريا تم شراؤها بطرق غير مشروعة أثناء فترة حظراستيراد الطيور: (5) طيور الحب، (4) طيور الدر الأسترالى و(1) طائر الروزيلا. كما نم تصنيف العترات المعزوله الى سالمونيلا تيفيميوريم وتم تأكيد العزل باستخدام تفاعل البلمره المتسلسل باستخدام جين (*inv* A). وكانت نتيجة اختبارات حساسية المضاد الحيوى أن المضادات الأكثر تاثيرا (100%) هى أموكسيسلين/ حمض الكلافولينك، سيبروفلوكساسين، جنتاميسين وأظهرت مقاومة (100%) لكل من دوكسيسيكلين وسلفا/ترايميثوبريم . وعند عمل رصد للجينات المقاومة لتتراسيكلين، سلفوناميد 1،2 أظهرت النتائج وجود (6) عينات ايجابية لجين المقاومة تتراسيكلين (أ)، (7) عينات ايجابية لجين المقاومة تتراسيكلين(ب)، (7) عينات ايجابية لجين المقاومة سلفوناميد (1) بينما ظهرت النتيجة سلبية لجين المقاومة تتراسيكلين (2) فى جميع العينات.