Prevalence of *Enterobacteriacea* in Wild Birds and Humans at Sharkia Province; With Special Reference to the Genetic Relationship between *E. coli* and *Salmonella* Isolates Determined bBy Protein Profile Analysis

Magda A. M. Amin, Mohamad N. M. Ali, Maysa A. I. Awadallah, Merwad, A. M. Amin, Ahmed, H. A., Rasha M. A. Gharieb, Rasha M. M. Abu-El-ezz, and Rehab E. Mohamad

Department of Zoonoses, Fac. Vet. Med., Zag. Univ. Egypt
magda_amin_2013@yahoo.com.

Abstract: The present study was carried out to evaluate the role of wild birds as a reservoir for some pathogenic bacteria of zoonotic importance that are pathogenic to man. The occurrence of some pathogenic bacteria in cloacal swabs of wild birds and stool samples of human live at the same region was also carried out. For this purpose, a total of 410 cloacal swabs were collected from different spp of wild birds that were shot at various locations in Sharkia Province, Egypt. The species included were cattle egrets (n=160), crows (n=100), wild ducks and little egrets (n=75, each). The samples were examined bacteriologically for the presence of *Enterobacteriaceae.* Identification of isolated microorganisms revealed the recovery of *Escherichia coli*, *Salmonella spp, Enterobacter cloacae*, *Enterobacter agglomerans*, *Enterobacter aerogens*, *Enterobacter hafnia, Citrobacter freundii*, *Citrobacter diversus*, *Klebsiella ozaenae*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Proteus mirabilis*, and *Proteus rettegri* at different percentages from the examined wild bird spp. No *Salmonella* spp were isolated from human's stool or cloacal swabs of Ibis. However, 17 *Salmonella* isolates belonging to 5 serotypes were isolated from Crows, 2 isolates "2 serotypes" from great egret, and only one isolate identified as *S. enteritidis* from wild duck. On the other hand, *E. coli* were isolated from 15 human stool samples (15%), and from the cloacal swabs of 5 Ibis (3.125%), 5 Crows (5%), 12 wild duck (16%), and 7 little egrets (9.33%). Serotypes O26:K69 were detected in six human's stool samples and 6 cloacal swabs of wild duck. On the other hand *E. coli* O111:K58 was recorded in 4 stool samples of human and 2 cloacal swabs of little egrets. Moreover, the enterotoxigenic *E. coli* O127:K63 (B8) was identified in the cloacal of 24 *E. coli* isolates recovered from examined sources at locality I swabs of 3 Ibis & 4 Wild ducks. Other serotypes identified in 5 human, 2 Ibis, 2 Wild ducks and 5 Great egrets were O55:K59, O114:K90, O119:K69, and O125:K70, respectively. The only serotype isolated from 5 crows was the enteropathogenic *E. coli* O86:K61. In addition, SDS-PAGE of whole cell protein profiling were carried out for six *E. coli* serotypes (2, O26:K60; 2, O111:K58 and 2, O127:K63) and for seven *Salmonella* isolates (2, *S. typhimurium*; 3, *S. enteritidis*; 1, *S. anatum* and 1, *S. Virchow*). For SDS-PAGE of protein profiles of *E. coli* isolates, a high degree of similarity was concentrated in region between 15-131KDa, while those profiles of Salmonella isolated produce patterns with molecular masses of 17-129KDa. This study emphasized that wild birds are reservoirs for some zoonotic bacteria within family of Enterobactericeae.
[Magda A. M. Amin, Mohamad N. M. Ali, Maysa A. I. Awadallah, Merwad, A. M. Amin, Ahmed, H. A., Rasha M. A. Gharieb, Rasha M. M. Abu-El-ezz, and Rehab E. Mohamad**Prevalence of *Enterobacteriacea* in Wild Birds and Humans at Sharkia Province; With Special Reference to the Genetic Relationship between *E. coli* and *Salmonella* Isolates Determined bBy Protein Profile Analysis.** *J Am Sci* 2013;9(4):173-183]. (ISSN: 1545-1003). http://www.americanscience.org. 24

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1. Introduction

Wild birds have the potential to disperse pathogenic microorganisms they carry either biologically or mechanically **(Clark, 2003 and Hubalek, 2004).** The migratory birds could become long-distance vectors for a wide range of microorganisms that can be transmissible to humans **(Nuttall, 1997).** This creates the potential for establishment of novel foci of emerging or re-emerging communicable bacterial diseases along bird migration routes **(NIAID, 2002).**

Internationally, there is an increasing awareness amongst biologist to the need of gathering information on wild birds' diseases **(Cork 1994).** Consequently, studies have concentrated on migratory and flocking bird species such as waterfowl **(Fallacara et al., 2001; Ballweber, 2004; Hubalek, 2004 and Clark & Hall, 2006).**

There are unique characteristics in waterfowl that may enhance the conditions conductive to the development of microorganisms and ultimately promote their transmission **(Morishita, 2004).** Waterfowl tend to aggregate in flocks around staging and feeding areas, as a consequence, large numbers of birds are concentrated spatially and temporally. This causes an increased opportunity for infection by exposure to contaminated environments **(Clark & Hall, 2006).** Additionally, the highly mobile behavior of waterfowl and their utilization of agricultural areas increase the possibility of cross transmission of

microorganisms to other individuals or species, by direct contact or faecal contamination.

