Pet birds, their role in the transmission of some bacterial pathogens.

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Abstract: Housing "pet birds" has recently become one of the popular hobbies among Egyptian families. Pet birds represent potential reservoirs of several bacterial pathogens of zoonotic significance. Thus, the present study aimed to survey the main popular pet birds' shops in Zagazig city, Sharkia, Egypt for *Listeria spp, Staphylococci spp,* E.*coli,* and *Salmonella spp*. A total of eighty five samples (50 birds, 15 feed samples, 15 litter samples and 5 water samples) were examined, the results revealed that E.*coli* was the most prevalent bacterial species with a prevalence rate of 36/70 (51.4%) from the examined 50 birds, 15 feed and 5 water samples, followed by Coagulase Positive *Staphylococci* (CPS) and Coagulase Negative *Staphylococci* (CNS) with prevalence rates of 12/50 (24%), and 6/50 (12%), respectively from the examined 50 bird samples , *Salmonella spp* with a prevalence rate of 8/85 (9.4%) from the examined 50 birds, 15 feed,15 litter, and 5 water samples while the least recorded prevalence rate was for *Listeria spp,* 2/85 (2.4%) from the examined 50 birds, 15 feed, 15 litter, and 5 water samples. Studying the antimicrobial resistance profiles of isolates against 7 antimicrobial agents revealed that 30/36 (83%) of E.*coli* isolates, 7/8(87.5%) salmonella isolates, 7/18 (38.9%) of staphylococci isolates, and 2/2(100%) of listeria isolates demonstrated multidrug resistance patterns against 4 antimicrobial groups. Polymerase Chain Reaction (PCR) was also applied to investigate the presence of virulence and antimicrobial resistance genes. The alarming findings highlighted the importance of monitoring pet birds' facilities and raising public awareness for biosecurity practices to mitigate the imposed public health risk.

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1. Introduction

Pet birds are those which feed on different seeds or grains and could be kept legally under captivity. The recently increased popularity of housing pet birds has raised the significance of pet bird zoonosis and pet bird medicine **(Veladiano *et al.*, 2016)**. The most popular pet birds were passeriformes (canaries and finches etc.) and, psittaciformes (budgerigars, parrots and parakeets...etc.). **Lopes *et al.*,** (**2014)** had emphasized the importance of pet birds in the transmission of main zoonotic pathogenic bacteria as E.*coli, salmonella* spp., *listeria spp.* and *staphylococci spp.* Moreover**, (Alley *et al.*, 2002)** reported that the human risk for salmonellosis was in association with passerine birds' infection.

Bacteria may play the role of primary or secondary disease causal agents, birds are exposed to bacterial infection via environmental contact or when these bacteria exist as resident flora, their pathogenicity is determined by the interaction between host factors which may include the state of the bird's defense system, general health condition, the existence of a concurrent disease, environmental factors as external stressors, and pathogen factors as virulence factors, **(Doneley, 2009)**.

Pathogen factors include its virulence and the infective dose to which the bird is exposed. Once, bacterial pathogens have entered the body, they have to survive the natural host defenses and compete with nonpathogenic flora. This requires the ability to colonize the site of infection, determined by the physical characteristics of the microorganism as the flagella, adherence factors, and enzymes. Pathogenic bacteria also possess virulence factors that enable them to cause primary damage to targeted organs, such as invasion and secretion of cytotoxins or enterotoxins **(Wade, 2003).**

Traditionally veterinarians used antibiotics in treatment of sick birds, especially when the illness was supported by the isolation of bacteria. **(Doneley, 2009)**.

Antimicrobial resistance is the mechanism by which bacteria can resist the action of chemotherapeutic agents, WHO referred the recent widespread of antimicrobial resistance bacterial infection to the indiscriminate use of drugs in human and veterinary medicine **(Siqueira *et al.*, 2017).**

Thus, the purpose of this study was to investigate the prevalence, virulence and antimicrobial resistance profiles in bacteria with zoonotic potentials isolated from the most popularly housed pet birds.

2. Materials and Methods

I- Samples' collection

Samples' collection was planned to screen the main shops of pet birds (five shops) in Zagazig city, Sharkia governorate, Egypt. From each shop, 10 apparently diseased birds (5 Budgerigars, 3 Canaries, and 2 Cocktails), 3 litter samples, 3 feed samples, and one water sample were collected. A total of 85 samples involved internal organs (liver, trachea, lung, heart, and intestine) from each of 50 apparently diseased birds, 15 feed samples, 15 litter samples and 5 water samples were collected. Samples were transferred to RLQP Sharkia laboratory for bacteriological examination.

II- Bacterial isolation and identification

Isolation of *staphylococcus* species was applied according to (**ISO/IEC 6888-1:1999-AMD/2003)**, isolation of E.*coli* was according to (**Kreig *et al.*, 1984),** isolation of *salmonella* species was according to (**ISO/IEC 6579: 2017)**, and for *listeria* species isolation was according to (**ISO/IEC 11290:2017).**

III- Serological identification

Salmonella isolates were serotyped according to **(Patrick** and **Francois, 2007)**, serotyping of E. *coli* was carried according to **(Ewing, 1986)**, typing of *staphylococci* isolates was carried out by using Integral system stafilococchi kit (NCCLS 2004), and Oxoid Listeria Test Kit (Oxoid, Basingstoke, Hampshire, England) was used for identification of Listeria species **(Pagotto *et al.*, 2001).**

