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Detection and Importance of Some Pathogenic Bacteria in Psittascine Birds with Special Reference to the Virulence Genes

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ABSTRACT

Psittascine birds are potential carriers and/or transmitters of some pathogenic bacteria, which have an important impact on human health. The scope of this study was to survey, isolate and identify some pathogenic bacteria in psittascine birds with special regard to plasmid detection of some virulent and resistance genes. A total of 120 samples (85 from different birds, 35 samples from their surrounding) were collected for microbiological investigations. All isolates were submitted to antimicrobial susceptibility testing and discussed in details. *E.* coli was the predominant isolate in all examined birds (34.11%) followed by *Staphylococcus* spp. (32.9%) and *Salmonella* spp. (12.9%). *E.* coli of O78 was the most prevalent serotype detected in all samples also; *Salmonella Typhymurium* was the most frequent serovar in all recovered *Salmonella* spp. isolates. Plasmid detection of *crl* and *eae*A virulence genes of *E.* coli isolates were found in (100% and 33.3%, respectively). Also, all examined isolates of *Salmonella* spp. and *Staphylococcus* spp. showed the presence of (*inv*A, *stn* and *fim*H) and (*clf*A and *ica*A) virulence genes in (100%, for each), respectively. Meanwhile, screening for antibiotic resistance genes (*dfr*A, *tet*A, *bla*$_{TEM}$, *qnr*A, *nor*A and *bla*Z) of *E.* coli, *Salmonella* spp. and *Staphylococcus* spp., respectively were detected on all relative plasmid profiles (100%, for each). In conclusion, several bacterial pathogens were isolated from psittascine birds so, it is recommended to apply good biosecurity management and good hygiene with competent and quality veterinary care in birds/ pets breeding, with preventive guidelines. Also, due to the zoonotic nature of some bacteria, it is very important that pet bird owners should be trained well to practice good hygiene when handling / breeding their pets to limit the risk of zoonotic diseases.

INTRODUCTION

Pet birds (which are members of psittasciform group of birds) are the source of recreation for human especially children because of its sociable and affectionate nature, intelligence, bright colors and ability to imitate with human voices.

The term "Pet bird" designates birds housed and bred for an exclusively ornamental use. This category includes mainly Passeriformes or songbirds (e.g. canaries, finches, sparrows) and psittaciformes or love birds (e.g. parrots, parakeets, budgerigars). Budgerigars, canaries, parakeets or parrots are regularly sold at high prices, "which is a lucrative business for pet shops or local breeders" (Seeley *et al*., 2014). Small passerines, canaries, and finches are social birds often bred and housed in flock aviaries. The food habits of these pets in seeking food on the ground could lead to their contamination by droppings from infected ones. These birds also probably encounter a higher risk, since they often spend a relatively long duration at the feeding site. An accident infection of wild birds may create due to the contaminated environment especially in domestic pigeons and colonial water birds (Tizzard, 2004). These infected birds may transmit infections to humans, either directly as a result of handling, or indirectly.

Pigeons live in urban and rural areas in close contact with humans and other animals and their population growth is increasing. They act as a reservoir for several pathogenic and zoonotic agents that can be transmitted to poultry, wildlife, domesticated pets, and/or humans via excreta, secretions, or dust from feathers. In addition, ingestion of infected pigeons by wild and domestic animals can also transmit these pathogenic agents to it (Suphoronski *et al*., 2015).

The management of psittacine birds provides many challenges increasing the potential for disease outbreaks. Pet birds suffered from many bacterial diseases with often involvement of normal flora or environmental pathogens in response to stress and immunosuppression that pose threats in a flock situation. Bacterial enteritis as well as bacterial respiratory disease is often a spontaneous stress associated disease caused mainly by *E.* coli *spp., Klebsiella* spp., *Salmonella* spp., *Pasteurella* spp., *Pseudomonas* spp., *Aeromonas* spp., *Citrobacter* spp., *Enterobacter* spp. and *Mycoplasma* spp. (Akhter *et al*., 2010). Kirk *et al*., (2002) reported that most of paratyphoid infections caused by enteric Salmonellae (*Salmonella* Typhimurium, *Salmonella* Enteritidis). Also, Cabllero *et al*., (2015) mentioned that *Campylobacter* spp. and *Salmonella* spp. also, Listeriosis and enterobacteriosis are most common zoonoses could be transmitted by pigeons.

Antimicrobial resistance is of a global concern in the public health and veterinary medicine (Szmolka and-Nagy, 2013). Siqueira *et al*., (2017) explained that the antimicrobial resistance is the mechanism by which bacteria can resist the action of antimicrobial agents. World Health Organization referred the recent widespread of multi-drug resistant bacteria (MDR) to the indiscriminate use of drugs in human and veterinary medicine**.** However, scarce information about antimicrobial resistance and diseases in pet birds, there are reports indicated that free-living birds can act as potential disseminators of resistant serotypes of *E.* coli and *Salmonella* spp. against some antimicrobials like: cephalosporins, ampicillin, streptomycin, sulfamethoxazole and tetracycline (Lopes *et al*., 2014).

Plasmids are important vehicles for rapid adaptation of bacterial populations to their environmental conditions. Plasmid-mediated gene transfer plays an important role not only in the mobilization and dissemination of antibiotic resistance genes but also in the spread of degradative pathways and pathogenicity determinants of pathogens (Smalla *et al*., 2015). The recent advances in sequencing technologies provide an enormous potential for plasmid classification, diversity and evolution studies but numerous challenges still exist. High level multidrug resistance is normally associated with plasmids that encode specific resistance (Hall, 1997).