Crows and cattle egrets (white Ibis) are two species of wild birds that recently have increased significantly and have become highly adaptable to the urban environment **(Aruji et al., 2004).** They may carry causal agents of highly pathogenic microorganisms with zoonotic infectivity and their fecal shedding may contaminate the environment **(Maruyama et al., 1990; Refsum et al., 2002 and Fukuyama et al., 2003).**

An association with transmission from wild birds to humans was identified for 10 pathogens. Wild birds including migratory species may play a significant role in the epidemiology of enteric zoonotic bacteria including *Escherichia coli* and *Salmonella* spp **(Tsiodras et al., 2008).**

Application of advanced molecular diagnostic testing during the recent years has led to the identification of microbial agents known to affect humans and birds including some zoonotic bacterial spp. such as *E. coli,* **(Ejidokun et al., 2006, Makino et al., 2000)** and *Salmonella typhimurium* **(Kapperud et al., 1998).** Pathogens associated with wild and migratory birds may be transmitted to humans via several routes. Wild birds such as ducks, little egrets and crows can contaminate water with feces, nasal discharges, and respiratory secretions harboring microrganisms of Family *Enterobactericeae*, resulting in a waterborne human infection after direct contact with aquatic environments **(Reed et al., 2003).**

The present work was undertaken to assess the extent to which wild ducks, crows, little egrets and cattle egrets are acting as reservoirs for some pathogenic microorganisms especially those of the family *Enterobacteriaceae*. Also, the current work aimed to explore the possibility of cross transmission of pathogenic *Enterobacteriaceae* to humans residing the same areas where wild birds were found. Protein profile analysis of *E. coli* and *Salmonella* serotypes were applied to determine the genetic relatedness of the isolated strains from different sources including wild birds and humans.

2. Material and Methods

I- Sampling

Cloacal swabs were collected from 410 different spp of wild birds that were shot during their roaming at different locations at Sharkia Province, Egypt. The sampled species were cattle egrets (n=160), crows (n=100), wild ducks and little egrets (n=75, each). Moreover, 100 stool samples of human residing the same region where birds were caught were also collected. All samples were examined for the presence of the members of the Family *Enterobacteriaceae*.

I.1. Cloacal swabs: were obtained by inserting a sterile culture swab with (buffered peptone water, **Oxoid, CM509)** into the cloaca and gently rotating the tip against the mucosa. Swabs were then immediately returned to the sleeve. Samples were labeled with respect to the species and kept at ambient temperature until inoculation.

I.2. Stool samples of human: stool cups were distributed one day before collection and the investigated persons were instructed to collect the next day's stool **(Vadivellu et al., 1989)**. A swab was taken from each stool sample using a sterile swab and then immersed in sterile buffered peptone water tubes under aseptic condition **(Sadoma, 1997)**. The tubes were labeled with respect to name, age, & sex.

II. Isolation and identification of *Enterobacteriaceae*:

Cloacal swabs of birds & stool samples of human, collected into buffered peptone water, were incubated at 37° C for 24 hrs. On the next day, a loopful from each tube was streaked onto MacConkey agar **(Oxoid, CM7)** and incubated at 37° C for 18-24 hrs for isolation of different species of *Enterobacteriaceae*. At the same time, 1ml from each tube was transferred to Rappaport Vasiliadis medium **(Oxoid, CM669)** and incubated for 24 hrs at 42° C for enrichment of *Salmonella* spp. A loopful from RV medium was then streaked onto Xylose Lysine Deoxycholate agar (XLD) **(Oxoid, CM469)** and incubated for 24 hrs at 37° C.

For isolation of *E. coli*, 1 ml from buffered peptone water enriched samples was transferred to MacConkey broth and incubated for 24 hrs at 37° C, then a loopful from this broth was streaked onto EMB agar and incubated for 24 hrs at 37° C. Characteristic pink and pale colonies on MacConkey agar plates, Colonies morphologically similar to those of *Salmonella* spp on XLD agar, and shiny metallic colonies on EMB agar were purified on nutrient agar slants and incubated at 37° C for 18-24 hours for further identifications as the following:

II.1. Microscopical examination:

II.2. Biochemical identification (Borrow and Feltham 1993)

Oxidase test, Indole test, triple sugar iron agar **(Oxoid, CM277)**, Methyl red test, Vogues proskauer test, Citrate utilization test, Urea hydrolysis test, Sugar fermentation test, H_2S production, and Decarboxylation of arginine, ornithine and lysine were performed for each isolate.