Table (1): Primers sequences, target genes, amplicon sizes and cycling conditions

Test target	Target gene	Primers sequences (5´-3´)	Amplified segment (bp)	Reference
Staphylococci species **Conserved gene**	**16S rRNA[1]**	CCTATAAGACTGGGATAACTTCGGG CTTTGAGTTTCAACCTTGCGGTCG	791	**(Mason *et al.*, 2001)**
Listeria monocytogenes **Conserved gene**	**16S rRNA[1]**	GGA CCGGGGCTA ATA CCG AAT GAT AA TTC ATG TAGGCG AGT TGC AGC CTA	1200	**(Kumar *et al.*, 2015)**
E.coli **Conserved virulence gene**	***pho*A[1]**	CGATTCTGGAAATGGCAAAAG CGTGATCAGCGGTGACTATGAC	720	**(Hu *et al.*, 2011)**
Salmonella spp **Conserved virulence gene**	***inv*A[1]**	GTGAAATTATCGCCACGTTCGGGCAA TCATCGCACCGTCAAAGGAACC	284	**(Oliveira *et al.*, 2003)**
E.*coli* **virulence gene**	***eae*A**	ATG CTT AGT GCT GGT TTA GG GCC TTC ATC ATT TCG CTT TC	248	**(Bisi-Johnson *et al.*, 2011)**
E.*coli* **virulence gene**	***fim*H**	TGCAGAACGGATAAGCCGTGG GCAGTCACCTGCCCTCCGGTA	508	**(Ghanbarpour and Salehi, 2010)**
E.*coli, salmonella species* **Mobile colistine resistance gene**	***Mcr*1**	CGGT CAGTCCGTTTGTTC CTTGGTCGGTCTGTAGGG	308	**(Newton-Foot *et al.* 2017)**
Staphylococci species **Methicillin resistance gene**	***mec*A**	GTA GAA ATG ACT GAA CGT CCG ATA A CCA ATT CCA CAT TGT TTC GGT CTA A	310	**(McClure *et al.*, 2006)**
Staphylococci species **Virulence gene**	***ica*A**	CCT AAC TAA CGA AAG GTA G AAG ATA TAG CGA TAA GTG C	1315	**(Ciftci *et al.*, 2009)**
Listeria monocytogenes **Virulence gene**	***inl*A**	ACG AGT AAC GGG ACA AAT GC CCC GAC AGT GGT GCT AGA TT	800	**(Liu *et al.*, 2007)**
Listeria monocytogenes **Macrolide resistance gene**	***mef*A**	AGTATCATTAATCACTAGTGC TTCTTCTGGTACTAAAAGTGG	345	**(Morvan *et al.*, 2010)**

1: conserved genes for species confirmation, *pho*A*:* Alkaline phosphastase gene, *inv*A: invasion of the host epithelial cells, *eae*A: Intimin / attaching and effacing gene, *Mcr*1: mobile colistin resistance gene, *fim*H: adhesion, *mec*A: methicillin resistance gene, *ica*A: intercellular adhesion cluster. *In*l A: internalinA, *mef*A: macrolide resistance gene.

IV- Antimicrobial susceptibility testing

The susceptibility of isolated strains to four commonly used antimicrobial agents was performed by the standard Kirby–Bauer disc diffusion method **(Quinn *et al.*, 1994)** and the results were interpreted according to **(CLSI, 2015)**.The selection of antibiotics involved in the study based on the feedback collected from shop owners describing their use of the

commercially available medications as (gentamycin, quinolones, macrolides), availability of commercial preparation (trimethoprim sulphamethazole) prescribed for pet birds, and some scholar articles with therapeutic recommendation for chloramphenicol, tetracycline, gentamycin, sulpha**.**

V- Molecular detection of antibiotic resistance genes and virulence genes

DNA extraction

DNA extraction was performed using the QIAamp DNA Mini kit (Qiagen, Germany, GmbH).

Oligonucleotide Primer

The used primers were supplied from **Metabion (Germany)**, **Table (1).**

PCR amplification

Primers were utilized in a 25- µl reaction containing 12.5 µl of Emerald Amp Max PCR Master Mix (Takara, Japan). The reaction was performed in an Applied biosystem 2720 thermal cycler.

Analysis of the PCR Products

The products of PCR were separated by electrophoresis on 1.5% agarose gel (Applichem, Germany, GmbH). Gelpilot 100 bp and 100 bp plus DNA Ladders (Qiagen, Germany, GmbH) and gene ruler 100 bp ladder (Fermentas, Germany) were used to determine the fragment sizes. The gel was photographed by a gel documentation system (Alpha Innotech, Biometra) and the data was analyzed through computer software.

3. Results

I. Isolation of bacteria with zoonotic attributes

Thirty six out of seventy examined samples (51.4%) were positive for E.*coli*, of which 31/50 (62%) were isolated from pet birds, 4/15 (26.7%) from feed and 1/5(20%) from water samples. Moreover, examination of 50 birds for *staphylococci spp.* revealed the isolation of 12/50 (24%) Coagulase Positive *Staphylococci* (CPS), 6/50 (12%) Coagulase Negative *Staphylococci* (CNS). Examination of samples also revealed the isolation of 8/85 (9.4%) *Salmonella* isolates, of which 4/50 (8%) isolates were isolated from pet birds, 3/15(20%) from litter samples and 1/15(6.7%) feed samples, while salmonella was not isolated from the examined (5) water samples. The study also revealed the isolation of 2 (2.4%) *listeria monocytogenes* isolates out of total examined 85 samples (50 birds, 15 feed, 15 litter and 5 water) notably *listeria monocytogenes* isolates were only isolated from 2/50 (4%) pet birds while no isolation was recorded from feed, water or litter samples, **Table (2).**

PCR was applied in order to confirm the isolated strains, in this regards PCR targeted the relevant species conserved genes as demonstrated in **Figures (1, 2, 3, and 4)**.