As there is less knowledge has been documented in psittascine birds in Egypt, the purpose of this study was to investigate the prevalence, genetic diversity of plasmid

virulence and antibiotic resistance genes of most isolated bacteria with zoonotic potential in most popular housed psittacine (pet) birds and their roles in disseminating the infection especially for pet bird's owners.

MATERIALS AND METHODS

Samples:

The study was conducted during the period from December 2018 till August 2019. A total eighty-five samples from different psittascine birds [Pigeons (n=40)**,** budgerigars (n= 30), parrots (n= 15)] and 35 from their surrounding environment were collected. The examined birds showed some signs of illness like: diarrhoea, loss of appetite, loss of feathers, weakness and some cases were died. The samples were collected from five retail outlets in Ismailia and Sharkia Governorates, Egypt. Cloacal swabs with wet vent and/or fresh droplets also, (liver, lung and intestine) samples were collected from diseased birds. In addition, thirty-five environmental samples include [litter (n=15), water (n=10) and feedstuff (n=10)]. All collected samples were transported in an icebox within 2-3 h and transferred to the laboratory for bacteriological analysis.

Bacterial Isolation and Identification:

a- Isolation of *E.* coli was carried out according to (MacFaddin, 2000).

b- Isolation of *Salmonella* species was carried out according to (ISO/IEC 6579: 2017).

c- Isolation of *Staphylococcus* spp. carried out according to (ISO/IEC 6888-1:1999-AMD/2003).

d- Identification of the recovered isolates by biochemical tests (Quinn *et al*., 1994).

e- The recovered isolates of *Staphylococcus* spp. were confirmed using Integral system stafilococchi kit (NCCLS, 2004).

Serological Identification:

E. coli isolates were serotyped according to (Kok *et al*., 1996) and *Salmonella* spp. isolates were serotyped according to (Patrick and Francois, 2007).

Antimicrobial Susceptibility Testing:

The susceptibility of isolated strains against a panel of seven commonly used antimicrobial agents was performed using the standard Kirby–Bauer disc diffusion method (Quinn *et al*., 1994). The results were interpreted according to (CLSI, 2015). The antibiotics involved in the study were selected based on the feedback collected from shop owners describing their use of the commercially available medications prescribed for pet birds. The antimicrobials were used: Trimethoprim/sulfamethoxazole, tetracycline, ampicillin, norfloxacin, erythromycin, ciprofloxacin and gentamycin.

Plasmid Screening of Some Virulence and Antibiotic Resistance Genes:

Plasmid DNA from MDR isolates of *E.* coli (no=6), *Staphylococcus* spp. (no=5) and *Salmonella* spp. (no=5) were isolated using QIAprep Spin Miniprep Kit (QIAGEN GmbH, Hilden, Germany). Screening for the presence of a selection of some antibiotic resistance and virulence plasmid-associated genes was carried out by PCR amplification using specific primers and different cycling conditions as shown in table (1). The PCR products were tested for positive amplification by agarose gel electrophoresis. For each PCR experiment, appropriate positive and negative controls were included.

Table 1: Primers sequences, target genes and amplicon sizes of some virulence and antibiotic resistance genes of different bacterial isolates

Test target	Target gene	Primers sequences	Amplified segment (bp)	Reference
E. coli virulence genes	*Crl*	TTTCGATTGTCTGGCTGTATG	250	Bisi-Johnson *et al.*, (2011)
		CTTCAGATTCAGCGTCGTC		
	eaeA	ATG CTT AGT GCT GGT TTA GG	248	Grape *et al.*, (2007)
		GCC TTC ATC ATT TCG CTT TC		
Salmonella spp. virulence genes	*invA*	GTGAAATTATCGCCACGTTCGGGCAA	284	Oliveira *et al.*, (2003)
		TCATCGCACCGTCAAAGGAACC		
	Stn	TTG TGT CGC TAT CAC TGG CAA CC	617	Murugkar *et al.*, (2003) Hojati *et al.*, (2013)
		ATT CGT AAC CCG CTC TCG TCC		
	fimH	GTGCCAATTCCTCTTACCGTT	164	
		TGGAATAATCGTACCGTTGCG		
Staph spp. virulence genes	*clfA*	TCA ACA AAG AAC AAC AAA ATG C	638	Wada *et al.*, (2010)
		GCT TTC GGT GCT TGA GAT TC		
	icaA	CCT AAC TAA CGA AAG GTA G	1315	Ciftci *et al.*, (2009)
		AAG ATA TAG CGA TAA GTG C		
E. coli Resistant Genes	*dfrA*	TGGTAGCTATATCGAAGAATGGAGT	425	Ghanbarpour and Salehi, (2010)
		TATGTTAGAGGCGAAGTCTTGGGTA		
Salmonella spp. Resistant Genes	*bla*TEM	ATCAGCAATAAACCAGC	516	Colom *et al.*, 2003
		CCCCGAAGAACGTTTTC		
	*tet*A(A)	GGTTCACTCGAACGACGTCA	576	Randall *et al.*, (2004)
		CTGTCCGACAAGTTGCATGA		
	qnrA	ATTTCTCACGCCAGGATTTG	516	Robicsek *et al.*, (2006)
		GATCGGCAAAGGTTAGGTCA		
Staph spp. Resistant genes	*nor*A	TTCACCAAGCCATCAAAAAG	620	Duran *et al.*, (2012)
		CTTGCCTTTCTCCAGCAATA		
	blaZ	ACTTCAACACCTGCTGCTTTC	173	
		TGACCACTTTTATCAGCAACC		
		ATGGACAACCCGACAGAAGC		

RESULTS

Bacterial Finding:

Bacteriological examination of all examined psittacine birds revealed that *E.* coli was the predominant isolate in all bird samples (34.11%) as shown in table (2). *E.* coli was isolated in all examined budgerigars 53.3% (16/30), 46.7% (7/15) in parrots, 12.5% (6/40) in pigeons. *Staphylococcus* spp. was recovered in a total percentage of (32.9%). It was isolated in budgerigars, parrots and pigeons birds in percentages of (63.3%, 40% and 7.5%), respectively. The birds were examined also, for *Salmonella* spp., which was found in a prevalence rate of (12.9%); where it was recovered in (20%, 16.7% and 7.5%) from parrots, budgerigars and pigeons bird, respectively.

