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Prevalence of Zoonotic *Escherichia coli* and *Salmonellae* in Wild Birds and Humans in Egypt with Emphasis on RAPD-PCR Fingerprinting of *E. coli*

Maysa A. Awadallah, Abdallah M. Merwad and Rehab E. Mohamed

Department of Zoonoses, Faculty of Veterinary Medicine,
Zagazig University, Zagazig,44511, Egypt

Abstract: This study investigated the prevalence of *Escherichia coli (E. coli*)and salmonellae in 400 cloacal swabs of wild birds including cattle egrets, doves, sparrows and quails (100, each);and 150 stool samples of diarrheic and non-diarrheic humans (75, each), in Sharkia Province, Egypt. The prevalence of *E. coli* and salmonellae showed no significant differences among examined wild birds (P>0.05). There was a significant difference for *E. coli* incidence in diarrheic and non-diarrheic humans (P<0.05); while for *Salmonella* spp. no significant variation was found (P>0.05). Twenty one isolates of *E. coli* were serotyped from wild birds and human. Six *Salmonella* isolates from wild birds were serotyped into *Salmonella enteritidis(S. enteritidis),* S. *typhimurium*, *S. haifa*, *S. chester* and *S. muenster*, while those two isolates of human were identified into *S. typhimurium* and *S. entertidis*. Eight *E. coli* serotypes; belonged to O127: K63, O128:K67and O26:K60 strains from wild birds and human; were subjected to RAPD-PCR. A maximum similarity (66.7%) was found between O127:K63 from quails and O26:K60 of sparrow origin and the two isolates from doves (O26:K60 and O127:K63). A higher similarity (62.5%) was observed between O128:K67 strain from human and O26:K60 from doves. This evidenced the zoonotic transmission of *E. coli* strains and salmonellae from wild birds.

Key words: Wild birds · Enterobactericeae · Zoonoses · Genetic diversity

INTRODUCTION

Wild and migratory birds generally cross one or more national boundaries and use various habitats including marshes, grain stores, pasture and other water bodies [1]. This will create novel foci of emerging or reemerging bacterial diseases that posing public health hazards, along bird migration routes [2]. These wild birds including crows and sparrows could acquire salmonellae and *Escherichia coli* [3,4], respectively; by feeding on raw sewage and garbage and may spread these agents to humans directly or by contaminating the commercial poultry operations.

However, cattle egrets play a major role in the natural selection process and in controlling the agriculture and domestic life enemies such as rodent, mollusks and arthropods. Their close contact with farmers may transmit enteric bacterial pathogens including *E. coli*, salmonellae and other viral agents that threaten both poultry industry and public health [5]. The quail winters in African lands; and then migrates northwards first to the lands of North Africa and Egypt during the months of March and April. The common custom in Egypt was to catch these quails in nets and then consumed as a cheapest protein source that may cause food poisoning [6].

The role of wild birds as reservoir hosts for some zoonotic pathogens within family *Enterobactericeae* were previously investigated in many studies: Refusm *et al*. [7] in Norway; Aruji *et al*. [8] and Kobayashi *et al*. [9] in Japan; Yong *et al*. [10] in Malaysia; Phalen *et al*. [11] in USA ; Ahmed *et al*. [12] in Baghdad ; and El-Sheshtawy and Moursi [13], Medani *et al*. [14], Hedawy and El-Shorbagy [15], Enany *et al*. [16] and Maha *et al*. [17] in Egypt.

The molecular differentiation of different *E. coli* strains could give guidance for epidemiological studies of sources of infection and disease transmission. A random amplified polymorphic DNA polymerase chain reaction (RAPD-PCR) is quicker and more effective procedure to

Corresponding Author: Abdallah M. Merwad, Department of Zoonoses, Faculty of Veterinary Medicine, Zagazig University, Zagazig, 44511, Egypt Tel: +20 0552555876, Fax: +20 0552284283, Mob +20 01008079363.

differentiate variant isolates of *E. coli* [18]. The distinctive DNA patterns generated by RAPD for each *E. coli* isolate reflects genetic diversity present in a bird species [19].