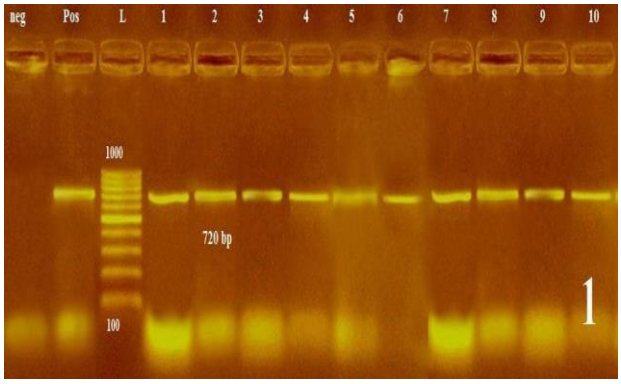

Figure (1): *pho*A gene for E.*coli*
Lane L: DNA molecular size marker 100-1000 bp
Lane (Pos): Positive control
Lane (Neg): Negative control
Lane 1-10: Positive for *pho*A gene at 720 bp

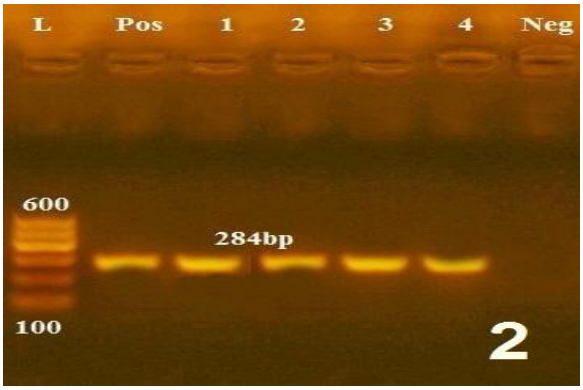

Figure (2): *inv*A gene for *salmonella spp*
Lane L: DNA molecular size marker 100-600 bp
Lane (Pos): Positive control
Lane (Neg):Negative control
Lane 1-4: Positive for *inv*A gene at 284 bp

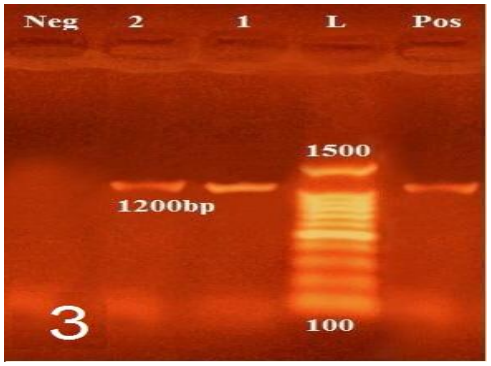

Figure (3):16S rRNA gene of *Listeria spp.*
Lane L: DNA molecular size marker 100-1500 bp
Lane (Pos): Positive control
Lane (Neg): Negative control
Lane 1, 2: Positive for 16S rRNA gene at 1200 bp

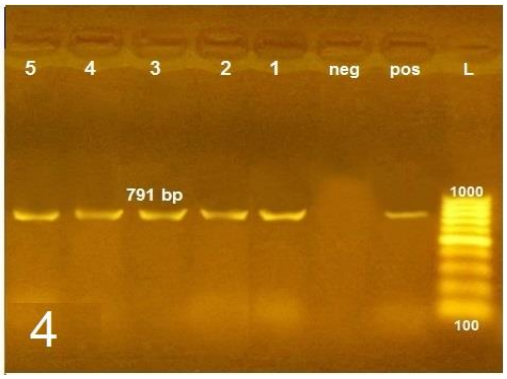

Figure (4): 16S rRNA gene of *staphylococcus spp.*
Lane L: DNA molecular size marker 100-1000 bp
Lane (Pos): Positive control
Lane (Neg): Negative control
Lane 1-5: Positive for 16S rRNA gene at 791 bp

Table (2): Prevalence rate of bacterial isolates

Samples		Prevalence of Bacterial Isolates									
		CPS		CNS		E.*coli*		*Salmonella spp*		*Listeria spp*	
Type	Number	Number	Prevalence	Number	Prevalence	Number	Prevalence	Number	Prevalence	Number	Prevalence
Birds	50	12/50	24%	6/50	12%	31/50	62%	4/50	8%	2/50	4%
Litter	15	-	-	-	-	-	-	3/15	20%	0/15	0%
Feed	15	-	-	-	-	4/15	26.7%	1/15	6.7%	0/15	0%
Water	5	-	-	-	-	1/5	20%	0/5	0%	0/15	0%
Total	85	12/50	24%	6/50	12%	36/70	51.4%	8/85	9.4%	2/85	2.4%

For CPS, and CNS isolation, out of total 50 pet birds were examined
For E.*coli* isolation out of total 70 samples were examined (50 pet birds, 15 feed , and 5 water samples)
For *Listeria spp* and *salmonella spp* isolation out of total 85 samples were examined (50 pet birds, 15 feed, 15 litter and 5 water samples)

