

Prevalence of some bacterial pathogens in wild birds

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Abstract

This study was designed to detect the different types of bacterial pathogens that can be present in different species and ages of some wild birds in Egypt. Two hundred cloacal and tracheal swabs collected from apparently healthy free-living and captive wild birds were bacteriologically examined for detection of different bacterial pathogens. The results revealed isolation rate as follows: *Escherichia coli* (*E.coli*) (9%), *Staphylococcus aureus* (*Staph. Aureus*) (2%), *Citrobacter spp.* (1.5%), *Klebsiella spp.* (1.5%), *Pseudomonas spp.* (1%) and *Salmonella spp.* (1%), however, *Enterobacter spp.*, *Shigella spp.* and *Proteus spp.* (0.5%). No *Pasteurella spp.* was isolated. Serotyping of 18 isolates of *E. coli* revealed 9 different serotypes while *Salmonella* isolates serotyped as *S. GIVE* and *S. Santiago*. The antimicrobial patterns of all the bacterial isolates were studied and the antimicrobial resistances (AMR) profiles of the each were recorded which of great concern to public and animal health. It was concluded that most of bacterial pathogen isolated from wild birds carry variable antibiotic resistance patterns and backed that to the widespread use of antibiotics to treat diseases and to promote growth by the livestock.

Key words: Wild birds, Egypt, Bacteria, Antimicrobial resistance.

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Introduction

Wild birds are known to carry and transmit over 40 diseases to humans and livestock (Shannon *et al.*, 2009). Recent studies have also highlighted wild birds as sources of pathogens associated with human disease problems. The development and spread of antibiotic resistance in clinical practice have been increasing since the early 1960s and wild birds are currently viewed as a major threat to the public health on a global level (Levy and Marshall, 2004) due to their ability to fly freely and cover long distances during annual migrations, they potentially play a role in the epidemiology of human-associated zoonoses (Hussein *et al.*, 2007). Recent studies reported that antibiotic-resistant bacteria were present in many parts of the world among wild bird species which found in remote habitats (Hernández *et al.*, 2012)

Wildlife may play a critical role as reservoirs for pathogenic enteric bacteria and

zoonotic diseases. Numerous wild bird species

are attracted to untreated sewage, garbage dumps, manure, and other sources of enteric pathogens. As a consequence, a number of enteric bacteria such as *E. coli*, *Salmonella spp.*, *C. jejuni*, *C. coli*, *C. lari* and *Helicobacter canadensis* (Moore *et al.*, 2002 and Fogarty *et al.*, 2003) have been isolated from intestinal samples of wild birds. Although potentially pathogenic fecal bacteria have been isolated from some species, recent reviews (Reed *et al.*, 2003) suggested that the role wild birds in human diseases was largely understudied and that much work remains to determine the role of wild birds on zoonotic transmission of enteropathogens.

Wild birds were important with regard to antibiotic resistance in several different ways; as sentinels, mirroring human activity and its impact on the environment because of the diverse ecological niches of birds and as they

bacteria, as a reservoir and melting pot of antibiotic-resistant bacteria and resistance genes, as potential spreaders of antibiotic resistance through the ability to migrate long distances in short periods of time and as a possible source of antibiotic resistant bacteria colonizing and/or infecting human being.

The presence of AMR in wildlife has implications for public health, food safety and potable water source protection among others (Smith *et al.*, 2014). Recent studies reported that antibiotic-resistant bacteria are present in many parts of the globe, including among wild bird species found in remote habitats (Hernández *et al.*, 2012). Spread of antibiotic-resistant bacteria to remote areas that are reached mainly by migrating birds could also influence bacterial communities in these fragile ecosystems, as antimicrobial substances are part of the cross-talk of bacteria (Allen *et al.*, 2010). There are indications of spread of antibiotic resistance through migration of wild birds and of transmission between humans and wild birds and vice versa. As previously suggested, thorough spatial and temporal studies of antimicrobial drug resistance in different natural habitats of wild birds are warranted (Hernandez *et al.*, 2010 and Gilliver *et al.*, 1999).

Materials and Methods

Samples

Samples were collected from 200 wild birds representing 8 different species: 40 white chicken valley, 40 pigeons, 40 pelican, 20 helmeted guinea fowl, 20 fezzan, 20 peacock, 20 ostrich and 20 white anz. Cloacal and tracheal swabs were collected from each bird for bacteriological examination.

Isolation of the bacterial pathogens

Salmonella detection was done according to the protocol of ISO-6579:2002 standard. *E. coli* and other *Enterobacteriaceae* (*Klebsiella*, *Citrobacter* and *Enterobacter spp.*) isolation was done according to Swayne *et al.* (1998). *Staphylococcus* isolation was done as Holt *et al.* (1994). Also, *Pseudomonas spp.*, *Shigella spp.*,

Proteus spp. and *Pasteurella spp.* were isolated according to Quinn *et al.* (1994).