II. 3.Serotyping of *Salmonella* and *E. coli* isolates: was done at **Food Analysis center, Faculty of Veterinary Medicine, Benha University, Egypt.**

II.3.1. Serotyping of *Salmonella* spp:

Isolates proved biochemically to be *Salmonella* were subjected to serological identification according to Kauffman's White Scheme **(Kauffman, 1974)** by using rapid diagnostic *Salmonella* antisera sets **(Welcome Diagnostic, a Division of the Welcome Foundation Limited, Dartford England DA15 AH).**

II.3.2. Serotyping of *E. coli*

The isolates were serologically identified according to **Kok et al. (1996)** by using rapid diagnostic *E. coli* antisera sets **(DIFCO Laboratories, Detroit Michigan 48232-7058, USA)** for diagnosis of the Enteropathogenic types.

II.4. SDS-PAGE of whole cell proteins profiling:

It was done on six *E. coli* serotypes (2, O_{26}:K_{60}, 2, O_{111}:K_{58}, and 2, O_{127}:K_{63}) and seven *Salmonella* isolates (2, *S. typhimurium*; 3, *S. enteritidis*; 1, *S. anatum* and 1, *S. Virchow*) that were recovered from different sources. The total protein of the isolates to be examined was prepared as described by **Kishor et al. (1996)**. Total protein analysis was carried out by using **SDS-PAGE as the following:**

Extracted protein of purified bacterial preparation was resolved on discontinuous buffer system composed of 10% (w/v) acrylamide running gel and 4% shaking get **(Sambrook et al., 1989)**. Electrophoresis was carried out at a constant voltage (100v) until tracking dye (Bromophenol blue) moved to the bottom of the gel. Wide range protein marker (10 KDa to 200 KDa), Page Ruler protein Ladder; Fermentas) was used. The gel was stained with SDS-PAGE gel stain (Gel Code Blue Stain, PIERSE, USA). Molecular weight of each protein band was calculated with reference to a standard curve derived from the migration pattern of the protein molecules weight marker.

Cluster analysis:

Different fragments on the gel were numbered sequentially and the presence or absence of fragments in each isolate was scored (Present 1, absent 0) for comparison with each other. Cluster analysis of whole cell proteins was performed according to the genetic distance method of **Nei, 1972**.

3. Results

Table (1): Prevalence of *Enterobacteriaceae* from different species of wild birds shot at different location at Sharkia Province, Egypt in the period from June 2010-June 2012.

Bacterial isolates	Isolates from each wild birds' species				
	Cattle egrets n=160	Crows n=100	Wild ducks n= 75	Little egrets n=75	Total n=410
Escherichia coli	5[a](3.125)[b]	5(5)	12(16)	7(9.33)	29(7.07)
Salmonella	0(0)	17(17)	5(6.66)	8(10.66)	30(7.32)
Enterobacter aerogenes	5(3.125)	1(1)	0(0)	0(0)	6(1.46)
Enterobacter cloacae	0(0)	0(0)	1(1.33)	0(0)	1(0.24)
Enterobacter hafnia	1(0.625)	1(1)	1(1.33)	1(1.33)	4(0.98)
Citrobacter freundii	1(0.625)	0(0)	2(2.66)	0(0)	3(0.73)
Citrobacter diversus	1(0.625)	0(0)	0(0)	0(0)	1(0.24)
Klebsiella ozaenae	1(0.625)	2(2)	1(1.33)	1(1.33)	5(1.22)
Klebsiella pneumonia	3(1.875)	0(0)	2(2.66)	1(1.33)	6(1.46)
Proteus mirabilis	2(1.25)	0(0)	4(5.33)	2(2.66)	8(1.95)
Proteus vulgaris	7(4.375)	0(0)	4(5.33)	1(1.33)	12(2.93)
Proteus rettegri	4(2.5)	0(0)	2(2.66)	2(2.66)	8(1.95)
Total	30(18.75)	26(26)	34(45.33)	23(30.66)	113(27.56)

n: Number of examined wild birds. a: Number of infected bird species in the consensus of examined one.
b:Percentage infected bird species in the consensus of examined one.

Table (2): Prevalence of *Enterobacteriaceae* in 100 stool samples of human residing at the same locations where wild birds were shot.

Isolated bacterial spp.	No. of positive samples	%
E. coli	15	15
Salmonella spp.	0	0
Enterobacter agglomerans	1	1
Enterobacter aerogenes	2	2
Enterobacter cloacae	3	3
Enterobacter hafnia	1	1
Citrobacter freundii	2	2
Citrobacter diversus	0	0
Klebsiella ozaenae	2	2
Klebsiella pneumonia	0	0
Proteus mirabilis	0	0
Proteus vulgaris	0	0
Proteus rettegri	0	0
Total	26	26

Table (3): Serotyping of *E. coli* isolates from human stool & cloacal swabs of different species of wild birds.