II. Serotyping of bacterial isolates

Serotyping of E.*coli* isolates revealed six serotypes, of which the most predominant serotype was O78 (15/36), followed by O1:H7, O128: H2 was (6/36) each, then O91: H21, O44: H18 and O2: H6 was (3/36) each, **table (3).**

Table 3: Serotyping of E. *coli* isolates

Serotype	Number of isolates
O91: H21	3/36
O128: H2	6/36
O1: H7	6/36
O78	15/36
O44: H18	3/36
O2: H6	3/36
Total	36/36

Serotyping of the total detected 8 salmonella isolates revealed the detection of 3 serotypes, which were *Salmonella* Typhymurium (6/8), *Salmonella* Kentucky and *Salmonella* Inganda (1/8) each**, table (4).**

Typing the isolated staphylococci was applied by using the Integral system stafilococchi kit: ISSK, **table (5).**

III- Studying the phenotypic antimicrobial resistance profiles of isolates

The study revealed that E. *coli* isolates demonstrated resistance to colistin, tetracycline and gentamycin with rates of (100%, 100%, and 91.7%, respectively) and that 8/8 (100%) of *salmonella* isolates demonstrated multidrug resistance phenotypic patterns against (colistin, sulfamethoxazole-trimethoprim and tetracycline). It was also observed that 18/18 (100%), 15/18 (83.3%) and, 9/18 (50%) of *staphylococcus* isolates demonstrated resistance against oxacillin, erythromycin, and tetracycline, respectively, and that 2/2 (100%) *listeria monocytogenes* strain showed resistance against erythromycin, tylosin and oxacillin, **table (6).**

Table 4: Serotyping of *Salmonella* serovars

Serovars	Antigenic structure		Number of isolates
	O	H	
Salmonella Typhimurium	4,5	i: 1,2	6 /8
Salmonella Kentucky	8,20	i: Z6	1 /8
Salmonella Inganda	6,7	Z10: 1,5	1/8
Total			8/8

Table (5): Typing staphylococci isolates

Coagulase Type	Species	Number of isolates
Coagulase Positive	*Staphylococcus aureus*	12/18
Coagulase Negative	*Staphylococcus warneri*	3/18
	Staphylococcus xylosus	3/18
Total		18/18

Table (6): Phenotypic resistance profile of isolates

Bacterial isolates / **Chemotherapeutic agent***	**E.*coli***		***Salmonella spp.***		***Staphylococcus spp.***		***Listeria monocytogenes***	
	No. of Resistant isolates	**Resistance rate**	**No. of Resistant isolates.**	**Resistance rate**	**No. of Resistant isolates**	**Resistance rate**	**No. of Resistant isolates**	**Resistance rate**
Colistin	36/36	100%	8/8	100%	-	-	-	-
Sulfamethoxazole-trimethoprim	30/36	83.3%	8/8	100%	-	-	-	-
Erythromycin	-	-	-	-	15/18	83.3%	2/2	100%
Oxacillin	-	-	-	-	18/18	100%	2/2	100%
Gentamycin	33/36	91.7%	7/8	87.5%	-	-	-	-
Tetracycline	36/36	100%	8/8	100%	9/18	50%	1/2	50%
Tylosin	-	-	-	-	7/18	38.9%	2/2	100%

(*) The percentage was calculated with reference to the total number of isolates from each type of bacteria.

IV-Investigation of genotypic virulence attributes of isolates by PCR

Ten E.*coli* isolates with multidrug resistance phenotypic attributes were randomly selected and tested by PCR for the acquisition of *fim*H, and *eae*A virulence genes, while the results revealed that all 36/36(100%) of the tested E.*coli* isolates were positive for *fim*H genes, **figure (6)**, PCR failed to detect *eae*A gene in the tested E.*coli* isolates **figure (5).**

Testing the presence of *inl*A virulence gene in the two *Listeria monocytogenes* isolates involved in the study was confirmed by PCR, **figure (7).**

The presence of *ica*A virulence gene was proven by PCR in 5 randomly selected multidrug resistance *staphylococcus spp* isolates, **figure (8).**

The virulence of Salmonella isolates was confirmed by the detection of the conserved virulence *inv*A gene in 4 tested salmonella isolates, as demonstrated in **figure (2).**

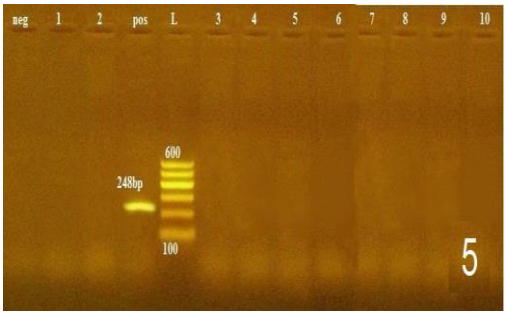

Figure (5): *eae*A virulence gene for E.*coli*
Lane L: DNA molecular size marker 100-600 bp
Lane (Pos): Positive control
Lane (Neg): Negative control
Lane 1-10: Negative for *eae*A gene at 284 bp