Biochemical identification:

Biochemical identification was carried out according to the character of each organism, using protocols of MacFaddin (2000).

Serotyping of *Es.coli* and *Salmonella* isolates:

E.coli isolates were serotyped by slide agglutination test (Lee *et al.*, 2009) using standard *Escherichia coli* antisera (Sifin and Denka Seiken Comp.) Typing of *Salmonella* isolates was performed as Kauffmann (1957).

Antibiotic susceptibility test:

It was performed using disc diffusion method as described by NCCLS (2008) against a range of 15 antibiotic discs from Oxoid (Basingstoke, UK): amoxicillin (30 µg), colistin sulphate (10 µg), ciprofloxacin (5 µg), doxycycline (30 µg), enrofloxacin (5 µg), gentamycin (10 µg), tetracycline (30 µg), neomycin (30 µg), streptomycin (10µg), norfloxacin(10 µg), chloramphenicol (30 µg), Nitrofurantoin (300 IU), levofloxacin (5 IU), ceftriaxone (30 IU), and nalidixic acid (30 µg). Inhibition zones were measured and interpreted as resistant (R), intermediate (I) or susceptible (S).

Results

Results of this study revealed isolation of numerous bacterial isolates from wild birds with different ages and species (table 1). The prevalence of *E.coli*, *Staph. aureus*, *Citrobacter spp.*, *Klebsiella spp.*, *Pseudomonas spp.*, *Salmonella spp.*, *Enterobacter spp.*, *Shigella spp.* and *Proteus spp.* were arranged in the rate of 9, 2, 1.5, 1.5, 1, 1, 0.5, 0.5 and 0.5%; respectively. *Pasteurella spp.* was not detected.

The eighteen *E. coli* isolates were serotyped into 9 different serotypes as follow: 5 from 40 white chicken valley (2 of O119, 2 of O159, 1 of O63), 4 from 40 pigeons (2 of O128, 2 of O28 ac), 3 from 40 pelican (2 of O44, 1 of O27), 2 from 20 peacock (1 of O27 and 1 of O169), 1 from 20 helmeted guinea fowl (O44), 1 from 20 fezzan (O91), 1

from 20 ostrich (O27) and 1 from 20 white anz. (O63). The highest isolated serotypes were O44 and O27 (16.6% each) as shown in table (2). Serotyping of *Salmonella* isolates

which were identified into two serotypes; *S. Give* isolated from cloacal swabs of pigeons and *S. Santiago* isolated from cloacal swabs of Pelicans (Table 3)

Table (1): Prevalence of some bacterial pathogen in the examined wild birds in Egypt.

Species of birds	Examined samples		Isolates								
	Types	No.	<i>E. coli</i>	<i>Salmonella</i>	<i>Shigella</i>	<i>Staph. aureus</i>	<i>Citrobacter</i>	<i>Enterococcus</i>	<i>Proteus</i>	<i>Pseudomonas</i>	<i>Klebsiella</i>
White chicken valley	T	20	2	0	0	0	1	1	0	0	1
	c	20	3	0	0	0	0	0	0	0	0
	Total	40	5	0	0	0	1	1	0	0	1
Pigeons	T	20	0	0	0	1	0	0	0	0	0
	C	20	4	1	0	0	2	0	1	0	0
	Total	40	4	1	0	1	2	0	1	0	0
Pelican	T	10	0	0	0	0	0	0	0	0	0
	C	10	3	1	1	1	0	0	0	1	0
	Total	20	3	1	1	1	0	0	0	1	0
Ostrich	T	10	-	0	0	0	0	0	0	0	0
	C	10	1	0	0	1	0	0	0	0	0
	Total	20	1	0	0	1	0	0	0	0	0
Helmeted guinea fowl	T	10	-	0	0	0	0	0	0	0	0
	C	10	1	0	0	0	0	0	0	1	0
	Total	20	1	0	0	0	0	0	0	1	0
Peacock	T	10	2	0	0	0	0	0	0	0	0
	C	10	-	0	0	0	0	0	0	0	0
	Total	20	2	0	0	0	0	0	0	0	0
Fezzan	T	10	0	0	0	0	0	0	0	0	0
	C	10	1	0	0	0	0	0	0	0	1
	Total	20	1	0	0	0	0	0	0	0	1
White Anz.	T	10	0	0	0	0	0	0	0	0	1
	C	10	1	0	0	1	0	0	0	0	0
	Total	20	1	0	0	1	0	0	0	0	1
Total		200	18 (9%)	2 (1%)	1 (0.5%)	4 (2%)	3 (1.5%)	1 (0.5%)	1 (0.5%)	2 (1%)	3 (1.5%)

