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Monitoring the antimicrobial resistance of Salmonella and *Escherichia coli* in chickens and migratory birds

By
Nehal, M. Nabil*, Reem, M. Reda*, Maram, M. Tawakol*
*Reference Laboratory for Veterinary Quality Control on Poultry Production, Animal Health Research Institute, Agricultural Research Center, Nadi El-Seid Street, Dokki, Giza 12618, Egypt.

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ABSTRACT

Antimicrobial resistance is considered as one of the most important issue that has a negative impact on poultry industry worldwide as well as of public health concerns. In this study, antimicrobial resistance pattern of *Salmonella* species and *Escherichia coli,* isolated from broiler chickens and migratory birds in Egypt, was investigated. A total of 250 diseased broiler chickens and 100 migratory birds (five bird species) were collected. All collected samples were then subjected to *Salmonella* and *E. coli* isolation, identification and antimicrobial susceptibility pattern. *Salmonella* and *E. coli* were isolated from broiler chickens with an incidence of (14%) and (40%) respectively, and from migratory birds with an incidence of (10%) and (30%) respectively. Based on serotyping, three serotypes were detected from broiler chickens (*S. typhimurium*, *S.kentucky* and *S. enteritidis*) and four Salmonella serotypes from migratory birds (*S. typhimurium*, *S.* kentucky, *S. infantis* and *S. enteritidis*). Twelve *E. coli* serotypes found in samples collected from broiler chickens (O1, O6, O55, O86, O91, O124, O125, O128, O144, O158, O159 and O166) while *E. coli* serotypes obtained from migratory birds, were found as: O1, O2, O26, O55, O78, O91, O113, O121, O124, O 128, O146 and O158.Overall,the antimicrobial susceptibility testing of isolated *Salmonella* and *E. coli* from broiler chickens and migratory birds showed resistance to most of the used antimicrobial agents; *Salmonella* isolates obtained from broiler chicken farms were found totally resistant to amoxicillin, ampicillin-sulbactam, and tetracycline. *E. coli* isolates obtained from broiler chicken farms showed resistance ranged from 25% - 95%. *Salmonella* and *E. coli* isolates retrieved from migratory birds showed resistance which ranged from 60% - 100% and 66.7 % - 96.7% respectively. This study highlights the spread of antimicrobial resistance *Salmonella* and *E. coli* in both domestic chicken and migratory birds. Hence, strict biosecurity measurements and continuous monitoring of antimicrobial resistance among poultry population should be implemented in addition to avoid the misuse of antimicrobial agents.

Corresponding Author: Nehal, M. Nabil, Reference Laboratory for Veterinary Quality Control on Poultry Production, Animal Health Research Institute, Agricultural Research Center, Nadi El-Seid Street, Dokki, Giza 12618, Egypt
E-mail address:
DOI:

INTRODUCTION

Avian salmonellosis and colibacillosis are on the top list of most commonly detected bacterial diseases in poultry all over the globe **(Ibrahim et al. 2021 Lutful Kabir 2010 and Van Boeckel et al. 2019)** and caused by pathogenic *Salmonella* species and *E. coli* (**Ejeh et al. 2017**). On the other hand, *Salmonella* is a another important bacterial pathogenposinga negative impaction poultry production due to high mortality rates in young birds and chronic carriers in adult birds (**Soliman et al. 2018).** *E. coli* is a Gram-negative bacterium and acts as normal inhabitant in the intestinal tract with the exception of few strains which harbor virulence genes and have the ability to cause diarrhea (**El-Demerdash et al. 2021)** and colibacillosis (**Jahantigh and Dizaji (2015)** inbroiler, commercial layers and breeders (**Koutsianos et al. 2021).** Those strains usually associated with high morbidity and mortality in poultry species (**Roseliza et al. 2016**).

The resistance of poultry pathogens to antimicrobial agents has shown to result in treatment failure producing economic losses (**Nhung et al. 2017**). Several strains of Salmonella and E. *coli* were described previously with multiple resistances to antibiotics (**Ejeh et al. 2017**).

The emergence of antimicrobial resistance of *E. coli* has been described previously mainly due to the misuse of antimicrobial agents (**Ibrahim et al. 2019**). In addition to the negative impact on poultry, antimicrobial resistance threatens the public health via zoonotic strains.