Examined samples	Total No. of *E. coli* isolates	The identified serotypes	No.	%	Strain characterization
Human stool	15	O26:K60(B6)	6	40	EHEC
		O55:K59(B5)	5	33.33	EPEC
		O111:K58(B9)	4	26.66	EHEC
Cloacal swabs of little egrets	5	O127:K63(B8)	3	60	ETEC
		O114:K90	2	40	EPEC
Cloacal swabs of crows	5	O86:K61 (B7)	5	100	EPEC
Cloacal swabs of wild ducks	12	O127:K63(B8)	4	33.33	ETEC
		O26:K60(B6)	6	50	EHEC
		O119:K69(B19)	2	16.66	EPEC
Cloacal swabs of little egrets	7	O125:K70(B15)	5	71.42	ETEC
		O111:K58(B9)	2	28.57	EHEC

Table (4): Serotyping of *Salmonella* isolates cloacal swabs of crows, wild ducks and little egrets:

Examined Species of Wild birds	Total No. of *E. coli* isolates	The identified se Retypes	No.	%	Antigenic structure	
					O	H
Crows	17	*S. muenster*	3	17.64	3,10,15,34	e,h : 1,5
		S. typhimurium	5	29.41	1,4,5,12	i : 1,2
		S. Virchow	2	11.76	6,7,14	r : 1,2
		S. anatum	4	23.53	3,10,15,34	e,h : 1,6
		S. enteritidis	3	17.65	1,9,12	g,m : 1,7
Wild ducks	5	*S. enteritidis*	5	100	1,9,12	g,m : 1,7
Little egrets	8	*S. typhimurium*	4	50	1,4,5,12	i : 1,2
		S. enteritidis	4	50	1,9,12	g,m : 1,7

**Salmonella* could not be detected in stool sample of human and cloacal swabs of Ibis.

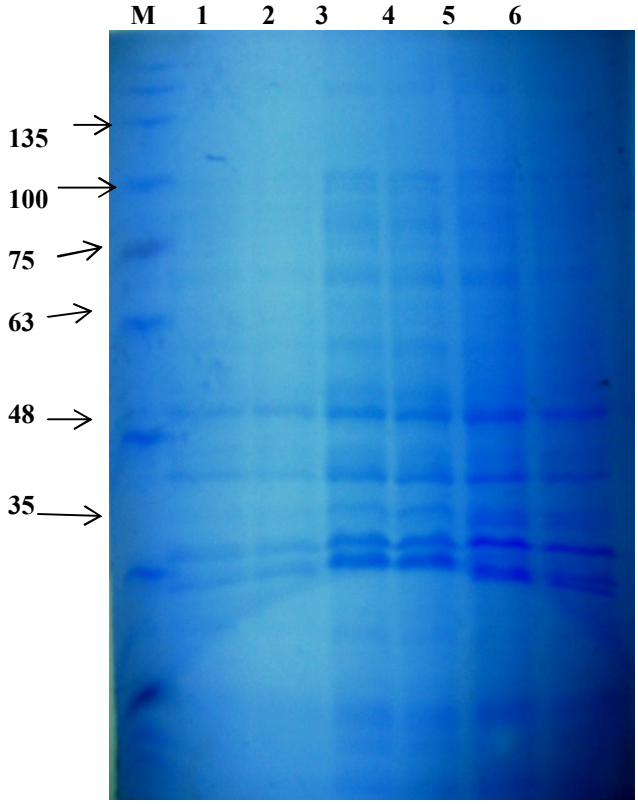

Fig (1): SDS-PAGE of whole cell protein of six *E. coli* isolates recovered from different sources
Lanes (Sources); M (Molecular marker); 1- O26:K60 from human, 2- O26:K60 from wild ducks; 3- O127:K63 from wild ducks; 4- O127:K63 from human; 5- O111:K58 from little egrets; 6- O111:K58 from human

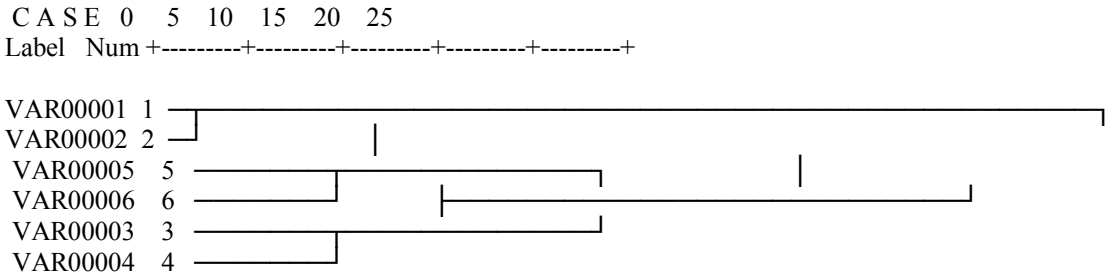

Fig (2): The dendrogram based on whole cell protein profiles of *E. coli* isolates