The objectives of this study were to investigate prevalence of *E. coli* and *Salmonella* spp. in wild birds & human; and also to trace the epidemiological relationship among *E. coli* strains isolated from different wild birds and humans using RAPD-PCR fingerprinting.

MATERIALS AND METHODS

Sample Collection and Prepration: Cloacal swabs were collected from 400 wild birds (one hundred from each bird species) including; cattle egrets, sparrows, doves and quails from different localities in Sharkia Province, Egypt from January to December, 2012. Also, diarrheic and non-diarrheic humans, who inhabit the surrounding environment of wild birds, were asked to obtain stool samples. One hundred and fifty stool samples were collected from diarrheic and non-diarrheic (75, each). All samples were inoculated into a sterile buffered peptone water and transferred to the laboratory with a minimum time of delay.

Isolation of *Escherichia coli*: The inoculated cloacal and stool swabs, into buffered peptone water (BPW), were incubated at 37°C for 18-24 hrs. One ml from the pre-enriched sample in BPW was transferred to 5 ml MacConkey broth and incubated at 37°C for 18-24 hrs. A loopful from the enrichment broth was streaked directly onto Eosin methylene blue (EMB) agar then incubated at 37°C for 18-24 hrs. The metallic shiny colonies from each plate were picked up and purified on nutrient agar plate and incubated at 37°C for 24-48 hrs.

Isolation of Salmonellae: From the pre-enriched sample in BPW, 0.1 ml was transferred to 9.9 ml Rappaport Vassiliadis enrichment broth and incubated at 40°C for 24 hrs. A loopful from the enriched cultured broth was streaked onto Xylose lysine deoxycholate (XLD) agar and incubated at 37°C for 18-24 hrs. Red colonies with black centers were picked up and purified by streaking onto nutrient agar plates, then incubated at 37°C for 18-24 hrs. All purified colonies were streaked onto nutrient agar slants and incubated at 37°C for 18-24 hrs for biochemical identification [20].

Serological Identification: Twenty one *E. coli* isolates, including 14 isolates from wild birds and 7 strains from human, were serologically identified using rapid diagnostic *E. coli* antisera sets (DIFCO Laboratories, Detroit Michigan 48232-7058, USA) according to Kok *et al*. [21]. While, eight *Salmonella* isolates (6 from wild birds and 2 from human) were identified according to Kauffman white scheme [22] using rapid diagnostic *Salmonella* antisera sets (Welcome Diagnostic, a Division of the Welcome Foundation Limited, Dartford England DA15 AH).

Table 1: Oligonucleotide sequence of selected primers.

No.	Primer sequence	Size
1	5'-TCC CAG CAGT- 3'	10-mer
2	5' -GTC GTC GTCT- 3'	10-mer
3	5' -ACG GGA CCTG-3'	10-mer
4	5'-GTT AGT GCGG- 3'	10-mer
5	5'-AAG AGC CCGT- 3'	10-mer

Genomic DNA Isolation: Genomic DNA of eight *E. coli* serotypes (O127: K63, O128: K67& O26:K60) from quails, doves, sparrows and human was extracted using QIAamp DNA Mini QIAcube Kit according to the manufacturer´s instructions. The primers and their nucleotide sequences (Alpha DNA, Canada) were listed (Table 1). The Sequences of primers were selected according to Gomes *et al*. [19] and Schmidt *et al*. [23].