Table 2: Prevalence of some pathogenic bacteria in psittascine birds

Bacterial isolates / Bird	*E. coli*	*Staphylococcus spp.*	*Salmonella spp.*
Budgerigars (30)	16 (53.3%)	19 (63.3 %)	5 (16.7%)
Parrots (15)	7 (46.7%)	6 (40%)	3 (20%)
Pigeons (40)	6 (12.5%)	3 (7.5%)	3 (7.5%)
Total birds (85)	29 (34.11%)	28 (32.9%)	11 (12.9%)

Bacteriological examination of thirty-five environmental samples (litter, feed stuffs and water samples) detected the presence of *E.* coli, *Staphylococcus* spp. and *Salmonella* spp. in 34.3%, 20% and 5.7%, respectively as shown in table (3).

Table 3: Incidence of bacterial species from the surrounding environment

Environmental Samples	Bacterial isolates		
	***E.* coli spp.**	***Staphylococcus* spp.**	***Salmonella* spp.**
Litter (15)	6	4	1
Feed stuff (10)	4	2	1
Water (10)	2	1	-
Total (35)	12 (34.3%)	7 (20%)	2 (5.7%)

Identification of *Staphylococcus* spp.

The isolated strains Staphylococcus *spp.* of were identified using the Integral system stafilococchi kit: ISSK, as shown in table (4). Most isolates (19/35) were found as coagulase positive staph (CPS) which was *S.* aureus while others were of coagulase negative staph (CNS); 4/35 of *S. warneri*, 5/35 of *S. xylosus* and 7/35 of *S. hominis*.

Table 4: Serotyping of *Staphylococcus* spp isolates.

Serotypes		No.of isolates (35)
CPS	***S. aureus***	19/35
CNS	***S. warneri***	4/35
	S. xylosus	5/35
	S. hominis	7/35
Total		35/35

CPS= Coagulase Positive Staph CNS= Coagulase Negative Staph

Serotyping of *E.* coli and *Salmonella* spp.

The Serotyping of forty-one *E.* coli isolates revealed five different serotypes. The most predominant serotype was O78 (11/41), followed by O128: H2 (10/41), O127:H6 (9/41), O146: H21 (7/41) and (4/41) of isolates were O2: H6 of total *E.* coli isolates table (5).

Table 5: Serotyping of *E.* coli isolates

Serotypes	No. of isolates (41)
O78	11
O128:H2	10
O127 : H6	9
O146 : H21	7
O2 : H6	4
Total	41/41

Serotyping of the total detected 13 Salmonella *spp.* isolates revealed the detection of 5 serotypes, which were *Salmonella* Typhymurium (7/13), while *Salmonella* Virginia (3/13), *Salmonella* Abortusequi (2/13) and *Salmonella* Agona (1/13), table (6).

Table 6: Serotyping of *Salmonella* serovars isolates

Serovare	Antigenic structure		No. of isolates (13)
	O	H	
Salmonella typhimurium	4,5	i:1,2	7
Salmonella Virginia	6,10	-	3
Salmonella abortusequi	8,20	i,z6	2
Salmonella agona	4,12	-	1
Total	13/13		

Phenotypic Profiles of Antimicrobial Resistance of Isolated Bacteria:

The sensitivity patterns of different isolated bacteria against selected antibiotics were so variable; as shown in table (7). The isolates of *E.* coli were highly resistant to erythromycin (97.6%) then trimethoprim/ sulfamethoxazole, tetracycline and norfloxacin (92.7% for each), then ampicillin (80.5%), ciprofloxacin (73.2%) and gentamycin (19.5%). However, isolates of *Salmonella* spp. demonstrated high resistance against tetracycline and ampicillin (100%); meanwhile, gentamycin was the most sensitive drug. In addition, *Staphylococcus* spp showed multidrug resistance (MDR) profile against tetracycline and ampicillin (100% for each), norfloxacin (97.1%) then trimethoprim-sulfamethoxazole (85.7%), erythromycin (71.4%), ciprofloxacin (65.7%) and gentamycin (57.1%).

Table 7: Antibiotic resistance pattern of pathogenic bacteria in psittascine birds

Antibiotic group	Antibiotics	*E. coli spp.* (41)		*Salmonella spp.* (13)		*Staphylococcus spp.* (35)	
		No. *	Resistance rate	No. *	Resistance rate	No. *	Resistance rate
Trimethoprime	**Trimethoprim/ Sulfamethoxazole**	38	92.7%	10	76.9%	30	85.7%
Tetracycline	**Tetracycline**	38	92.7%	13	100%	35	100%
Penicillin	**Ampicillin**	33	80.5%	13	100%	35	100%
Quinolone	**Norfloxacin**	38	92.7%	9	69.2%	34	97.1%
	Ciprofoxacin	30	73.2%	9	69.2%	23	65.7%
Macrolide	**Erythromycin**	40	97.6%	10	76.9%	25	71.4%
Aminoglycoside	**Gentamycin**	8	19.5%	5	38.5%	20	57.1%

No. = Number of Resistant isolates

The resistance rate was calculated according to the total number of each species

Genotypic Detection of Plasmid Virulence Attributes:

The *crl* and *eae*A virulence genes of six MDR *E.* coli isolates were detected on plasmid profile with PCR. The *crl* gene was found on a plasmid of all examined isolates (100%) (Fig.1); while *eae*A gene was shown on a plasmid of 2/6 (33.3%) only of all isolates, (Fig. 2). For five MDR *Salmonella* spp. isolates, the plasmid profile of (*inv*A, *stn* and *fim*H) was confirmed with PCR in all examined isolates 5/5 (100%) for each, (Figs. 3, 4 and 5). Also, the conserved virulence (*clf*A and *ica*A) genes of five MDR *Staphylococcus* spp. isolates were detected in all isolates (100%), (Figs. 6 and 7).