T= Tracheal swabs C= Cloacal swabs

Table (2): Serotyping of isolated *E. coli*

Serotype	Origin	Sampling type	No. of isolates	Total number of isolates	% of positive*
O119	White chicken valley	Tracheal swab	2	2	11.1
O159	White chicken valley	Cloacal swab	2	2	11.1
O63	White chicken valley	Cloacal swab	1	2	11.1
	White Anz.	Cloacal swab	1		
O128	Pigeon	Cloacal swab	2	2	11.1
O28 ac	Pigeon	Cloacal swab	1	2	11.1
	Pigeon	Tracheal swab	1		
O44	Helmeted guinea fowl	Cloacal swab	1	3	16.6
	Pelican	Cloacal swab	2		
O27	Pelican	Cloacal swab	1	3	16.6
	Ostrich	Cloacal swab	1		
	Peacock	Tracheal swab	1		
O169	Peacock	Tracheal swab	1	1	5.6
O91	Fezzan	Cloacal swab	1	1	5.6

*The percentage according to the total number of *E. coli* isolates.

Table (3): Serotyping of isolated *Salmonella* species

Serotype	origin	Sampling type	No. of positive	% of positive*
<i>S. Give</i>	Pigeon	Cloacal swa	1	50
<i>S. Santiago</i>	Pelican	Cloacal swa	1	50

*The percentage according to the total number of *Salmonella* isolates.

The results of antibiotic resistance analysis of *E.coli* showed that 17 strains (94.4%) carried resistance phenotypes to two or more antimicrobial agents. The most commonly reported resistance phenotypes were against tetracycline and colistin (83.3%); followed by amoxicillin (61.1) then streptomycin and nalidixic acid (55.6). Both *Salmonella* strains showed resistance to more than two

antimicrobial agents norfloxacin, neomycin and ceftriaxone. Three of four *Staph.aureus* isolates (75%) showed resistance phenotypes to more than two antimicrobial agents as nalidixic acid (100%) followed by tetracycline and doxycycline (75%) then colistin, streptomycin, ceftriaxone and enrofloxacin (50%). Both strains of *Ps.aeruginosa* showed 100% resistance phenotypes to the fifteen tested antimicrobial agents. The strains of both *Enterobacter* and *Shigella* showed only resistance to tetracycline, while *Proteus* showed resistance to only nalidixic acid. Three strains of *Klebsiella* showed resistance against amoxicillin and neomycin while the *Citrobacter spp.* strains showed resistance against colistin and tetracycline in percentage 100 and 25%; respectively (tables 4 and 5).

Table (4): Antibiotic resistance profiles of the bacterial pathogens isolated from wild birds in Egypt.

Antimicrobial Drug	Total No. (%) of isolates resistant								
	<i>E. coli</i> N=18	<i>Salmonella</i> N=2	<i>Shigella</i> N=1	<i>Staphylococcus</i> N=4	<i>Citrobacter</i> N=3	<i>Enterobacter</i> N=1	<i>Proteus</i> N=1	<i>Pseudomonas</i> N=2	<i>Klebsiella</i> N=3
Ciprofloxacin	9(50)	1(50)	0(0)	0(0)	0(0)	0(0)	0(0)	2(100)	0(0)
Neomycin	9(50)	2(100)	0(0)	1(25)	0(0)	0(0)	0(0)	2(100)	3(100)
Chloramphenicol	7(38.9)	0(0)	0(0)	1(25)	0(0)	0(0)	0(0)	2(100)	0(0)
Doxycycline	8(44.4)	0(0)	0(0)	3(75)	0(0)	0(0)	0(0)	2(100)	0(0)
Tetracycline	15(83.3)	1(50)	1(100)	3(75)	1(33.3)	1(100)	0(0)	2(100)	0(0)
Ievofloxacin	8(44.4)	1(50)	0(0)	1(25)	0(0)	0(0)	0(0)	2(100)	0(0)
Nitrofurantoin	2(11.1)	0(0)	0(0)	1(25)	0(0)	0(0)	0(0)	2(100)	0(0)
Enrofloxacin	3(16.7)	0(0)	0(0)	2(50)	0(0)	0(0)	0(0)	2(100)	0(0)
Ceftriaxone	0(0)	2(100)	0(0)	2(50)	0(0)	0(0)	0(0)	2(100)	0(0)
Gentamicin	3(16.7)	1(50)	0(0)	0(0)	0(0)	0(0)	0(0)	2(100)	0(0)
Streptomycin	10(55.6)	1(50)	0(0)	2(50)	0(0)	0(0)	0(0)	2(100)	0(0)
Nalidixic acid	10(55.6)	1(50)	0(0)	4(100)	0(0)	0(0)	1(100)	2(100)	0(0)
Norfloxacin	8(44.4)	2(100)	0(0)	1(25)	0(0)	0(0)	0(0)	2(100)	0(0)
Colistin	15(83.3)	1(50)	0(0)	2(50)	3(100)	0(0)	0(0)	2(100)	0(0)
Amoxicillin	11(61.1)	1(50)	0(0)	0(0)	0(0)	0(0)	0(0)	2(100)	3(100)