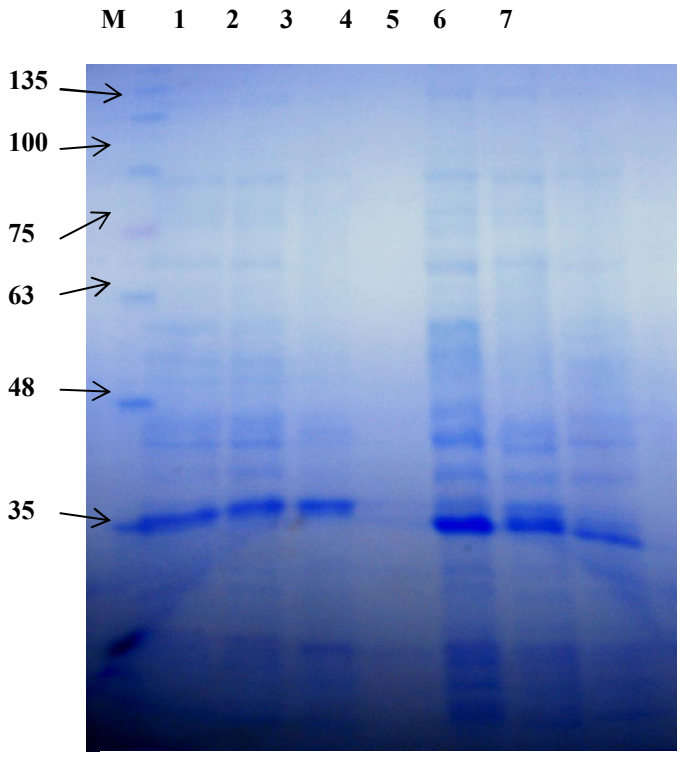

Fig. 3 SDS-PAGE of whole cell protein of seven *Salmonella* isolates recovered from different sources
Lanes (Sources); M (Molecular marker); 1- *S. enteritidis* from little egrets; 2- *S. enteritidis* from crow; 3- *S. typhimurium* from little egrets; 4- *S. typhimurium* from crow; 5- *S.anatum* from crow; 6- *S. enteritidis* from wild duck; 7- *S. virchow* from crow

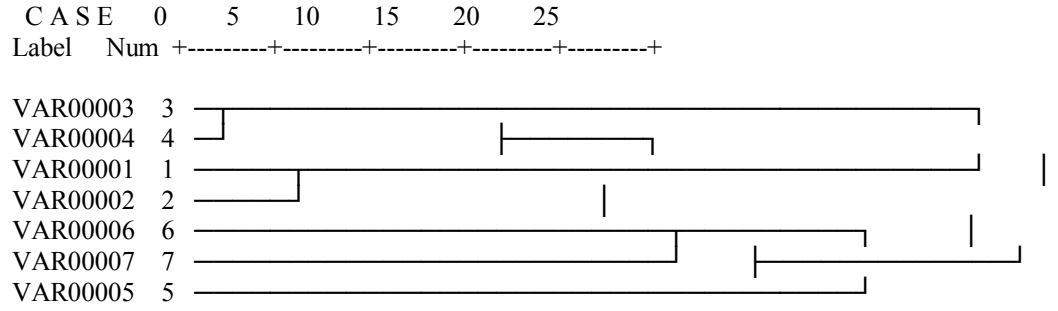

Fig (4): The dendrogram based on whole cell protein profiles of E. coli isolates

4. Discussion

Human medicine often does not delve deeply into the role of animals in the transmission of zoonotic agents and veterinary medicine does not cover the clinical aspects of human diseases. However, to effectively and completely cover the area of infections associated with wild birds; involvement of both physicians and veterinarians especially those dealing with avian species is required **(Grant and Olsen, 1999).** Unfortunately, one recent study demonstrated that communication between physicians and veterinarians about zoonotic diseases is largely absent. Therefore, one important factor that could potentially explain the paucity of available data regarding the transmission of bacterial pathogens from wild birds to humans could be the lack of communication between physicians and ornithologists.

Among *Enterobacteriaceae*, *E. coli* and *Salmonella spp.* are the most potential pathogens affecting humans, animals and birds concerning zoonoses and food poisoning. Previously conducted studies revealed that *E. coli* and *Salmonellae* have been isolated from free- living birds **(Carven et al., 2000; Makino et al., 2000; Kirk et al., 2002; Schmidt et al., 2000; Refsum et al., 2002 and Fukuyama et al., 2003)**

Results shown in **Table 1**, revealed the isolation of many spp of the family *Enterobacteriaceae* from 113 (27.56%) cloacal swabs out of 410 different wild birds' spp. examined. The isolated *Enterobacteriaceae* were *E. coli (7.07%), Salmonella spp* (7.32%), *Ent. aerogenes* (1.46%), *Ent. cloacae* (0.24%), *Ent. hafnia* (0.98%), *Cit. freundii* (0.73%), *Cit. diversus* (0.24%), *Kl. ozaenae* (1.22%), *Kl. pneumoniae* (1.46%), *Pr. mirabilis* (1.95%), *Pr. vulgaris* (2.93%), and *Pr. rettegri* (1.95%). Higher prevalence (97%) of *Enterobacteriaceae* in many seabirds of the families [Alcidae (28), Laridae (49), Podicipedidae (6), and Misc (6)] along the Pacific coast, USA was reported by **Steele et al. (2005).** The *Enterobacteriaceae* they isolated were *E. coli* (89%), *Salmonella* (1%), *Ent. Cloacae* (6%), *Cit. freundii* (19%), *Kl. pneumonia* (21%), *P. mirabilis* (6%), and other *Proteus* spp. (36%).