Genotypic Detection of Plasmid Resistance Attributes:

As showed in (Fig. 8), the *dfr*A gene was present in all examined MDR *E.* coli isolates on their plasmid profile with PCR. Also, the *bla*$_{TEM}$, *tet*A and *qnr*A resistance genes were similarly detected in all plasmid profiles of five examined MDR *Salmonella* spp. isolates, (100%, for each) (Figs. 9, 10 and 11). In addition, *nor*A and *bla*Z resistance genes of five MDR *Staphylococcus* spp. examined isolates were detected on all relative plasmid profiles (100%, for each) (Figs. 12 and 13). It is clear that there was positive relative relation between the presence of different antibiotic resistance genes of each examined bacterial species and their genes on plasmid profile as shown in Tables 8, 9 and 10.

Table 8: Phenotypic and genotypic resistance profiles of *E*. coli isolates

NO.	Sample source	Serotype	Antimicrobial resistance profile	Virulence gene		Resistance gene
				*eae*A	*Crl*	*dfr*A
1	Pigeon	O78	T, SXT, NOR, AM,CIP,CN	+	+	+
2	Budgerigars	O127: H6	T, SXT, NOR, AM,CIP,CN	+	+	+
3	Parrot	O128:H2	T, SXT, NOR, AM,CIP,CN	-	+	+
4	Feed	O2: H6	T, SXT, NOR, AM,CIP,CN	-	+	+
5	Water	O127:H6	T, SXT, NOR, AM,CIP,CN	-	+	+
6	Litter	O2: H6	T, SXT, NOR, AM,CIP,CN	-	+	+

Table 9: Phenotypic and genotypic resistance profiles of *Salmonella* spp. isolates

NO.	Sample source	*Salmonella* serovars	Antimicrobial resistance profile	Virulence gene			Resistance gene	
				*inv*A	*stn*	*fim*H	*tet*A	*qnr*A
1	Pigeon	*S. typhimurium*	E,T,SXT,NOR,AM,CIP,CN	+	+	+	+	+
2	Budgerig-ars	*S .typhimurium*	E,T,SXT,NOR,AM,CIP,CN	+	+	+	+	+
3	Parrot	*S. agona*	E,T,SXT,NOR,AM,CIP,CN	+	+	+	+	+
4	Feed	*S. typhimurium*	E,T,SXT,NOR,AM,CIP,CN	+	+	+	+	+
5	Litter	*S. abortusequi*	E,T,SXT,NOR,AM,CIP,CN	+	+	+	+	+

Table 10: Phenotypic and genotypic resistance profiles of *Staphylococcus* spp. isolates

NO.	Sample source	Staph serotype	Antimicrobial resistance profile	Virulence gene		Resistance gene	
				*clf*A	*ica*A	*nor*A	*bla*Z
1	Pigeon	*S. aureus*	E,T,SXT,NOR,AM,CIP,CN	+	+	+	+
2	Budgerigars	*S. warneri*	E,T,SXT,NOR,AM,CIP,CN	+	+	+	+
3	Parrot	*S. xylosus*	E,T,SXT,NOR,AM,CIP,CN	+	+	+	+
4	Feed	*S. aureus*	E,T,SXT,NOR,AM,CIP,CN	+	+	+	+
5	Litter	*S. aureus*	E,T,SXT,NOR,AM,CIP,CN	+	+	+	+

SXT: Trimethoprim/sulfamethoxazole, T: Tetracycline, AM: Ampicillin, NOR: Norflxacin, CIP: Ciprofoxacin, CN: Gentamycin

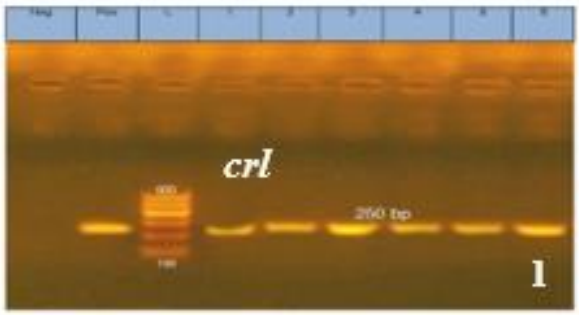

Figure (1): *crl* virulence gene for *E.coli*
Lane L: DNA molecular size marker 100-600 bp
Lane (Pos): Positive control
Lane (Neg): Negative control
Lane 1-6: positive for gene *crl* at 250 bp

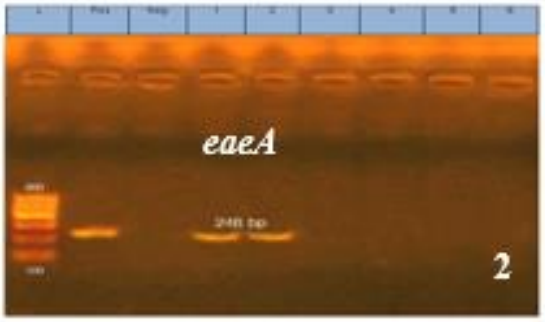

Figure (2): *eaeA* virulence gene for *E.coli*
Lane L: DNA molecular size marker 100-600 bp
Lane (Pos): Positive control
Lane (Neg): Negative control
Lane 1-6: positive for *eae*A gene at 248 bp.