N = Number of positive samples, (%) percentage of positive samples

Table (5): Antimicrobial resistance profiles of bacterial pathogens isolated from wild birds in Egypt

Bacteria	Recognized phenotypes	Antibiotic resistance profiles	No. of antibiotics	No. of isolates	% of isolates
<i>Salmonella</i>	Multi-resistance	NOR-N-CRO	3	1	50
		NOR-N-CRO-CIP-LEV-TE-CN-S-NA-AX-CT	11	1	50
<i>E. coli</i>	Multi-resistance	CT	1	1	6.5
		CT-C-DO	3	2	11.1
		TE-S-CT	3	1	6.5
		TE-CT-AX	3	2	11.1
		TE-CT-F	3	1	6.5
		TE-CT-F-AX	4	1	6.5
		S-LEV-TE-C-NA-CT-DO	7	1	6.5
		N-CIP-TE-C-NA-AX-CT-DO	8	1	6.5
		NOR-N-CIP-LEV-TE-S-NA-AX-CT	9	2	11.1
		NOR-N-CIP-LEV-TE-S-NA-AX-CT-ENR	10	3	16.6
		NOR-N-CIP-CT-TE-S-NA-DO-C-CN	10	1	6.5
		NOR-N-CIP-LEV-TE-S-NA-AX-DO-C-CN	11	2	11.1
		<i>Shigella</i>		TE	1
F-NA	2			1	25
<i>Staph. aureus</i>	Multi-resistance	C-NA-DO-TE	3	1	25
		LEV-S-ENR-NA-CT-DO-CRO-TE	8	1	25
		NOR-S-ENR-NA-CT-DO-N-CRO-TE	9	1	25
<i>Citrobacter</i>		CT	1	1	33.3
		TE-CT	2	2	66.7
<i>Enterobacter spp.</i>		TE	1	1	100
<i>Proteus spp.</i>		NA	1	1	100
<i>Klebsiella spp.</i>		C-AX	2	3	100
<i>Pseudomonas spp.</i>	Multiresistance	Cip-N-C-Do-TE-LEV-ENR-N-CRO-CN-S-NA-NOR-CT-AX	15	1	100

Cip:Ciprofloxacin. N:Neomycin. C, chloramphenicol. Do:Doxycycline, TE:Tetracycline. Levo: Levofloxacin. ENR:Enrofloxacin. F:Nitrofurantoin. CRO:Ceftriaxone.CN:Gentamicin. S: Streptomycin; NA: Nalidixic acid. NOR: Norfloxacin. CT:Colistin ;AX: Amoxicillin

Discussion

Billions of birds travel between continents twice a year in only a few weeks (Berthold, 1993). During these migrations, birds have the potential to distribute widely pathogenic microorganisms (Reed et al., 2003), so we try to survey the most important bacterial pathogens among 200 birds which represent 8 different species: 40 white chicken valley, 40 pigeons, 40 pelican, 20 helmeted guinea fowl, 20 fezzan, 20 peacock, 20 ostrich and 20 white Anz. The migratory bird species are important to public health because they can be reservoirs for different types of pathogenic microorganisms (Reed et al., 2003, Ahmed et al., 2011 and Pardal et al., 2012).

Due to their migration across national and intercontinental borders these birds can become long-range vectors for several pathogenic microorganisms (Grenfell and Dobson, 1995). A high percentage of pathogens are more often isolated in migratory birds compared with other animal species (Tsiodras et al., 2008) and the potential for transport and dissemination of these pathogens by wild birds is of increasing public health concern (Rappole and Hubalek, 2003 and Tsiodras et al., 2008).

The Results of our study revealed 9 bacterial types isolated from different species of wild birdsthat included 35 isolates, which involved :18 isolates of *Escherichia coli* (9%), 4 of *Staphy.aureus* (2%), 3 of

Citrobacter spp. (1.5%), 3 of *Klebsiella* spp. (1.5%), 2 of *Pseudomonas aeruginosa* (1%), 2 of *Salmonella* spp. (1%), 1 of *Enterobacter* spp. (0.5%), 1 of *Shigella* spp. (0.5), and 1 of *proteus* spp. that's completely differ from Euden (1990) who reported a total lack of isolations from 78 examined raptors but Goodenough and Stallwood, (2010) found that blue tit and great tit hosted *Pseudomonas aeruginosa*, *P. fluorescens*, *P. putida*, *Staphylococcus hyicus*, *E. cloacae*, *Keratinolytic bacteria Pseudomonas stutzeri* and *Bacillus subtilis*. The study of Lister et al. (2009) explained the arising of multidrug-resistant phenotype in *P. aeruginosa* could be through the acquisition of multiple imported resistance mechanisms on mobile genetic elements, a combination of imported and chromosomally encoded resistance mechanisms, accumulation of multiple chromosomal changes over time, and/or a single mutational event leading to the overexpression of a multidrug resistance mechanism, i.e., an efflux pump.