RAPD-PCR of *Escherichia coli*: The amplification reactions were carried out according to Gomes *et al*. [19] with minor modifications. Ten µl of DNA template was initially denatured at 95°C for 5 min. A final volume of PCR reaction (40 µl) included 25 µl of PCR master mix, 100 pmol of each primer and completed by deionized water. Temperature cycling was programmed as follows: 1) 94°C for 30 sec, 35°C for 1 min and 72°C for 5 min for 40 cycles; 2) 72°C for 10 min and 3) 10°C for 3 min. The amplicons were analyzed on agarose gel consisted of 2% agarose and 5µL of ethidium bromide in 1X Tris-Acetate EDTA buffer. The amplified products were electrophoresed at 100 volts for 1 hr.; then were visualized under ultraviolet transilluminator and photographed.

Analysis of RAPD Data and Dendrogramming: The data was analyzed using Molecular Evolutionary Genetics Analysis Version 5 (MEGA 5) software, obtaining joint clusters dendrograph.

Statistical Analysis: The Chi square test was analyzed with SPSS software version 20 (SPSS for Windows, SPSS Inc. Chicago, USA).

RESULTS

As shown in Table 2, *E. coli* and salmonellae were isolated in the rate of 48 % and 10.75%, respectively. The prevalence rates of *E. coli* and salmonellae revealed no significant differences between investigated wild bird species (P>0.05). However, the prevalence of *E. coli* showed a significant difference in diarrheic and non - diarrheic humans (P<0.05) (Table 3). For the incidence rate of salmonellae, there was no significant difference in diarrheic and non-diarrheic humans (P>0.05). The serotypes of *E. coli* and *Salmonella* spp. from wild birds and humans were illustrated in Tables (4&5).

Table 2: Prevalence of *E. coli* and *Salmonella* spp. in wild birds collected from Sharkia, Egypt.

	Bacterial Isolates	
Wild birds (100 cloacal swabs /each bird species)	*E. coli* No. (%)*(Total/ bird= 100)	*Salmonella spp.* No. (%)
Quails	47[!]	9
Doves	49[!]	13
Sparrows	45[!]	13
Cattle egrets	51[!]	8
Total (400)	192 (48)	43 (10.75)

*: The number and percentage of each infected bird species has the same value.

!: There were no significant differences in prevalence of *E. coli* and salmonellae among examined wild birds (P>0.05).

Table 3: Prevalence of *E. coli* and *Salmonella* spp. in diarrheic and non-diarrheic humans inhabiting surrounding environment of wild birds.

	Diarrheic (75)		Non - Diarrheic (75)		Total (150)	
Bacterial Isolates	No. Positive	%	No. positive	%	No. positive	%
E. coli	48[*]	64	36[*]	48	84	56
Salmonella spp.	7[!]	9.3	6[!]	8	13	8.7

*: There was a significant difference for prevalence of *E. coli* among diarrheic and non-diarrheic humans (P<0.05).

!: There was no significant variation for prevalence of salmonellae in diarrheic and non-diarrheic groups (P>0.05).

Table 4: Serotyping of twenty one E. *coli* isolates from wild birds and human.

	Sources of E. coli Serotypes								
	Wild bird species					Human			
	Quails	Doves	Sparrows	Cattle egrets		Diarrhoeic	Non-diarrhoeic		
E. coli Serotypes	No.	No.	No.	No.	Total	No.	No.	Total	Total
O26:K60	1	2	1	-	4	-	-	-	4
O55:K59	-	-	1	-	1	-	-	-	1
O78:K80	-	-	-	-	-	1	-	1	1
O86:K61	1	-	-	-	1	-	-	-	1
O111:K58	-	-	1	-	1	1	-	1	2
O114:K90	1	-	-	-	1	-	-	-	1
O119:K69	-	-	-	2	2	-	-	-	2
O125:K70	-	1	-	-	1	-	1	1	2
O126:K71	-	-	-	-	-	1	-	1	1
O127:K63	1	1	-	-	2	-	1	1	3
O128:K67	-	-	1	-	1	1	-	1	2
Un typable	-	-	-	-	-	-	1	1	1
Total	4	4	4	2	14	4	3	7	21

Table 5: Serotyping and antigenic formula of eight identified *Salmonella* isolates from wild birds and humans.