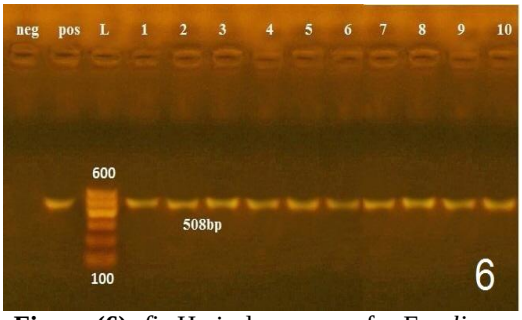

Figure (6): *fim*H virulence gene for E.*coli*
Lane L: DNA molecular size marker 100-600 bp
Lane (Pos): Positive control
Lane (Neg): Negative control
Lane 1-10: Positive for *fim*H gene at 508 bp

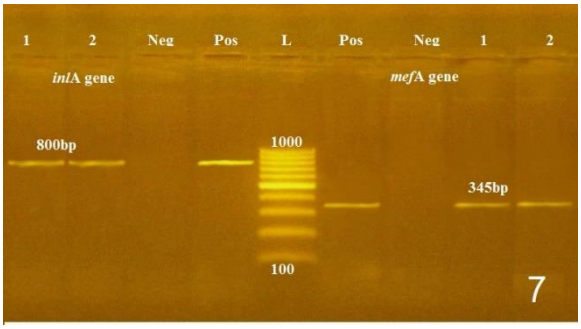

Figure (7): *inl*A, and *mef*A gene of *listeria* spp.
Lane L: DNA molecular size marker 100-1000 bp
Lane (Pos): Positive control
Lane (Neg): Negative control
Lane 1,2 L: Positive for *inl*A gene at 800 bp
Lane 1,2 R: Positive for *mef*A gene at 345 bp

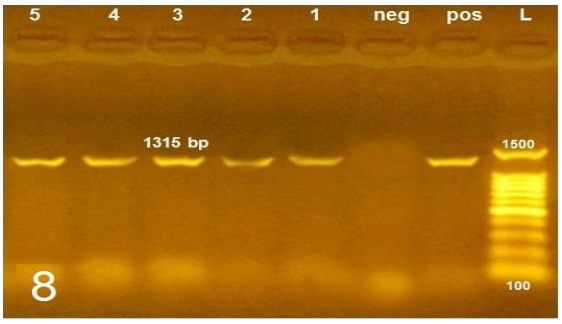

Figure (8): *ica*A gene for *staphylococci spp.*
Lane L: DNA molecular size marker 100-1500 bp
Lane (Pos): Positive control
Lane (Neg): Negative control
Lane 1-5: Positive for *ica*A gene at 1315 bp

IV-Investigation of genotypic resistance attributes of isolates by PCR

The presence of *mcr*1 gene of colistin resistance was confirmed in all 10/10 (100%) tested E.*coli* and in 4/4(100%) of tested *salmonella* isolates**, figure (9).**

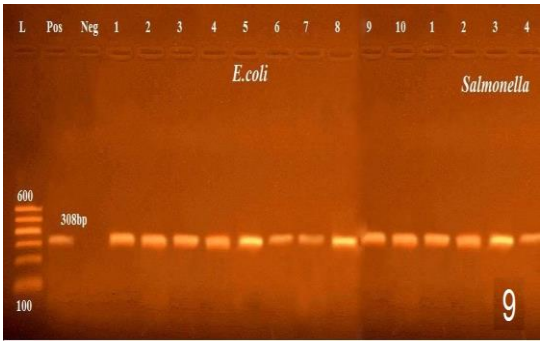

Figure (9): *mcr*1 mobile colistin resistance gene
Lane L: DNA molecular size marker 100-600 bp
Lane (Pos): Positive control
Lane (Neg): Negative control
Lane 1-10 L: Positive for *mcr*1 gene of E.*coli*
Lane 1-4 R: Positive for *mcr*1 gene of *Salmonella spp*

PCR also confirmed the detection of *mef*A resistance gene of macrolides in 2/2 (100%) *Listeria monocytogenes* isolates, **figure (7).**

Methicillin Resistance *Staphylococci* / MRSA was confirmed by PCR through the detection of *mec*A resistance gene in 4/5(80%) of tested isolates, **figure (10).**

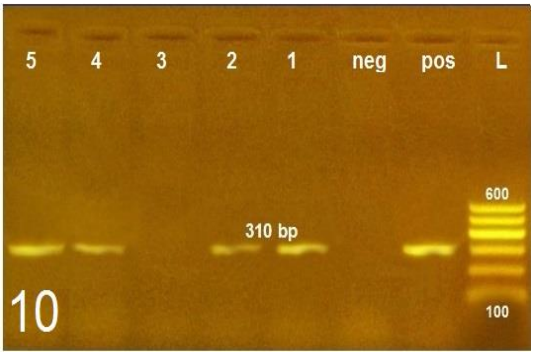

Figure (10): *mec*A methicillin resistance gene
Lane L: DNA molecular size marker 100-600 bp
Lane (Pos): Positive control
Lane (Neg): Negative control
Lane 1, 2, 4, 5: Positive for *mec*A gene at 310 bp
Lane 3: Negative for ***mec***A gene