Away from domestic bird species, migratory birds are considered as potential source of pathogenic microorganisms spread either biologically or mechanically (**Benskin et al. 2009)**. Migratory birds have shown an important role in the ecology and circulation of different bacterial pathogens for example: *Salmonella enterica, Campylobacter jejuni, Pasteurella multocida* and *Mycobacterium avium* **(Hubálek 2004)**. Migratory birds can transmit antimicrobial resistant bacteria to domestic birds and then to humans, as well as from one country to another (**Elsohaby et al. 2021 Islam et al. 2021[a]**). (**Bonnedahl and Järhult 2014**). Therefore, this study aims to investigate the present status of antimicrobial resistance of *Salmonella* and *E. coli* among both domestic poultry (chickens) and migratory bird species in Egypt. Here we applied different methods commonly used in official antimicrobial resistance monitoring programs. This in turn will increase our knowledge in understanding the resistance profile in different migratory birds: Common teal (Anas crecca) Northern pintail (Anas Acuta), Northern Northern shoveler (Spatula clypeata), Common pochard (Aythyaferina) and Mallard ducks (Anas Platyrhynchos)) obtained from live bird markets (LBMs) in Egypt.

MATERIAL and METHODS

2.1. Sampling

A total of 250 diseased broiler chickens (30 to 35 days of age), showing diarrhea, inability to move, ruffled feather with higher mortality rates, were collected from Dakahlia Governorate, Egypt. Five individual chickens were collected from each farm and subjected to postmortem examinations. Liver and spleen were collected for isolation of *Salmonella* and *E. coli* isolation meanwhile cecum tissues were collected for Salmonella isolation only. All samples were obtained aseptically and shipped directly to the Reference Laboratory for Veterinary Quality Control on Poultry Production (RLQP), Animal Health Research Institute (AHRI) for further laboratory examinations. In addition, a total of 100 cloacal swabs were collected from five species of 100 migratory birds from live bird markets (LBMs) in Dakahlia and Damietta Governorates (60 birds from Dakahlia and 40 birds from Damietta) **(Table 1).** Swab samples were collected on non-selective media (Buffered Peptone Water) for *Salmonella* and *E. coli* isolation and identification.

Table (1) Number and bird species of the included migratory birds in this study.

Migratory bird Species	No. of birds	No. of birds from each Governorate
Common teal (*Anas crecca*)	12	Dakahlia (8)- Damietta (4)
Northern pintail (*Anas Acuta*)	21	Dakahlia (11)- Damietta (10)
Northern Northern shoveler (*Spatula clypeata*)	9	Dakahlia (4)- Damietta (5)
Common pochard (*Aythyaferina*)	20	Dakahlia (10)- Damietta (10)
Mallard ducks (*Anas Platyrhynchos*)	38	Dakahlia (27)- Damietta (11)

2.2. Bacteriological examinations

The obtained tissue samples and cloacal swabs were subjected to Salmonella isolation according to **ISO 6579 (2017)** and serotyped according to Kauffman- white scheme **(Kauffman, 1974)** to detect somatic (O) and flagellar (H) antigens **(Cruickshank et al. 1975) and (WHO 2007).** Also the collected tissue samples and cloacal swabs were subjected to *E. coli* isolation and identification as described previously by **Edward and Ewing (1986),** and further serotyped according to **Kok et al. (1996).**

2.3. Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed using agar disc diffusion method on Muller Hinton agar plates as described elsewhere **Finegold and Martin (1982)**. Ciprofloxacin (5µg), Ampicillin - sulbactam (20µg), tetracycline (30µg), amoxicillin (25 µg), erythromycin (15 µg) and doxycycline (30µg) were used. The inhibition zones diameters were measured and assessed according to the manufactures´ instructions (Oxoid), and then were categorized into susceptible or resistant according to the CLSI guidelines (**CLSI 2016**).

3. RESULTS

3.1. Incidence of *Salmonella* and *E. coli* isolation, identification and serotyping in broiler chickens

Salmonella and *E. coli* were isolated from the randomly collected chickens with an incidence of (14%) and (40%) respectively (**table 2**).