In previous reports **(Adesiyun et al., 1998; Fallacara et al., 2001; Kirk et al., 2002),** the isolation rates of *Salmonella spp.* from free living birds were usually low. **Kobayashi et al., (2006)** isolated *Salmonella spp.* from 19 cloacal swabs out of 328 wild birds (5.8%) in Japan. However, **Fallacara et al., 2001** recorded *E. coli* in 67% of water fowl residing in metropolitan parks in Central Ohio that was greatly higher than that reported in the current study. Nevertheless, some reports on the free- living birds showed low isolation rates of *E. coli* from omnivorous passerines & carnivorous non-passerines (1-5% and 15%, respectively) **(Glunder, 1981; Brittingham et al., 1988, and Marfin and Gubler, 2001)** . Lower isolation rates of *E. coli* and *Salmonella* in the current study may be contributed to limited species of wild birds investigated and different volume of intestinal samples examined.

Concerning the public health hazard, crows adapt to environment scavenging for they fed on food located on the ground which has been contaminated by infectious droppings from another infected animals such as rats or fish and become infected or carrier of pathogens. Such pathogens are *Salmonella* and enteropathogenic *E. coli* which represent a zoonotic risk **(Angus, 1983 and Meier, 2007)**.

As shown in **Table 1,** the isolation rate of *Enterobacteriaceae* from 100 cloacal swabs of crows was 26% (26/100). All crows examined in this study were clinically healthy and the enterobacteria isolated were biologically classified into 5 species *"E. coli*, *Salmonella spp*, *Ent. aerogenes*, *Ent. hafnia*, and *Kl. ozaenae*". Of the 5 species, *Salmonella* spp (17%) was the bacterium most isolated that followed by *E. coli* (5%).

Higher isolation rate of *Enterobacteriaceae* from cloacal swabs of crows (79.1%) was previously reported by **Aruji et al., 2004** at Ueno Zoo, Tokyo. *E. coli, P. mirabilis, Kl. pneumonia, Ent. aerogenes, Ent, cloacae, Kl. oxytoca, Ent. agglomerans and Pseudomonas maltophila* were isolated at the rates of 21.6, 5.8, 2.1, 1.2, 1.2, 0.2, 0.2, and 0.2%, respectively. However, *Salmonella* spp were not detected in their study. Higher isolation rate of *Salmonella spp.* 44.3% (47 out of 106 examined crows) were previously reported by **Yong et al., (2008)** in Bangsar, Kuala Lumpur, Malaysia.

The world population of cattle egrets has increases significantly since 1983 and these birds are frequently observed in close contact with people **(Shawn, 2000).** This has led to concern that ibis may transmit pathogens that threaten not only the poultry industry, but also public health. It is clear from table 1, that ibis act as a reservoir for many Enterobacteriaceae such as *E. coli, Ent. aerogenes* (3.125%, each), *Ent, hafnia, Cit. freundii, Cit. diversus, Kl. ozaenae* (0.625%, each), *Kl. pneumonia* (1.875%), *Pr. mirabilis* (1.25%), *Pr. vulgaris* (4.375%), and *Pr. rettegri* (2.5%).

In Egypt, **Maha et al., 2010** isolated E. coli (43.6%), Salmonella spp (14.5%), Enterobacter spp (21.8%), Citrobacter spp (18.1%/0, Klebsiella pneumonia (16.3%), *Pr. mirabilis* (7.2%) from 55 apparently healthy Ibis at Sharkia province. Moreover, **El. Sheshtawy and Moursi (2005)** in Ismailia and **Hedawy and El-Shorbagy (2007)** in Sohag, Egypt isolated *E. coli* and *Salmonella* from

cattle egrets at higher rates than those reported in the current study. *Salmonella* spp and *E. coli* were isolated from wild duck with percentages of 9.33 and 10.66, respectively. Nearly similar isolation rates of 14.4 and 8.9%, respectively were reported by **El-Attar et al. (1997)** in Sinai, Egypt.

Wild birds may carry or be infected with bacteria that can adversely affect the health of livestock animals and human beings, since birds inhabit places where human being live, migrate between waste treatment plants, grain stores and livestock pastures, making them important vehicles for the spread of zoonotic infection **(Kobayashi et al., 2006)**. **Table 2,** illustrates the prevalence of *Enterobacteriaceae* from stool samples (n = 100) of human residing at the same location where wild birds were shot. *E. coli*, *Ent. Agglomerans*, *Ent. Aerogenes*, *Ent. Cloacae*, *Ent. Hafnia*, *Cit. freundii* and *Cit. diversus* were isolated from 15, 1, 2, 3, 1, 2, 2 samples out of the total examined.

Escherichia coli has been classified historically as enteropathogenic *E. coli* (EPEC) along with serotypes O_{55}, O_{111}, and O_{127} among others and has been implicated as a major cause of acute and persistent infantile diarrhea in developing parts of the world **(Levine, 1987)**. Wild birds have been implicated as sources of various enteric pathogens of humans **(Girwood *et al.,* 1985**, **Healing *et al.,* 1992)**, including *Salmonella* and *E. coli* belonging to serotype O86.