Table (7): Phenotypic and genotypic resistance profiles of E. *coli* serotypes

No.	Sample Source	Serotype	Antimicrobial resistance profile	Confirmatory gene (*pho*A)	Virulence genes		Antibiotic resistance genes (*Mcr*1)
					*eae*A	*fim*H	
1	Budgerigar	O78	$CT^{1,}SXT^{2}, T^{3,}CN^{4}$	+	-	+	+
2		O1:H7	$CT^{1,}SXT^{2}, T^{3,}CN^{4}$	+	-	+	+
3		O128:H2	$CT^{1,}SXT^{2}, T^{3,}CN^{4}$	+	-	+	+
4	Canaries	O2:H6	$CT^{1,}SXT^{2}, T^{3,}CN^{4}$	+	-	+	+
5		O78	$CT^{1,}SXT^{2}, T^{3,}CN^{4}$	+	-	+	+
6		O1:H7	$CT^{1,}SXT^{2}, T^{3,}CN^{4}$	+	-	+	+
7	Cocktail	O128:H2	$CT^{1,}SXT^{2}, T^{3,}CN^{4}$	+	-	+	+
8		O78	$CT^{1,}SXT^{2}, T^{3,}CN^{4}$	+	-	+	+
9	Feed	O91:H21	$CT^{1,}SXT^{2}, T^{3,}CN^{4}$	+	-	+	+
10	Water	O44:H18	$CT^{1,}SXT^{2}, T^{3,}CN^{4}$	+	-	+	+

1: Colistin, 2: Sulphamethazole Trimethoprime, 3: Tetracyclin, 4: Gentamycin.

Table (8): Phenotypic and genotypic resistance profiles of *Salmonella serovars*

No.	Source	Serovars	Antimicrobial resistance profile	Virulence gene (*inv*A)	Antibiotic resistance genes (*Mcr*1)
1	Canaries	***S. Typhimurium***	$CT^{1,}SXT^{2}$, $T^{3,}CN^{4}$	+	+
2	Cocktail	***S. Kentucky***	$CT^{1,}SXT^{2}$, $T^{3,}CN^{4}$	+	+
3	Litter	***S. Typhimurium***	$CT^{1,}SXT^{2}$, $T^{3,}CN^{4}$	+	+
4	Feed	***S. Inganda***	$CT^{1,}$ SXT^{2}, $T^{3,}$ CN^{4}	+	+

1: Colistin, 2: Sulphamethazole Trimethoprime, 3: Tetracyclin, 4: Gentamycin.

Table (9): Phenotypic and genotypic resistance profiles of *Listeria monocytogenes* isolates

No.	Source	Serotype	Antimicrobial resistance profile	Confirmatory gene (16S rRNA)	Virulence gene (*inl*A)	Antibiotic resistance genes (*mef*A)
1	Budgerigar	***Listeria monocytogenes***	TY^{1},E^{2}, T^{3},OX^{4}	+	+	+
2			TY^{1},E^{2}, T^{3},OX^{4}	+	+	+

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

No.	Source	Serotype	Antimicrobial resistance profile	Confirmatory gene (16S rRNA)	Virulence gene (*ica*A)	Antibiotic resistance genes (*mec*A)
1	Budgerigar	***Staphylococcus aureus***	E^{1},OX^{2}, T^{3},TY^{4}	+	+	+
2	Cocktail	***Staphylococcus xylosus***	E^{1},OX^{2}, T^{3},TY^{4}	+	+	+
3	Budgerigar	***Staphylococcus aureus***	E^{1},OX^{2}, T^{3},TY^{4}	+	+	-
4	Canaries		E^{1},OX^{2}, T^{3},TY^{4}	+	+	+
5	Canaries	***Staphylococcus warneri***	E^{1},OX^{2}, T^{3},TY^{4}	+	+	+

1: Erythromycin, 2: Oxacillin, 3: Tetracyclin, 4: Tylosin.

4. Discussion

Differentiation between pathogenic and nonpathogenic Escherichia *coli* is difficult, as Escherichia *coli* are frequently the causative agents of secondary infection in birds consequently are associated with stress, malnutrition, poor hygiene and hypovitaminosis A **(Marietto *et al*. 2007),** while the role of Escherichia *coli* as commensals in healthy pet birds is controversial, *Salmonella spp.* have never been considered as part of birds' normal intestinal flora.

In the present study, a total of 70 samples were examined for E.*coli* isolation (50 pet birds, 15 feed, and 5 water samples), the result revealed the isolation of E.*coli* from 36/70 samples with a prevalence rate of (51.4%), this result was higher than that of **(Giacopello *et al*., 2013)** who isolated (35.2%) E.*coli* form canaries, while much lower prevalence rate was recorded by **(Siqueira *et al*., 2017)** who recorded a prevalence rate of (19%). It's significant to screen the circulation of pathogens in the environment, this can be obtained by serotyping and genotypic assays, serotyping applied in the current investigation revealed that E. *coli* isolates belonged to 6 serogroups: O78, O1: H7, O128: H2, O91: H21, O44: H18 and O2: H6. This result accorded with the result of sero-identification of pathogenic E.*coli* reported by **(ISU/CFSPH, 2016)** which recorded that avian septicemic E. *coli* usually belong to a limited number O serogroups (O1, O2, and O78).

Pet birds can acquire infection with *salmonella* through ingestion of contaminated feed, and water, through maternal route by vertical egg transmission or by contamination and penetration of egg shell.