Figure (3): *inv*A virulence gene for *salmonella*
Lane L: DNA molecular size marker 100-600 bp
Lane (Pos): Positive control
Lane (Neg): Negative control
Lane 1-5: positive for *inv*A gene at 284 bp

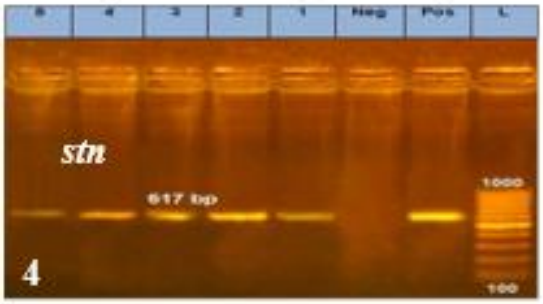

Figure (4): *stn* virulence gene for *salmonella*
Lane L: DNA molecular size marker 100-1000 bp
Lane (Pos): Positive control
Lane (Neg): Negative control
Lane 1-5: positive for *stn* gene at 617 bp

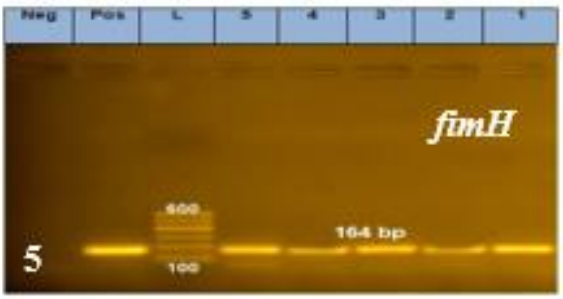

Figure (5): *fim*H virulence gene for *salmonella*
Lane L: DNA molecular size marker 100-600 bp
Lane (Pos): Positive control
Lane (Neg): Negative control
Lane 1-5: positive for *fim*H gene at 164 bp

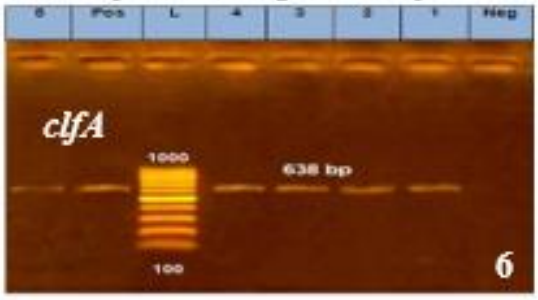

Figure (6): *clf*A virulence gene for *staph.spp.*
Lane L: DNA molecular size marker 100-1000 bp
Lane (Pos): Positive control
Lane (Neg): Negative control
Lane 1-5: positive for *clf*A gene at 638 bp

Figure (7): *ica*A virulence gene for *staph.spp.*
Lane L: DNA molecular size marker 100-1500 bp
Lane (Pos): Positive control
Lane (Neg): Negative control
Lane 1-5: positive for *ica*A gene at 1315 bp

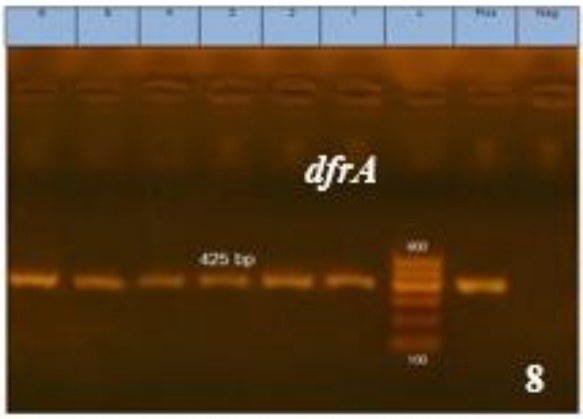

Figure (8): *dfr*A resistant gene for *E. coli*
Lane L: DNA molecular size marker 100-600 bp
Lane (Pos): Positive control
Lane (Neg): Negative control
Lane 1-6: positive for *dfrA* gene at 425 bp

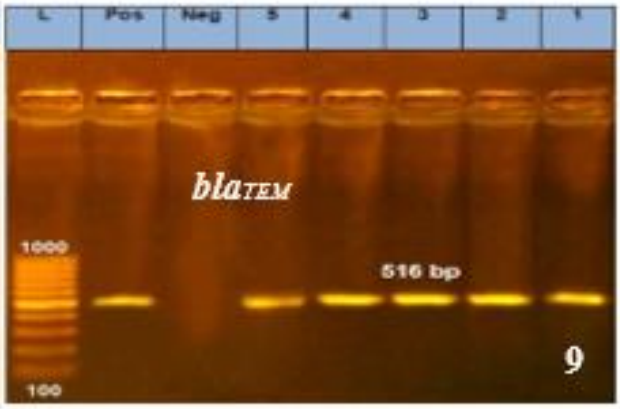

Figure (9): *bla*TEM resistant gene for *Salmonella*
Lane L: DNA molecular size marker 100-1000 bp
Lane (Pos): Positive control
Lane (Neg): Negative control
Lane 1-5: positive for *bla*TEM gene at 516 bp

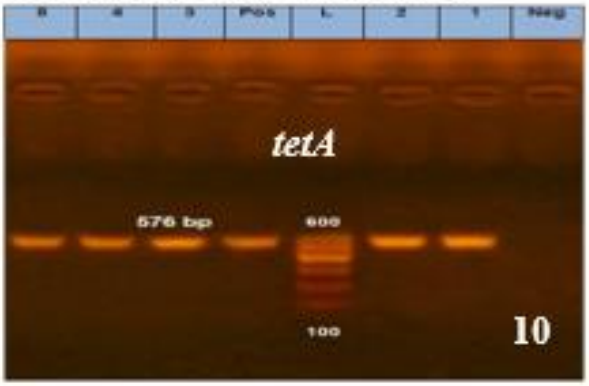

Figure (10): *tet*A resistant gene for *Salmonella*
Lane L: DNA molecular size marker 100-600 bp
Lane (Pos): Positive control
Lane (Neg): Negative control
Lane 1-5: positive for *tet*A gene at 576bp