Table, 3 shows different serotypes of *E. coli* isolated from human stool and cloacal swabs of different species of wild birds. The identified serotypes in human were O_{26}:K_{60} (n=6), O_{111}:K_{58} (n=4) and O_{55}:K_{59} (n=5). One third of the isolated *E. coli* in man was enteropathogenic groups. Similar prevalence of enteropathogenic *E. coli* in human beings in Egypt was previously recorded in Tanta and Assiut Provinces in Egypt **Mohamed et al., 1992 and Sonbol et al., 1994,** and in Iran **Aslani and Alikhani, 2009.**

On the other hand, the identified serotypes in; ibis were O_{127}: K_{63} (n=2) and O_{114}:K_{90} (n=2); crows were O_{86}:K_{61} (n=5); wild ducks were O_{127}: K_{63} (n=4), O_{26}:K_{60} (n=6) and O_{119}:K_{69} (n=2); little egrets were O_{125}:K_{70} (n=5) and O_{111}:K_{58} (n=2). **Aruji et al., (2004)** identified *E. coli* serotypes O_{77}, O_{118}, O_{30}, O_{114}, O_{144}, O_8, and O_{60} in the examined crows in Japan. In Scotland, recent observations showed high mortality in wild birds of the family Fringillidae (siskins, green finches, chaffinches), as well as sparrows and pheasants **(Pennycott *et al.,* 1998)**. Postmortem analysis of these birds showed that death was due to either *Salmonella enterica* serovar *typhimurium* DT40 or *E. coli* O_{86}:K_{61}. Also, *E. coli* O86:K61 was recovered from selected tissues of 43 out of a total of 46 finches found dead in the Scottish Highlands during April-May of 1994 and 1995 **(Foster *et al.,* 1998).** The authors attributed the isolation of the aforementioned serotype from wild birds due to the provision of supplementary food, such as peanuts, for wild birds in gardens during the winter months. This has been cited as a possible cause of the spread of infection within the wild bird population.

The pathogenic potential of *E. coli* O_{86}:K_{61} strains isolated from the tissues of dead birds in Scotland was studied by **La Ragione *et al.* (2002)**. Their results showed that the potential exists for this pathogen to be zoonotic because the tested strains possess several putative virulence factors associated with human and animal diseases.

Practices that encourage large numbers of birds to congregate in urban areas may increase the zoonotic risk. Suitable precautions should be taken when focally contaminated material is handled. In addition to posing a threat to other wild birds and people, infected populations of wild birds may also act as reservoirs for domestic livestock and companion animals.

Salmonella infections can be transmitted in many ways, and the importance of different modes of transmission varies with the strain of *Salmonella*, behavioral and feeding patterns of the bird species, and husbandry practices when human intervention becomes part of hatching and rearing processes. For wild birds and humans, contaminated foods are the primary source of infection; food and water become contaminated by fecal discharges from various sources. Individual infected birds can excrete *Salmonellae* for prolonged periods of time ranging from weeks to months. Prolonged use of sites by birds and high density of individuals at those sites can result in cycles of salmonellosis within those populations. Persistently contaminated environments result from a small percentage of birds which remain as lifelong carriers that intermittently excrete *Salmonellae* into the environment.

Table, 4 shows *Salmonella* serotypes identified in wild birds examined. Results clarify that 17 *Salmonella* serotypes were identified in 100 crows examined. The identified serotypes were *S. muenster* (3), *S. typhimurium* (5), *S. virchow* (2), *S. anatum* (4) and *S. enteritidis* (3).

On the other hand, *S. typhimurium* (4) and *S. enteritidis* (4) were recorded in the examined little egrets. Moreover, only *S. enteritidis* (5) were isolated from wild ducks. However, *Salmonella* could not be detected in cloacal swabs of Ibis and stool samples of human beings.

S. typhimurium was the predominant *Salmonella* serotypes (19 out of 328) in cloacal swabs of different

species of birds. However, serotypes of *S. muenhen, S. virchow, S. bareily, S. typhimurium and S. bovismorbificans* were isolated from the foot pad of the same species of birds **(Kobayashi et al., 2006)**

(Mohammed, 1986) in Egypt confirmed that *S. enteritidis* and *S. typhimurium* are the most predominant *Salmonella* serovar in both domestic and wild ducks Moreover, in another study in India, *S. typhimurium* was the predominant serovar from ducks. *S. anatum* represents one of the most predominant *Salmonella* serovars isolated from ducks in many countries and had been previously isolated from chicken intestinal contents **(Tran et al., 2004)**. The isolation of this serotype from crows should alert bird handlers to the potential hazard posed by contact with crows.

The pattern of multiple serotypes in crows indicates that infection was acquired from a wide variety of sources, as might be expected from eating prey. Avian carnivores consume both mammals and small birds so it is not clear just what the relative importance of each are **(Tizard, 2004)**.

Although the total numbers of bird samples were not described, 441 of 470 Salmonella isolates from wild birds were *S. typhimurium* in Norway between 1969 and 2000 **(Refsum et al., 2002)**. All 67 *Salmonella* isolates from wild cranes were *S. typhimurium* in Japan **(Homan et al., 2005)**. *S. muenhen* and *S. enteritidis* were found in 7 isolates of *Salmonella* from 82 wild birds in Spain **(Pennycott et al., 1998).** Other than *S. typhimurium*, *S. muenhen* (from a Great cormorant), *S. virchow* (from a feral pigeon), *S. bareily* (from a Black headed gull) were also isolated. These *Salmonellae* might not infect birds but might be carried from farm to farm and other places when the birds migrate.