The serotyping of the isolated Salmonella and *E. coli* revealed the detection of three Salmonella serovars (*S. typhimurium*, *S.kentucky* and *S. enteritidis*) and 12 *E. coli* serogroup (O1, O6, O55, O86, O91, O124, O125, O128, O144, O158, O159 and O166) (**Table 2**).

Table (2) number and serotyping of the isolated *Salmonella* and *E. coli* isolated from 250 broiler chickens

Salmonella		*E. coil*	
No. of isolates	Serovar	No. of isolates	Serogroup
35/250 (14%)	*S.* Typhimurium (20) *S.* Kentucky (10) *S.* Enteritidis (5)	100/250 (40%)	O6 (5)- O55 (10) O86 (10)- O91 (15) O125 (15)- O128 (5) O166 (5) O1 (15)- O124 (5)- O144 (5)- O158 (5) O159 (5)

2. Incidence of *Salmonella* and *E. coli* isolation, identification and serotyping in migratory birds

Among 100 tested migratory birds, Ten *Salmonella* isolates were reported; 7 isolates from examined birds in Dakahlia governorate and 3 isolates from Damietta governorate. The serotyping revealed the detection of 4 Salmonella serovar; *S. typhimurium*, *S. kentucky*, *S. infantis* and *S. enteritidis*. Thirty *E. coli* isolates were reported; 12 isolates from examined birds in Dakahlia governorate and 18 isolates from

Damietta governorate (Table 2). The serotyping revealed the detection of 12 serogroups; O1, O2, O26, O55, O78, O91, O113, O121, O124, O 128, O146 and O158 (**Table 3**).

Table (3) Number and serotyping of the isolated Salmonella and *E. coli* isolated from migratory birds

Bird spp.	Total no. and site of birds collection		Salmonella		*E. coil*	
			No. of isolates (10)	serovar	No. of Isolates (30)	serogroup
Common teal	12	8 (DK*)	0	-	3	O78 (2)-O128 (1)
		4 (DM**)	0	-	4	O1 (1)- O26 (1)- O124 (1) O146 (1)
Northern pintail	21	11(DK)	4	*S. typhimurium* (1) *S. kentucky* (1) *S. infantis* (1) *S. enteritidis* (1)	3	O121 (1)- O91 (1)- O55 (1)
		10 (DM)	1	*S. enteritidis* (1)	5	O1 (1)- O2 (1)- O113 (1) O91 (1) O158 (1)
Northern shoveler	9	4 (DK)	0	-	2	O128 (1)- O121 (1)
		5 (DM)	0	-	2	O2 (2)
Common pochard	20	10 (DK)	1	*S. enteritidis* (1)	1	O55 (1)
		10 (DM)	1	*S. infantis* (1)	3	O2 (1)- O78 (1)- O158 (1)
Mallard ducks	38	27 (DK)	2	*S. kentucky* (2)	3	O91 (1)- O128 (1)- O1 (1)
		11(DM)	1	*S. typhimurium* (1)	4	O1 (1)- O78 (1)- O146 (1) O158 (1)
Total		100	10/100 (10%)	-	30/100 (30%)	-

*=Dakahlia Governorate, **= Damietta Governorate

Overall, *S. typhimurium*, *S. kentucky* and *S. enteritidis* were isolated from both migratory birds and chickens in broiler farms meanwhile *S. infantis* was isolated only from migratory birds. Six *E. coli* serogroup (O1, O55, O91, O124, O128 and O158) were commonly isolated from both migratory birds and broiler farms.

3.3. Antimicrobial Susceptibility pattern of *Salmonella* and *E.coli* in migratory birds and chicken

Salmonella isolates obtained from broiler chicken were found totally resistant to amoxicillin, ampicillin-sulbactam and tetracycline. *E. coli* isolates obtained from broiler chicken farms showed resistance ranged from 25% - 95% (**Table 4**). *Salmonella* and *E. coli* isolates retrieved from migratory birds showed resistance which ranged from 60% - 100% and 66.7 % - 96.7% respectively. (**Table 5**).