Madadgar *et al*., (2009) reported that feed contamination may play an important role in the spread *of salmonella* serovars. In the present study, 8/85 (9.4%) of samples were positive for *salmonella* serovars, higher prevalence rate was reported by **(Rahmani *et al*., 2011)** who reported a prevalence rate of (16.1%), while lower prevalence rate was recorded by **(Giacopello *et al*., 2013** and **Siqueira *et al*. 2017)** who recorded a rate of (5.7%) and (1.12%), respectively. Isolation of *salmonella* serovars from pet birds was frequently recorded by conservationists, breeding and rehabilitation facilities **(Lopes *et al*. 2014).** Scientific records for the distribution rates of salmonella serovars in pet birds were published by other researchers amongst was (**Madadgar *et al*., 2009)** who reported the detection of *Salmonella* Typhymurium, this result was in agreement with the

results recorded in the present study thus, serotyping of the 8 isolated salmonella strains revealed the detection of (6/8) *Salmonella* Typhymurium, (1/8) *Salmonella* Kentucky and (1/8) *Salmonella* Inganda.

Results recorded in the current study revealed the isolation of 18/50 (36%) staphylococci species from the internal organs sampled from 50 pet birds of which 12/50 (24%) were coagulase positive *staphylococci* (CPS), and belonged to *staphylococcus aureus*, higher prevalence rate was recorded by **(Arabkhazaeli *et al*., 2016)** who recorded outbreaks caused by *staphylococcus aureus* in canaries with a prevalence rate of (83.3%). The current results also revealed that 6/50 (12%) of the staphylococci strains isolated from the 50 examined pet birds involved in the current study belonged to coagulase negative *staphylococci* (CNS) of which (3/6) *staphylococcus warneri* and (3/6) *Staphylococcus xylosus* were identified, higher prevalence rate for CNS was recorded by **(Huynh *et al*., 2014)** who recorded a prevalence rate of (33.2%).

Susceptibility of pet birds to infection by *listeria species* is increased by stress imposed from poor hygiene in the aviary (i.e. contaminated soil, environment, nest boxes, bedding, and feed) **(Shivaprasad *et al*., 2007).** The current study investigated the prevalence of *Listeria monocytogenes* in a total examined 85 samples (50 birds,15 feed, 15 litter, and 5 water), the results revealed their isolation from pet birds with a prevalence rate of 2/50 (4%), while the examination of (15)feed, (5) water, and (15) litter samples revealed their freedom from listeria , with an overall prevalence rate of 2/85(2%), higher prevalence rate (50%) of caged canaries was recorded by (**Akanbi *et al*., 2008).**

Confirmation of the obtained results was applied by using PCR for detecting the relevant 16S rRNA sequence in listeria and staphylococci isolates and also by detection of the conserved specific sequences like *inv*A gene for pathogenic salmonella and *pho*A that encodes the enzyme alkaline phosphatase in E.*coli*, in this regard **(Witte *et al*., 2018)** conducted PCR to confirm the results of isolation by detection of the 16S rRNA amplicon in *Listeria monocytogenes*, and *Staphylococci isolates*. Moreover, (**Mohamed** and **Sayed, 2017)** recorded that (100%) of their E.*coli* isolates were confirmed by been positive for *pho*A gene.

Antimicrobial resistance has become a global concern in the public health and veterinary medicine **(Szmolka and-Nagy, 2013).** The results recorded in the current study revealed that 36/36 (100%), 36/36 (100%), and 33/36(91.7%) of E. *coli* isolates demonstrated phenotypic resistance patterns to colistin, tetracycline, and gentamycin, respectively. The results also revealed that 8/8 (100%) of *Salmonella* species demonstrated phenotypic resistance patterns to colistin, sulfamethoxazole-trimethoprim, and tetracycline, respectively. Many studies recorded the detection of multidrug resistance to aminoglycosides, quinolones, sulfas and other groups in E. *coli* and *Salmonella spp.* isolated from pet birds **(Madadgar *et al*., 2009 and Ramalivhana *et al*., 2014)**, on the other hand low resistance rate to tetracycline (13.9%) in E. *coli* strains isolated from pet birds have been reported by other researchers as **(Blyton *et al*., 2014)**. In the same regards, **(Siqueira *et al*., 2017)** recorded resistance rates to sulfa group (67%) and tetracycline (50%) in E.*coli and Salmonella* isolates involved in their studies. **Marietto *et al*., (2007)** reported that *Salmonella* Typhimurium strains demonstrated (100%) resistance to sulfonamide and colistin.

Studying the phenotypic antimicrobial patterns of staphylococci isolates involved in the current study revealed the demonstration of high resistance rates against most of the tested antimicrobial agents (oxacillin 100%, erythromycin 83.3%, tetracycline 50% and tylosin 38.9%). Similarly, high resistance rate against oxacillin was recorded by **(Rueanghiran *et al*., 2017)**. Resistance pattern of *staphylococcus aureus* was studied by **(Nemati *et al*., 2008)** they recorded resistance to oxacillin with a rate of (12.4%) they also recorded resistance rates of (37%, 56.8%, and 32.1%) to erythromycin, tetracycline and tylosin, respectively.

Investigation of antimicrobial susceptibility profiles of the isolated *listeria monocytogenes* was also applied, the result revealed that 2/2 (100%) isolates showed resistance against (erythromycin, tylosin and oxacillin) and a resistance rate of 1/2 (50%) against tetracycline. In this regards, although high resistance rate to oxacillin (100%) was recorded by (**Gómez *et al*., 2014)**, they recorded low resistance rate to tetracycline (3.9%). In contrast, the results disagreed with the reports of **(OIE, 2014)** that recorded low resistance rates of *listeria monocytogenes* to tetracycline and erythromycin.