The general level of *Salmonellae* in most species of wild birds is low, but extra care with personal hygiene is warranted by people who handle these birds or materials soiled by bird feces. This consideration is not limited to situations where the disease is apparent, and it extends to routine maintenance during any contact with birds.

Protein profile analysis is an effective, reliable method to study the epidemiology, diversity, and zoonotic potential of bacterial strains **(Mohamed et al., 2004)**. Moreover, it has discriminating potential in typing and characterization of *E. coli* and *Salmonella* spp. **(Fantinatti et al., 1994** and **Vidotto et al., 1994)**.

Whole cell protein profile of 6 *E. coli* (O26:K60, O111:K58 & O127:K63; 2 isolates each) are shown in **Fig. 1**. The protein profiles were inspected visually & compared with each other. The protein profiles of all *E. coli* isolates exhibited different banding patterns; molecular weight varied in the low molecular weight. A particular high degree of similarity was concentrated in the region between 51-131 KDa (may be species specific). The genetic distance of the strains based on whole cell protein profiles of E. coli isolates was calculated and dendrograms were constructed using the POPGEN statistical program **(Fig. 2)**. The dendrogram shows four clusters, cluster I includes two genotypes (O_{26} from human and O_{26} from wild ducks), cluster II includes two genotypes (O_{111} from little egrets and O_{111} from human), cluster III includes one genotype (O_{127} from wild ducks) and finally cluster IV includes one genotype (O_{127} from human). In case of SDS – PAGE of whole cell protein extract of 7 *Salmonella* spp (*S. typhimurium* 2 isolates; *S. enteritidis* 3 isolates; *S. anatum* 1 isolate and *S. virchow* 1 isolate) produce patterns containing up to 38 discrete bands with molecular masses of 17-129 KDa **(Fig. 3)**. The whole cell protein profiles of *Salmonella* spp are fairly homogenous with some variability in the high molecular mass region. A dendrogram of the protein profiles revealed five clusters **(Fig. 4),** cluster I includes two genotypes (*S. typhimurium* from little egrets and *S. typhimurium* from crow), cluster II includes two genotypes (*S. enteritidis* from little egrets and *S. enteritidis* from crow), cluster III includes one genotype (*S. enteritidis* from wild ducks), cluster IV includes one genotype (*S. virchow* from crow) and cluster V includes one genotype (*S. anatum* from crow). The aforementioned results indicated homogeneity and possible genetic relatedness between the strains in the same cluster. This homogeneity proofs the possibility of transmitting the serotypes of the same cluster between the two sources.

The genetic relationships of *E. coli* O_{18}:K_1:H_7 isolates of human and avian origins was previously studied by **Moulin-Schouleur et al. (2006)** by Pulsed Field Gel Electrophoresis (PFGE). The authors reported that all but one of the avian and human isolates belonged to major phylogenetic group B2. These results demonstrate that very closely related clones can be recovered from extra intestinal infections in humans and chickens and suggest that avian pathogenic *E. coli* isolates of serotype O_{18}:K_1:H_7 are potential human pathogens.

Previously published studies showed also the homogeneity of *Salmonella* strains that indicated the existence of inter-relation between the strains from different sources. For instance, **Suh and Song (2006)** reported data that revealed a highly genetic homogeneity between *S. enteritidis* isolates from human and chicken except one isolate, which originated from chicken and showed a different DNA band pattern from others. Moreover, in Iran, **Morshed and Peighambari (2010)**

reported that *S. enteritidis* isolates from poultry-related sources were closely related to human *S. enteritidis* isolates.

In order to effectively decrease the risk of infections associated with wild birds, the public health and animal health sectors must collaborate in developing strategies to decrease human exposure to pathogens carried by wild birds. An in-depth comprehension of avian migration routes as well as further research using advanced molecular testing of the prevalence, pathogenesis, and clinical associations of several pathogens that are transmitted to humans from the various migratory bird species would lead to a better understanding of the transmission dynamics of diseases carried by avian species helping to predict future outbreaks of relevant human infections. The transfer of zoonotic bacterial pathogens from birds to human, human to human, and bird to bird represents risk for human & wild birds health that can largely be prevented considering that many enteric bacteria are spread primarily via the faecal oral route **(Flammer, 1999)**. The transfer of enteric bacteria can effectively be reduced with proper hygiene, husbandry, and disinfection. The efficacy of simple measures, such as hand washing is well documented **(Pittet et al., 2000)**. The current study recommended future studies on the role of other wild bird spp. in transmitting some bacterial agents posing zoonotic hazards, and also further studies are recommended toward studying virulence genes of *E. coli* and *Salmonella* isolates.

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Corresponding author
Magda A. M. Amin
Department of Zoonoses, Fac. Vet. Med., Zag. Univ. Egypt
magda_amin_2013@yahoo.com.

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