		Sources of *Salmonella* Serotypes						
		Wild bird species				Human		
Salmonella Serotypes	Antigenic formula	Quails No.	Doves No.	Sparrows No.	Cattle egrets No.	Diarrheic No.	Non-diarrheic No.	Total
S. entertidis	O1,9,12, H g,m : 1,7	-	-	2	-	-	1	3
S.typhimurium	O1,4,5,12, H i : 1,2	-	1	-	-	1	-	2
S. Haifa	O1,4,5,12, H Z_{10} : 1,2	-	-	-	1	-	-	1
S. chester	O1,4,12, H e,h : e,n,x	-	1	-	-	-	-	1
S. muenster	O3,10,15,34, H e,h : 1,5	1	-	-	-	-	-	1
Total		1	2	2	1	1	1	8

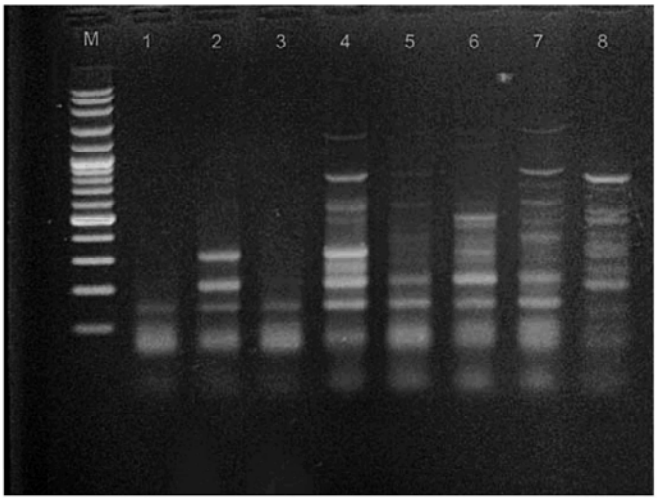

Fig. 1: RAPD-PCR profile of eight *E. coli* serotypes from wild birds and humans. M: Marker 100 base pair; lane1: O127:K63 (quail); lane2: O26:K60 (sparrow); lane3: O26:K60 (quail); lane4: O127:K63 (dove); lane5: O127:K63 (human); lane6: O26:K60 (dove); lane7: O128:K67 (human); lane8: O128:K67 (sparrow).

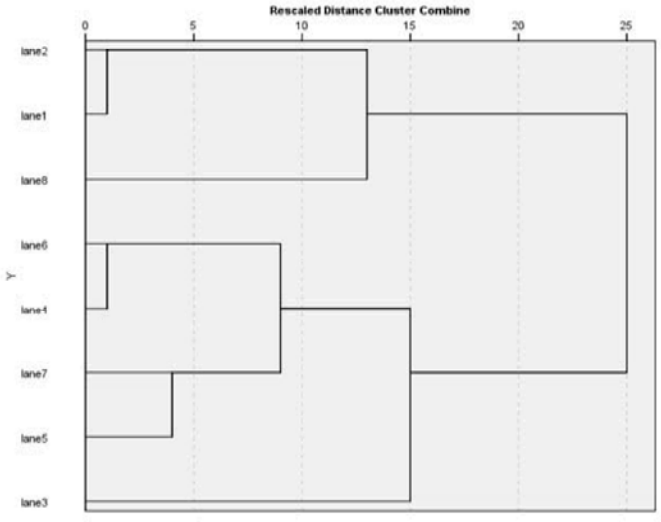

Fig. 2: Dendrogram for RAPD-PCR analysis of eight *E. coli* serotypes isolated from wild birds and human. lane1: O127:K63 (quail); lane2: O26:K60 (sparrow); lane3: O26:K60 (quail); lane4: O127:K63 (dove); lane5: O127:K63 (human); lane6: O26:K60 (dove); lane7: O128:K67 (human); lane8: O128:K67 (sparrow).