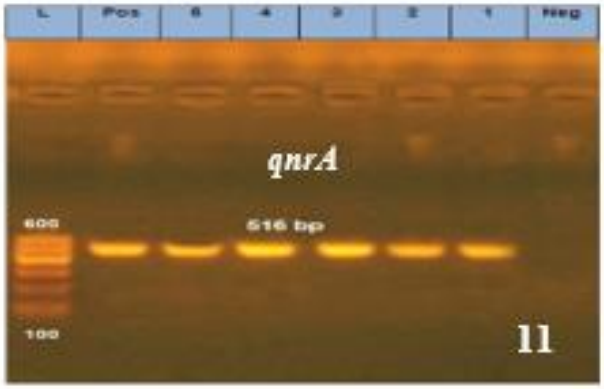

Figure (11): *qnr*A resistant gene for *Salmonella*
Lane L: DNA molecular size marker 100-600 bp
Lane (Pos): Positive control
Lane (Neg): Negative control
Lane 1-5: positive for *qnr*A gene at 516 bp

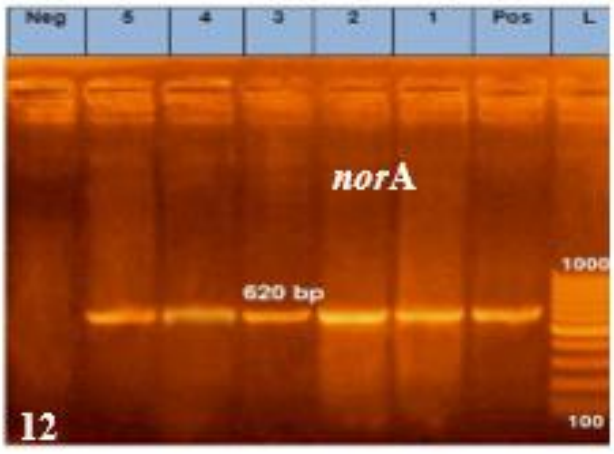

Figure (12): *nor*A resistant gene for *staph spp.*
Lane L: DNA molecular size marker 100-1000 bp
Lane (Pos): Positive control
Lane (Neg): Negative control
Lane 1-5: positive for *nor*A gene at 620 bp

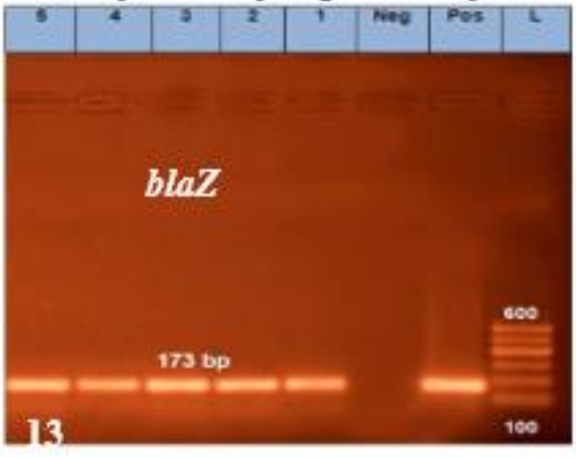

Figure (13): *blaZ* resistant gene for *staph.spp.*
Lane L: DNA molecular size marker 100-600 bp
Lane (Pos): Positive control
Lane (Neg): Negative control
Lane 1-5: positive for *blaZ* gene at 173bp

DISCUSSION

However, *E.* coli is commensal in the gastrointestinal tract of pet birds but they can also act as opportunistic pathogens. Also, *Staphylococcus* spp. is a pathogenic agent for different psittacine species (Hermans *et al.*, 2000). In the current study, the prevalence rate of *E.* coli from psittacine species and its surrounding was (34.11%). Also, *Staphylococcus* spp. was isolated in (32.9%) from psittacine species and in 20% from the surround environment. In the same way, Giacopello *et al*., (2013) isolated *E.* coli form canaries in percentage of (35.2%) and Medani *et al*., (2008) proved the presence of *E. coli* and *Staph.* Aureus from total 170 coloacal swabs from budgerigars in percentages of 53.33% and 70%, respectively. While much higher prevalence rate was recorded by (Akhter *et al*., 2010) who isolated *E*. coli (64.44%) and *Staphylococcus* spp. (46.67%) from many types of apparently healthy caged pet birds. The variation might be attributed to that the pets are kept indoors, feed contamination or recently bought birds are the most probable sources of infection to bird collections (Shima and Osborn, 1989).

Salmonellosis is an important worldwide zoonotic disease in wild birds causing great mortality. Recently, the prevalence of *Salmonella* spp. in wild birds has been increased significantly (Tizzard, 2004). In this study, *Salmonella* spp. was isolated from different pet birds and surrounding environment in lower percentage (12.9% and 5.7%), respectively. Rahmani *et al*., (2011) recorded *Salmonella* spp. in a similar percentage (16.1%) from canaries however; Suphoronski *et al*., (2015) detected *Salmonella* spp. in a relative percentage (22.32%) from different breeds of free-living and captive wild birds in Brazil. Lower prevalence rate (5.7% and 1.12%) of *Salmonella* spp. was reported by (Giacopello *et al*., 2013 and Siqueira *et al*. 2017), respectively. This could be attributed to various types and size of samples or using different methods for *Salmonella* spp. detection, or its geographic location and types of food consumed (Padungtod and Kaneene, 2006). It seems likely that some predisposing factors and environmental situations as breeding or shipping, could affect the severity of *Salmonella* Paratyphoid infections in pet birds (Raidal, 1998).