Trials to analyze and associate the observed high resistance rates and the circulation of bacterial species showing multidrug resistance against the most commonly used chemotherapeutic agents were applied by many researchers as **(Koochakzadeh *et al*., 2015)** who concluded that sub inhibitory concentrations of antibiotics may create an environment conducive to acquisition of resistance. Moreover, **(Rahmani *et al*., 2011)** raised the alarm against the indiscriminate use of antibiotics in avian medicine and the occurrence of multidrug resistance involved with failure in antimicrobial therapy.

Limited number of studies concerning the role of bacterial carriage for virulence factors in pet birds

compared with commercial birds were available **(Knobl** and **Menao 2010).** Consequently, studying the distribution of virulence genes could contribute in clarifying the pathogenesis of diseases in pet birds.

Studying the genotypic virulence attributes of the isolated bacterial species was applied by conventional PCR technique, the results revealed that 10/10 (100%) of tested E.*coli* possessed *fim*H gene, one of the virulence genes that contributes in bacterial adhesion, identical results was recorded by (**Nüesch *et al.*, 2018)** who found that *fim* gene was detected in all (100%) of their EHEC isolates. Similar result was recorded by **(Giacopello *et al*., 2013)** who recorded that (81.8%) of their E.*coli* isolates were positive for *fim*H gene.

In the present study *eae*A (Intimin / virulence gene for attaching and effacing) was not detected in the tested E.*coli* isolates, while this result was in agreement with that of **(Nüesch *et al*., 2018)** who recorded that none of the E.*coli* isolates collected from colisepticemic cases contained the *eae*A gene, the result disagreed with that of **(Sanches *et al.*, 2017)** who detected *eae*A gene with a rate of (5.74%). Moreover, (**Mohamed** and **Sayed, 2017)** recorded that (43.75%) of their studied isolates were positive for *eae*A gene.

PCR is traditionally conducted for detection of *inv*A gene which is considered the conserved virulence gene for genus salmonella, *inv*A gene codes for protein in the inner bacterial membrane which is important for invasion of the host epithelial cells in pathogenic salmonella species, the present results revealed that 8/8 (100%) of the studied isolates were positive for *inv*A gene, this finding agreed with that of **(Ashraf *et al*.,2013)** who detected *inv*A gene in all (100%) their studied salmonella isolates, lower detection rate of *inv*A gene was recorded by (**Sharma** and **Das, 2016)** who detected *inv*A gene in (55%) of *Salmonella* isolates involved in their study.

Studying the genotypic virulence attributes in the isolated staphylococci involved in the current study was applied by PCR for detection of *ica*A gene which acts for intercellular adhesion, the results revealed the detection of *ica*A gene in all 5/5 (100%) of the tested multidrug resistance *staphylococcus* isolates, similar result was recorded by **(El-Shekh *et al*., 2010)** who recorded detection of *ica* gene in (100%) of their *staphylococcus aureus* isolates. Lower detection rate of *ica*A gene (51.6%**)** was recorded by **(Prasanth** and **Saravanakumari, 2017).**

Internalin A (*Inl*A) gene facilitates the invasion of *listeria monocytogenes* into host cells, studying the presence of *Inl*A gene in the *listeria monocytogenes* isolated in the current study revealed that 2/2 (100%) *listeria monocytogenes* isolates were positive carriers for *inl*A virulence gene, other studies were in accordance with the recorded result as that of (**Pereira *et al*, 2018**) who detected (*Inl*A) gene in *listeria monocytogenes* with a prevalence rate of (95.6%).

Studying the antimicrobial genotypic attributes of the isolates was applied by PCR to investigate the presence of the mobile colistin resistance gene (*mcr*1), the results indicated positive detection in all 10/10 (100%) of the tested E.*coli* and in all 4/4 (100%) of tested *salmonella* isolates**,** similar result was recorded by (**Jayol *et al.*, 2018**) who detected (*mcr*1) gene in *Enterobacteriaceae* isolates from birds with a prevalence rate of (92.6%).

A worldwide public health concern is the emergence of methicillin-resistance *stapylococcus aureus* (MRSA), with resistant strains being isolated from pet birds **(Monecke *et al*., 2016)**. PCR evaluation for the presence of *mec*A gene in the studied staphylococci isolates showed that (4/5) 80% of the tested *staphylococci* isolates were positive for *mec*A gene (2/3 of CPS and 2/2 of CNS) while lower detection rate was recorded by **(Matias *et al*.,2018)** who recorded a rate of (22%), the current result agreed with that of **(Marsilio *et al*., 2018)** who found out that CNS isolates were considered an important *mec*A gene reservoir with the potential to transfer the gene to susceptible *staphylococcus* strains, including *Staphylococcus aureus*.

In the current study PCR confirmed the positivity of *mef*A macrolides resistance gene in 2/2 (100%) of the isolated *Listeria monocytogenes* strains, this was in agreement with the finding of (**Dhama *et al*., 2015)** who detected *mef*A gene in all (100%) their investigated listeria isolates. On the other hand, the result disagreed with that of (**Escolar *et al*., 2017**) who failed to find *mef*A gene in any of their studied *Listeria* strains that demonstrated intermediate resistance to erythromycin.

Conclusion

Indoor housing of pet birds has become more popular, thus may impose a potential significant risk of human infection by multi-host or nonspecific host bacterial strains that harbor zoonotic attributes and may play the role of a significant exposure interface for human in contact and to the surrounding environment. Therefore, the prevalence and the characterization of implicated pathogens is important to be monitored. Moreover, raising public awareness for good biosecurity practices is recommended.

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2/20/2019