Concerning RAPD-PCR fingerprinting, analysis of eight *E. coli* serotypes from wild birds and human using five RAPD primers yielded DNA profiles with number of DNA bands between 2 and 10 bands with molecular weight ranged from 50 to 1220 bp. (Fig. 1). Joint cluster dendrogram was generated from diverse RAPD patterns of *E. coli* serotypes (Fig. 2). Also, the analysis of RAPD patterns showed a maximum similarity of 66.7% between O127:K63 isolate from quails and O26:K60 isolate from sparrows, also between O26:K60 and O127:K63 isolates from doves. The highest percentage (62.5%) of similarity were observed between human strain (O128:K67) and dove strain (O26:K60). On the other hand the similarity coefficient was 0% between O128:K67 strain from human and O127:K63 isolate of quail origin.

DISCUSSION

Among bacterial pathogens transmitted by wild birds, *Enterobacteriaceae* especially *E. coli* and *Salmonella* spp. are the most potential pathogens causing food poisoning and posing a zoonotic hazard. In Table 2, there were no significant differences in prevalence of *E. coli* and *Salmonella* spp. among studied wild birds (P>0.05). The overall prevalence of *E. coli* was 48% in examined wild birds (Table 2). Nearly similar finding (38%) was previously recorded by Rogers [24] in California. Otherwise, Brittingham *et al*. [25] and Hedawy and El-Shorbagy [15] reported lower *E. coli* infection rates of 1 and 18.7% in free living birds, respectively. The variation in *E. coli* prevalence rates may be attributed to the species of wild bird examined, localities and bird feeding habits.

With respect to the overall incidence of *Salmonella* spp. (10.75%) in wild birds (Table 2), concordant percentage of 10.6% was obtained by Craven *et al*. [26]. On the other hand, in previous reports of Kobayashi *et al*. [9] and Vlahovic *et al*. [27], wild birds showed lower infection rates (5.8 and 7.4%) for salmonellae, respectively. Compared with other studies, higher prevalence of *Salmonella* spp. and *E. coli* in the current study may be associated with consumption of wild birds for polluted water as a result of leakage of human sewage into water canals. This was previously supported by Fricker [28], who stated that the ready availability of human waste disposal sites increases the frequency distribution of enteric bacteria in migratory birds.

The prevalence rate of *E. coli* in quails(47%), Medani *et al*. [14] recorded higher incidence (51%) of *E. coli* in quails Otherwise, lower percentages of 18.7 and 11.8% were cited by El-Sheshtawy and Moursi [13] and Hedawy and Wassel [29], respectively. Also, infection rate of salmonellae (9%) in quails was nearly similar to previous finding of Hedawy and Wassel [29]. Also, *E. coli* incidence rate (49%) in doves (Table 2) contrasted the findings of Enany *et al*. [16] and El-Sheshtawy and Moursi [13]. The prevalence rate of *Salmonella* spp. in doves in this study was in accordance with results of Vlahovic *et al*. [27] and Enany *et al*. [16] who detected *Salmonella* spp.with percentages of 14.3 and 18%, respectively. However, high infection rate (95%) was recorded in Baghdad [12]. Lower prevalence rates of salmonellae in doves were found to be 0.2% in Norway [7] and 8.3% in Egypt [13].

In Egypt, sparrows are considered as potential sources for environmental contamination with pathogenic bacteria such as *E. coli* and *Salmonella* spp. referred to their propensity to nest and roost near human activity such as dairy and poultry farms [30]. In Table 2, sparrows showed the higher *E. coli* prevalence compared with reports of El-Sheshtawy and Moursi [13] in Egypt (17.5%) and Vilela *et al*. [31] in Brazil (13.2%). Concerning incidence rate of salmonellae in sparrows in this study, higher infection rates of 100 % were cited by Ahmed *et al*. [12] and 22.5% by El-Sheshtawy and Moursi [13]. On the contrary, lower prevalence rates of 2, 2.1, 5.7 and 0.04% were reported by Shahata *et al*. [30], Refusm *et al*. [7], Kobayashi *et al*. [9] and Vilela *et al.* [31]; respectively. The higher prevalence of *E. coli* in sparrows in current study may result from the urban habits of those birds which are usually found feeding on grains in feed storage facilities and garbage dumps as was previously supported [31].