No *Pasteurella* spp. organisms were isolated during this work while *P. multocida* were isolated from eiders by Pedersen et al. (2003).

Problems attributed to coliform infections in birds and poultry are often caused by strains of *Escherichia coli* (Dho-Moulin and Fairbrother, 1999). Our findings stressed on the high prevalence of *Escherichia coli* isolation 9 % (18/200) that's agreed to some extent with the results of Maiko et al. (2009) who recorded the most prevalent species isolated from the flamingos were *Escherichia coli* 18 (48.6%), followed by *Pseudomonas aeruginosa* 4 (10.8%), *Proteus mirabilis* 3 (8.1%), *Citrobacter koseri* 2 (5.4%), and single isolates (2.7%) of *C. youngae*, *Enterobacter cloacae*, *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Proteus vulgaris* and *S. enterica* subsp. *arizonae*.

Brittingham et al. (1988) also determined the prevalence of six genera of

bacteria after examination of 387 cloacal swabs from 364 passerines and woodpeckers. The prevalence of *Escherichia coli* and *Salmonella* spp. were lower than our results and recorded as 1 and 0%; respectively while *Pseudomonas* spp. and *Staphylococcus* spp. were 22 and 15%; respectively which showed higher than ours which recorded as 1 and 2%; respectively.

Pedersen et al. (2006) detected *E. coli* in 326 of 406 pigeons (80.3%) which represented the double percent of our pigeon incidence (40%). Pigiņka (2009) recognized nine infectious agents from 66 birds. Widespread agent was *E.coli* that isolated from 44 birds and had 61.4% extensity from all isolated infections agents and represent more than 5 times our percentage. *Klebsiella* was found in percentage (3%) which was similar to our result. Four causes were detected as *Staphylococcus*, *Streptococcus*, *S. enteritidis*, *P. putida* with 1.4% extensity for each. Helena (2002) applied three studies and explained their results as they couldn't find *Salmonella* in black-headed gulls but other enteric bacteria such as *Escherichia coli*, *Hafnia* spp., *Klebsiella* spp., *Yersinia* spp. and *Citrobacter* were found in study 2 these bacterial species possibly belong to commences enteric flora of wild birds while The prevalence of *Salmonella* in black-headed gull recorded as 4.9 % and 2.7 % in studies 1 and 3, respectively and *S.Typhimurium* was the only serotype found.

The differences between the results explained by Clare et al.(2009) and Hernández et al. (2012) as that could be attributed to the variations in climate and environment of husbandry regions especially temperatures variation that effect on the bacterial growth.

Salmonella epizootics occurred much less frequently among raptors than among other avian species (Refsum et al., 2002) this result agreed to some extent with our result of

Salmonella isolation 1% (2 isolates from 200 wild birds) and serotyping of identified into two serotypes that isolated from cloacal swab, *S. Give* isolated from pigeon and *S. Santiago* from pelican. **Pohl et al. (1997)** reported that *S. Give* was found in 2 pigs in 1992, in 2 pigs and 1 horse in 1993, in 1 sample of animal feed in 1994, and in 1 sample from poultry in 1995. It was not detected in 1996. **Keymer (1972)** reported the prevalence as 1.9%, also another study of **Kirkpatrick and Trexler (1986)** found only 1.9% positive cases among 105 raptors. Therefore, the results of the present study show a near prevalence percentage when compared with previous reports but far from the results of **Cizek et al. (1994)** who isolated *Salmonella* from 25% of the examined birds (including house sparrows), and from 4.2% and 19.2% of the examined adult and young black-headed gulls, respectively while **Craven et al. (2000)** examined 25 wild bird and found 6 samples (24%) were positive for *Salmonella* spp. **Laura et al. (2014)** found that *Salmonella* spp. which isolated from 3 of the 56 captured birds (5.3%) serotyped as *S. Typhimurium* was isolated from one out of 13 night herons, *S. Livingstone* from one out of 8 blackcaps, and *S. Napoli* from 1 out of 6 river nightingales also, **Pennycott et al. (2006)** confirmed that *S. Typhimurium* was the most predominant serovar in the wild birds. **Kapperud et al. (1998)** suggested that serovar *Typhimurium* has established a reservoir in avian wildlife in Norway, and epidemiological and bacteriological evidence indicate that wild birds may transmit the infection to humans and to poultry. These reports indicate that strains of *Salmonella* spp. in wild birds could correlate with strains isolated from domestic animals and chickens. **Millán et al. (2004)** recorded the prevalence of *Salmonella* spp. which was 8.5% (7/82) in wild birds. The isolated serotypes were belonged to the species *S. enterica*, *S. Typhimurium*, *S. Enteritidis*, *S. Muenchen*, and serotype 6,14:z4, z23; (which