E. coli may be classified into several serotypes, according to the antigens which presents**,** its significance is to detect the pathogen circulation in the environment (Croxen *et al.*, 2013). In the current study, the most predominant serotypes of the recovered isolates of *E.* coli were O78 (11/41), then O128: H2 (10/41), O127:H6 (9/41), O146: H21 (7/41) and O2: H6 (4/41). Similar results were documented from wild birds in Egypt by (Hassan and Aml, 2014).

Different *Salmonella* serovars were identified in different species of pet birds (Lopes *et al*., 2014). As shown in table (6), serotyping of *Salmonella* spp. isolates proved that the most predominant serovar was *Salmonella* Typhymurium (7/13) followed by *Salmonella* Virginia (3/13), *Salmonella* Abortusequi (2/13) and *Salmonella* Agona (1/13). Madadgar *et al*., (2008) recorded also, that *Salmonella* Typhimurium was the main cause of high mortalities in flocks of canaries in Tehran and Australia attributing that to feed contamination of bird droppings with *Salmonella*. Similarly, Sousa *et al*, (2010) recorded that 80% of *Salmonella* spp. isolates were of *Salmonella* Typhimurium. Also, Georgiades and Iordanidis, (2002) reported that *Salmonella* Typhimurium was the most frequently isolated serotype in percentage of 75.5%. This result could conclude that contaminated feces might be a source of several zoonotic agents for other birds, animals and humans (Tanaka *et al.*, 2005). The zoonotic nature of *Salmonella typhimurium* and close contact of bird especially bird owners and immunosuppressed people strengthen its human public health importance (Madadgar *et al*., 2009).

Pigeons could play as reservoir for transmissible pathogens by air or contaminated food or water. The cause of spreading of bacterial disease in pigeons may due to the housing conditions of pigeons in Egypt which gives the chance for pigeons to come into close contact with wild and domesticated bird that enabling direct transfer of the infectious agents to take place especially when kept out to doors (Hassan and Amal, 2014).

Many researchers in different countries have documented common incidence of bacterial infections in pigeons with *E.* coli, *Streptococci* and *Salmonella* spp. (Herdt *et al.,* 1994). In the present study, *E. coli* was detected in lower percentage from pigeon samples (12.5%). The same incidence (12.1%) of EPEC and ETEC from feral pigeon droppings in Brazil was recorded by (Silva *et al.,* 2009). Similarly, Hassan and Amal, (2014) isolated *E.* coli in (21.8%) of all diseased and freshly dead pigeons. In addition, *Salmonella* spp. was isolated from pigeon samples in this study in percentage of (7.5%). Sousa *et al.,* (2010), Samah and Azhar, (2013) and Al-Aalim, (2017) documented *Salmonella* spp. in pigeon with rate of (8%, 9% and 7.9%), respectively.

Antibiotic resistance problem has problem has continued virtually unaltered until today (Matias *et al*., 2016). Multiple drug resistance (MDR) patterns have been previously reported in avian isolates and could be spread from animal origin into human population by direct contacts and through animal origin foods (Madadgar, 2008 and Mirzaie, 2010).

The phenotypic resistance profile patterns of different bacterial isolates were discussed in table (7). MDR patterns in pet birds to aminoglycosides, quinolones, macrolide and other groups in *E.* coli and *Salmonella* spp. were documented (Ramalivhana *et al*., 2014). In the current study, MDR of varying degrees (from 73.1% to 97.6%) for *E.* coli isolates were reported. High antibiotic resistance rate of *E.* coli isolates was recorded against erythromycin, trimethoprim-sulphamethaxzole, tetracycline and norfloxcin. In the same way, Hassan and Amal, (2014) stated high resistance rates of *E.* coli strains against trimethoprim-sulphmethoxazol (89.5%), ampicillin (84.2%), tetracycline (78.9%) and erythromycin (78.9%). Also, Hassan *et al.,* (2008) confirmed that most *E.* coli strains were highly resistant to different antibiotics like: amoxicillin, sulphamethoxazol, neomycin, oxytetracycline and trimethoprim.

For *Salmonella* spp., the isolates showed 100% resistance against each of tetracycline and ampicillin followed by trimethoprim-sulphmethoxazol and erythromycin (76.9%) then ciprofloxacin (69.2%). Similarly, Rahmani *et al*., (2011) recorded high resistance to tetracycline, and trimethoprim sulfamethoxazole for *Salmonella* spp. isolates. Also, Matias *et al*., (2016) found that *Salmonella* Typhimurium was multi-resistant especially for cephalosporin and quinolones groups**.**

Concerning to *Staphylococcus* spp. isolates, the present study reported high resistance rate to tetracycline and ampicillin (100% of each) and norfloxacin (97.1%). Similar antibiotic resistance for *Staphylococcus spp* in pet birds was stated by (Nemati *et al*., 2008). Generally, gentamycin then ciprofloxacin drugs were found to be least resistant drugs against all examined bacterial isolates in varying degrees. High sensitivity of gentamycin for different bacterial isolates was also documented from different birds in previous studies (Medani *et al*., 2008 and Kmet *et al.,* 2013).

There are a limited number of publications about the presence of virulence factors in psittacine birds in comparing with commercial avian. Virulence factors could be a first step in elucidating the pathogenesis of diseases in wild birds (Knobl and Menao, 2010). The detection of virulence factors of *E. coli* strains isolated from psittacine birds by molecular techniques may help to clarify the bacterial pathogenesis (Knobl *et al*., 2011). Some virulence factors are indeed involved in clinical cases of colibacillosis in psittacine birds (Saidenberg, 2009).