Salmonellosis in cattle egrets cause morbidity and mortality and have a potential public health threat [11]. Comparing the prevalence rates of *E. coli* and *Salmonella* spp. in cattle egrets in this study, Maha *et al*. [17] isolated *E. coli* and *Salmonella* spp. with similar percentages of 43.6 and 8%, respectively at Sharkia province, Egypt. Lower incidence of salmonellae (5%) in cattle egrets was reported [13]. Otherwise, higher prevalence rate (53.3%) was cited [11]. These results verified that wild birds may be regarded as true reservoirs in transmission of zoonotic *E. coli* and salmonellae due to their indirect contact with human habitations [12]. Thereby, fecal droppings of

wild birds have the potential to be heavily contaminated with some pathogenic bacteria that have a zoonotic hazard.

In Table 3, the study revealed a significant difference for *E. coli* prevalence in diarrheic and non diarrheic humans ($P<0.05$); while no significant variation was detected for salmonellae ($P>0.05$). With regard to the prevalence rate of *E. coli* (56%) in humans inhabiting the same surrounding environment of wild birds (Table 3), lower percentage (6%) was recorded [32]. So, the variation in the prevalence rate of *E. coli* from one study to another may be accounted for differences in number and health status of human cases, localities and hygienic measures. Moreover, the prevalence rate of *Salmonella* spp. (8.7%) in human in the present study disagreed with a finding of Soad and Wafaa [33] who cited a higher incidence (18%). However, lower infection rate of *Salmonella* (2%) was recorded in Egypt [34]. In Table 3, the infection rate of *E. coli* in non-diarrheic humans contradicted a higher prevalence (87%) cited by Rasha [34].

The incidence of salmonellae in diarrheic humans (Table 3) contrasted a lower percentage of 1.2% [34]. Comparing prevalence of *Salmonella* spp. (8%) in non-diarrheic humans in current study, lower percentage (2%) was reported [34].

Regarding to the twenty one *E. coli* serotypes from wild birds and humans (Table 4), the serotype *E. coli* O86 was identified from wild birds[33]. Otherwise, El-Sheshtawy and Moursi [13] isolated *E. coli* belonged to O2, O18, O45 and O78 serotypes from wild birds at Ismailia Province, Egypt. While, *Salmonella* isolates from wild birds were identified into *S. entertidis, S. typhimurium, S. haifa, S. chester* and *S. muenster* (Table 5). *Salmonella typhimurium* was identified from wild birds in Norway between 1969 and 2000 [7]. From zoonotic point of view, 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 droppings. Also, isolation of *S. typhimurium and S. entertidis* from human in this study was in accordance with a previous report in Israel [35].

Eight *E. coli* serotypes from sparrows, quails, doves and humans were subjected to RAPD-PCR finger printing. In this study, the DNA profiles using five RAPD primers (Fig. 1) was nearly similar to the finding reported by Salehi *et al*. [36] and Kilic *et al*. [37] whose results of RAPD-PCR of *E. coli* isolates, revealed 0-11 bands and 9 bands; respectively.

Also, It is interesting from this study (Fig. 2), the analysis of RAPD patterns using joint cluster dendrogram showed a higher similarity between *E. coli* serotype O128:K67 from human and the serotype *E. coli* O26:K60 from dove.

CONCLUSION

Wild birds are important reservoirs for dissemination of *E. coli, S. typhimurium* and *S. entertidis*. Also, the higher genetic similarity between two *E. coli* serotypes belonged to human O128:K67 isolate and dove O26:K60 strain supported an evidence of interspecies transmission of zoonotic *E. coli*.

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