belongs to subsp. IV or *houtenae*). **Refsum et al. (2002)** found *S. Typhimurium* serotype was almost the only one among 470 isolations from in wild birds in Norway. It has also been reported in captive raptors by **Wernery (1998)** versus 0% by us. **Mirzaie et al. (2010)** showed that 18 (3.8%) were positive for *Salmonella*, the most predominant serovars were *S. Typhimurium* and *S. Enteritidis* (9 and 8 cases each, respectively), whereas only 1 serovar belonged to *S. Montevideo*. Therefore, the results of the present study show a low prevalence when compared with previous reports. Birds suspected of having salmonellosis and any material contaminated by their feces should be handled carefully to prevent human exposure. **Quessy and Messier (1992)** believed that wild birds do not play a major role in the epidemiology of human salmonellosis. However, when large number of birds roosts at the same site, they may represent a potential health hazard to humans and other animals.

The only isolate of *Proteus* spp. (1/200) showed antimicrobial sensitivity to all antimicrobial used except nalidixic acid in contrary **Olinda et al. (2012)** who concluded that *Proteus* spp. appears to be a potential multi-resistant pathogen and causes severe lesions and diseases for wild birds in captivity as all the *Proteus* isolates were resistant to ampicillin, amoxicillin/clavulanic acid, aztreonam, cefepime, ceftazidime, ciprofloxacin, tetracycline, sulfamethoxazole/trimethoprim and gentamicin. **Maiko et al. (2009)** reported that the scientific data on the role of imported animals in the spread of multidrug-resistant (MDR) bacteria and resistance genes from one country to another was very limited. Also, little is known about the molecular basis of resistance in MDR bacteria isolated from imported animals, therefore our study was conducted to start decreasing the gap between the available data and the present cases of wild birds specially

in Egypt and draw an antibiotic resistance profile of each Egyptian isolates.

P.aeruginosa was a common avian pathogen, causing disease principally as a secondary invader (Gerlach, 1994). Our study isolated *Pseudomonas aeruginosa* with an incidence of 2%, that is in contrary with Awad-Alla et al. (2010) who could not isolate the organism from the internal organs of free living white ibis (Nipponianippon) and Hendawy and El-Shorbagy (2006) isolated *Pseudomonas aeruginosa* from ibises with an incidence of 20%, while the work of Mona et al. (2014) who collected 17 isolates of *P.aeruginosa* from free living birds (pigeons, ibises, hoopoes and crows) with an incidence of 3.63% (17/459 samples), 1/18 (5.5%), 2/22 (9.0%), 5/43(11.6%) and 9/70 (12.8%) respectively. Bailey et al. (2000) reported that *P. aeruginosa* which collected from 18 captive-bred kori bustards was the predominant isolate from all cases.

Additional isolates included *K. pneumoniae* (2), *E. coli* (2) and *Strept.viridans* (1) and the antibacterial resistance pattern of *P. aeruginosa* isolates on initial presentation was amoxicillin (6/6), amoxicillin-clavulanic acid (6/6), chloramphenicol (3/4), tetracycline (6/6), piperacillin (1/4), gentamicin (0/6), enrofloxacin (2/6) and carbenicillin (0/6). Those results agreed with our isolate results as they showed resistance against all the fifteen antimicrobial drugs used, and also agreed with another Egyptian study (Mona et al., 2014) which showed that 17 isolates of *P. aeruginosa* that collected from free living birds (pigeons, ibises, hoopoes and crows) with an incidence of 3.63% (17/459 samples). 1/18 (5.5%), 2/22 (9.0%), 5/43(11.6%) and 9/70 (12.8%) respectively produced multidrug resistance pattern.

Little information is available regarding serogrouping of *E. coli* in wild birds. According to the results of our study, 18 strains of *E.coli* were serotyped into 9 different O serotypes as follow : 3 isolates of O44, 3 of O27, 2 of O119, 2 of O159, 2 of O63, 2 of O 128, 2 of O28 ac ,1 of O169 and 1 of O91 while Hyun-Jeong et al. (2005) serotyped 79 isolates of *E. coli*, O serotypes appeared in 3 strains of O55, 3 of O158, 2 of O18, 1 of O6, 1 of O8, 1 of O28 ac and 1 of O125. The results showed that *E. coli* O157 hasn't been isolated from our examined wild birds and that disagree with other studies which has been isolated it from faeces collected from a garden bird feeding station in southwest Scotland, albeit at a low level (Foster et al., 2006). Pennycott et al. (2006) reported that *S. Typhimurium* DT40 and *E. coli* O86. Pedersen et al. (2006) detected *E. coli* in 326 of 406 pigeons (80.3%). Serogroup O86 has been identified as the cause of disease or mortality in birds (Foster et al., 1998), but other serogroups including O157 (Shere et al., 1998), O15, O18, O25, O45, O75, O152 (Morabito et al., 2001), and O128 (Schmidt et al., 2000) have been isolated from asymptomatic birds. Despite ambiguous evidence that the above serogroups cause disease in their avian carriers, there is concern that these *E. coli* serogroups are a risk to humans and cattle, and that birds are agents of transport and transmission.