The recent study showed the presence of *crl* virulence gene in all *E.* coli examined isolates (100%). *crl* is defined as curli expression gene which promotes bacterial adherence to the laminin and fibibronectin; activate plasminogen and help in chicken erythrocyte agglutination (Provence and Curtis, 1992). Similarly, Knobl *et al*., (2011) reported that the structural gene *crl* was present in all *E. coli* isolates (100%) from psittascine birds. In addition, attaching and effacing (*eaeA*) intimin virulance gene *E. coli* spp. was detected in this study in 2/6 (33.3%). In the same way, Mohamed and Sayed, (2017) recorded that (43.75%) of the isolates were positive for *eae*A gene. Meanwhile, Caballero *et al*., (2015) detected *eae* gene in lower percentage (13.3%) of *E.* coli from pigeon's isolates.

For *Salmonella* spp., the conserved virulence "*inv*A" gene was detected in all plasmid profiles of examined *Salmonella* isolates (100%) at amplicon size of 284 bp. It is the predominant necessary gene which could express virulence in the host, causing infection. This result was in agreement with (Shanmugasamy *et al*., 2011 and Enas *et al*., 2016) who reported the presence of *inv*A gene in all tested *Salmonella* spp. isolates from captive budgerigars. This implied that, the isolate that doesn't carry the gene may not be virulent and unable to invade epithelial cells causing disease (Enas *et al*., 2016). Moreover, *stn* virulence gene of *Salmonella* spp. was detected in all isolates by the presence of its amplified product at 617 bp. The same results were stated with (Murugkar *et al*., 2003 and Ziemer and Steadham, 2003) who revealed the presence of the *stn* gene in 100% of *Salmonella* isolates. In addition, the results revealed that 5/5 (100%) of tested *Salmonella* spp. isolates possessed *fimH* gene. It is one of the virulence genes that contribute in bacterial cell adhesion. Identical results from wild birds were recorded by (Thomas *et al*., 2017).

However, at present little information is available about virulence properties of *S. aureus* strains of psitascine bird origin. In the present study, *S. aureus* strains were investigated genotypically for various virulence determinants. The plasmid profile of *S. aureus* detects (*clf*A) and (*ica*A) virulence genes in (100%) of the examined isolates. El-Sayed *et al*., (2005) mentioned that *S. aureus* possesses various adhesion genes such as (*clf*A) which is known as the clumping factor. The capability of *S. Aureus* to adhere to extracellular matrix proteins is thought to be essential for colonization and the establishment of infections. In addition, *ica*A gene is the intercellular adhesion biofilm gene. Typical results were achieved with (El-Sayed *et al*., 2005 and El-Shekh *et al*., 2010) who recorded *clf*A and *icaA* genes in (100%) of *S. Aureus* isolates from different birds.

Plasmid-mediated gene transfer plays an important role not only in the mobilization and dissemination of antibiotic resistance genes but also in the spread of degradative pathways and pathogenicity determinants of pathogens (Smalla *et al*., 2015).

The results indicated that trimethoprim resistance gene (*dfr*A) was present in all (100%) MDR examined isolates of *E. coli*. Similarly, Shobrak and Abo-Amer, (2014) detected *dfrA* in 100% of the isolates. Moreover, *qnr* genes represent one of the most important and plasmid-mediated quinolone resistance (PMQR) mechanisms. It is admitted that resistance to quinolones results from both chromosomal and (PMQR) mechanisms. These genes encode pentapeptide repeat proteins that block the action of ciprofloxacin (CIP) on bacterial DNA gyrase and topoisomerase IV (Tran and Jacoby, 2002). In addition, *bla*$_{TEM}$ gene, a gene encoded for B- lactamases resistance and *tetA* (A) gene, a gene encoded for tetracycline resistance. The current study indicated that *bla*$_{TEM}$, *tetA* and *qnr*A resistance genes were detected in 100% of five *salmonella spp.* plasmid profiles. These results were nearly in coordinated with (Ezzat *et al*., 2019) who reported also the presence of *qnrA*, *bla*$_{TEM}$ and *tetA* genes in all examined MDR isolates of *Salmonella* spp. Also, Hur *et al*., (2011) reported that 19

out of the 21-penicillin resistant *Salmonella* spp. in Korea carried the *bla*$_{TEM}$ gene with a percentage of (90.5%) and Yemisi *et al*., (2014) stated that all of the twenty TET resistant *Salmonella* spp. isolates carried *tetA* gene (100%). On the other hand, all five examined isolates of MDR *Staphylococcus* spp. in this study carried (*bla*Z and *nor*A) genes (100%) on their plasmid profiles. These results agreed with (Matias *et al*., 2018) who detected high percentage of these resistance genes between isolates from wild birds.

Conclusion:

1. Some pathogenic bacteria like *E.* coli especially of O78, *Salomenella* Typhimurium and CNS *Staphylococcus* spp. were isolated from psittascine birds which could contaminate the environment causing a great risk to human public health due to close physical contact between birds and human. So, applying monitoring program to predict epizootic events should be reinforced.
2. Future studies with new approaches for virulence determinants are needed to appoint the clinical importance of these strains in psittaciformes, to establish the epidemiology of pathogens among wild birds.
3. Furthermore, the pet shops should be under the supervision of the General Authority for Veterinary Services and wildlife authority before and after license issue.
4. An alarm to the scientific community for antibiotic resistance phenomena due to the possibility of transmission of this resistance with mechanisms mutations in target genes and/or by determinants in plasmids which could be shed in the environment and may infect animals, then travel back through the food chain to humans. Hence, this problem should be carefully monitored.

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