Other studies addressed the incidence of nonpathogenic but emerging antibiotic-resistant strains of *E. coli* and the role of an avian reservoir in their distribution in the environment. Cole et al. (2005) showed an increased proportion of resistant *E. coli* isolates as more than 95% of isolates from migrating Canada Geese in Maryland were resistant to at least one of penicillin G, ampicillin, cephalothin and sulfathiazole, and many isolates had multiple resistance

(Middleton and Ambrose, 2005). Dolejská *et al.* (2007) suggested that the birds are an important reservoir of antibiotic-resistant bacteria reflecting their presence in the gulls' food or water took cloacal swabs from young black-headed Gulls at three breeding colonies in the Czech Republic and from 75 of the 257 birds sampled they isolated *Escherichia coli* showing resistance to tetracycline, cephalothin, streptomycin, sulphonamides and/or chloramphenicol. The relative incidences of resistance to each of these antibiotics were found to mirror the relative use of the compounds in human and veterinary medicine in the Czech Republic and the authors. Jorge *et al.* (2003) suggested that the natural occurrence of *Salmonella* in healthy birds during migration in Sweden may be low, as they detected only one *Salmonella* isolate serotyped as *S. Schleissheim*. Human salmonellosis caused by this serotype has been previously reported only in Turkey by Aksoycan (1983). These results agreed with our results and also with Hernandez *et al.* (2003) who found only one *Salmonella*-positive bird. Some studies suggested that wild birds may acquire *Salmonellae* after exposure to human-contaminated environments, or after scavenging on refuse tips and sewage sludge, and that wild bird that live away from such environments are unlikely to harbor *Salmonella* (Murray, 2000 and Tizard, 2004). Maiko *et al.* (2009) isolated seven isolates from Thirty-seven Gram-negative bacterial isolates that were obtained from the flamingos where (18.9%) showed multidrug resistance phenotypes, the most common being against: ampicillin, streptomycin, tetracycline, trimethoprim/ sulfamethoxazole and nalidixic acid.

This work highlights the role of wild birds in the dissemination of multidrug-resistant bacteria from one country to another through studying the reaction of each isolate against the anti-microbial agents commonly

used in our field. The antibiogram results of *E. coli* showed that 17 out of 18 isolates (94.4%) carry resistance to more than two antimicrobial agents. The most resistance were 15 of 18 isolates against tetracycline (83.3%), 15 of 18 against colistin (83.3%), 11 of 18 against amoxicillin (61.1%), 10 of 18 against streptomycin (55.6%), 10 of 18 against nalidixic acid (55.6%), 9 of 18 against ciprofloxacin (50%), 9 of 18 against neomycin (50%), 8 of 18 against doxycycline (44.4%), 8 of 18 against levofloxacin (44.4%), 8 of 18 against norfloxacin (44.4%), 7 of 18 against chloramphenicol (38.9%), 3 of 18 against enrofloxacin (16.7%), 3 of 18 against gentamicin (16.7%), 2 of 18 against nitrofurantoin (11.1%) while all the isolates sensitive for ceftriaxone. On the whole, our results revealed that the resistance of *E. coli* against tetracycline and ciprofloxacin were (83.3%) and (50%); respectively which disagree with Smith *et al.* (2014) who recovered 92 *Escherichia coli* isolates, all of them were susceptible to ciprofloxacin and only one isolate was resistant to tetracycline. In contrast, all of these isolates were resistant to rifampicin, oxacillin and penicillin while Hyun-Jeong *et al.* (2005) detected 47 from 79 strains that were highly tolerated against antimicrobial drugs such as tetracycline (40.5%) and carbenicillin (27.8%).

S. Give and *S. Santiago* which recovered from cloacal swabs of pigeons and pelican in our study showed 100% resistance against neomycin, ceftriaxone and norfloxacin while only one isolate (50%) showed resistance against tetracycline, ciprofloxacin, levofloxacin, colistin, gentamicin, streptomycin amoxicillin and nalidixic acid but both isolates (100%) were sensitive to doxycycline, chloramphenicol, enrofloxacin and nitrofurantoin. Those results agreed to some extent with Mirzaie *et al.* (2010) who recognized that 38.8% (7 of 18 of *Salmonella* isolates) were resistant to at least

one antibiotic. All the *Salmonella* isolates were sensitive to norfloxacin, flumequine, ampicillin and sultrim, and 35% were resistant to lincospectin (the most prevalent resistance). **Charlene et al. (2000)** found that 15 of 22 *Salmonella* isolates were sensitive to amikacin, apramycin, ceftiofur, ceftriaxone, cephalothin, ciprofloxacin, gentamicin, nalidixic acid, and trimethoprim. Seven isolates were resistant to sulfamethoxazole, four were also resistant to streptomycin and also observed resistance to other drugs, including ampicillin, tetracycline, chloramphenicol and kanamycin. In a separate study two of 28 isolates of *Salmonella* from black-headed Gulls *Larusridibundus* exhibited multiple resistances; one to sulfisoxazole, ampicillin and trimethoprim, and one to sulfisoxazole, ampicillin, streptomycin and chloramphenicol (**Palmgren et al., 2006**). **Cizeket al. (1994)** concluded that the drug resistance patterns and percentages were clearly different in *Salmonella* isolates cultured from nondomestic birds compared to those from poultry.

Antimicrobial drug resistance is relatively common place in poultry, but has also been described in bacteria isolated from wild birds (**Cole et al., 2005**). Arctic birds are known to contain multi-drug-resistant bacteria, indicating that migration behavior may be responsible for the introduction and transfer of drug-resistant bacteria to geographically remote areas (**Sjo"lund et al., 2008**). The increasing occurrence of antibiotic resistant microorganisms has fueled interest in the genetics and mechanisms of resistance evolved by bacteria to counteract the effect of antimicrobial agents. The fact that resistance genes do not respect phylogenetic, ecological or geographical boundaries implies that antimicrobial use and the resulting resistance in one ecological niche may have consequences for the

resistance situation in another niche (**Okeke and Edelman, 2001**).

This study determined the prevalence of bacterial pathogens in wild birds which may serve as a useful model for examining the spread of other disease organisms, both amongst birds, and from birds to other taxa and also the dissemination of antimicrobial resistance (AMR) which is a growing concern to public and animal health and concluded that most of bacterial pathogen isolated from wild birds carry variable antibiotic resistance patterns and backed that to the widespread use of antibiotics to treat diseases and to promote growth by the livestock.

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الملخص العربي

تواجه الطيور البرية أخطار صحية متعددة تنجم عن الإصابة بالفيروسات والبكتيريا والفطريات وقد جلبت هذه الطيور الأمراض الناشئة حديثاً والأشكال الجديدة من الأمراض الحيوانية القديمة زيادة الاهتمام العالمي بالطيور البرية لتأثيرها على الصحة العامة للإنسان.

وقد تم تصميم هذه الدراسة لتقديم لمحة عامة عن مسببات الأمراض البكتيرية الرئيسية المعزولة من الطيور البرية في مصر والتي لها القدرة على إحداث المرض في كل من الطيور والبشر من خلال تحديد مدى انتشار عشرة مسببات للأمراض البكتيرية الهامة (السالمونيلا، الإشريشيا كولاي، أنواعا لزائفة، الأمعانية، المكورات العنقودية، الكليسيلا، الباستوريلا، الشيجيلا، والانتيريوباكتري) في مسحات المذرقية والقصبة لهوائية من عدد مائتي طائر من الطيور البرية في مصر وكذلك نشر مقاومة مضادات لميكروبات (AMR) هو مصدر قلق متزايد على الصحة العامة والحيوانية.

وكانت معدلات الإيجابية من العزلات البكتيرية على النحو التالي: الإشريشيا القولونية (9٪)، المكورات العنقودية الذهبية (2٪)، السيتروباكتري (1.5٪)، الكليسيلا (1.5٪)، الزائفة الزنجارية (1٪)، السالمونيلا (1٪)، الأمعانية النيابية (0.5٪)، الشيجيلا (0.5٪)، والانتيريوباكتري (0.5٪)، في حين الباستوريلا (0٪).

بين 18 معزولة من الميكروب القولوني، ظهرت الأنماط المصلية الجسميه (O) في ثلاث سلالات من O44، ثلاث من O2، اثنان من O119، اثنان من O15، 9، اثنان من O63، اثنان من O128، اثنان من O28ac، ومعزولة واحدة من O169 و O91 وقد أظهرت النتائج أعلى مقاومة لمضادات الميكروبات ضد الكوليستين والتتراسيكلين (83.3٪) وضد الأموكسيسيلين بنسبة (61.1٪).

وقد تم عزل سلالتين من ميكروب السالمونيلا هما *S. Give* و *S. Santiago* من مسحات المذرقية من الحمام والبعج وأظهرت مقاومة 100٪ ضد النيومايسين، السيفاترياكسون والنورفلوكساسين.