Table (4) Antimicrobial Susceptibility pattern of Salmonella and *E.coli* isolated from broiler chickens

Isolates / Antimicrobial agent	Salmonella isolates (35)		*E. coli* isolates (100)	
	Sensitive	Resistant	Sensitive	Resistant
Amoxicillin	0 /35	35 /35	5/ 100 (5%)	95/ 100 (95%)
Ampicillin-sulbactam	0 /35	35 /35	30/ 100 (30%)	70/ 100 (70%)
Tetracycline	0 /35	35 /35	20/ 100(20%)	80/ 100 (80%)
Ciprofloxacin	20 /35	15 /35	75/ 100 (75%)	25/ 100 (25 %)
Doxycycline	30 /35	5/35	35/ 100 (35 %)	65/ 100 (65%)
Erythromycin	25 /35	10 /35	55/ 100 (55 %)	45/ 100 (45%)

Table (5) Antimicrobial Susceptibility pattern of *Salmonella* and *E.coli* isolated from migratory birds.

Isolates	Salmonella isolates (10)		*E. coli* isolates (30)	
Antimicrobial agent	Sensitive	Resistant	Sensitive	Resistant
Amoxicillin	3/ 10 (30%)*	7/ 10 (70%)	3/ 30 (10%)	27/ 30 (90%)
Ampicillin-sulbactam	4/ 10 (40%)	6/ 10 (60%)	6/ 30 (20%)	24/ 30 (80%)
Tetracycline	2/ 10 (20%)	8/ 10 (80%)	1/ 30 (3.33%)	29/ 30 (96.7%)
Ciprofloxacin	4/ 10 (40%)	6/10 (60%)	10/ 30 (33.3%)	20/ 30 (66.7 %)
Doxycycline	4/ 10 (40%)	6/ 10 (60%)	6/ 30 (20 %)	24/ 30 (80%)
Erythromycin	3/ 10 (30%)	7/ 10 (70%)	5/ 30 (16.7 %)	25/ 30 (83.3%)

DISCUSSION

Avian salmonellosis and colibacillosis are widely spread food-borne disease affecting birds and threaten poultry industry worldwide via their negative impact on meat and egg production as well as associated mortality**(Ibrahim et al. 2021) and (Lutful Kabir 2010).** Over the last decades, from 2000 to 2018, the proportion of antimicrobial resistance in chickens has been increased from 0.15 to 0.41 (**Van Boeckel et al. 2019**). Migratory birds have shown a clear important role in the interspecies transmission and intercontinental dissemination of antimicrobial resistant *Salmonella* (**Wei et al. 2020)** and act as reservoirs of antimicrobial resistant *E. coli* (**Islam et al. 2021**[b]).

In the present study, *Salmonella* was isolated from 250 broiler chickens with (14%). The findings in the current study agreed to some extent with **Diarrassouba et al. (2007) Adam et al. (2018) and Limawongpranee et al.(1999)** who isolated *Salmonella* from chickens with (11.2%), (11.6%) and (14.3%) respectively. Previous studies conducted by **Lebert et al. (2018) and Abd El-Ghany et al. (2012)** isolated Salmonella from chickens in Ontario with (0.3%)and from Egypt with (7.03%) respectively and these percentages were lower than this study. Three Salmonella serotypes were reported from chicken farms in this study; (*S. typhimurium*, *S.kentucky* and *S. enteritidis*). These results matched with a study conducted by **Abd El-Ghany et al. (2012)** who isolated *S. typhimurium*, *S. enteritidis* and *S. kentucky* from chickens in Egypt, **and Limawongpranee et al. (1999)** who isolated *S. enteritidis* from chickens in Japan. *Salmonella*, isolated from broiler chickens, showed antimicrobial resistance to amoxicillin, ampicillin-sulbactam, tetracycline, ciprofloxacin, doxycycline and erythromycin (**table 4**). These findings agreed to some extent with previous studies performed by **Adam et al. (2018)** who recorded resistance of all Salmonella isolates against amoxicillin, ampicillin and erythromycin, and **Das et al. (2021)** who reported resistance to ampicillin (98.8%), tetracycline (94.2%) and ciprofloxacin (50%) respectively.

Further, *E. coli* was isolated from 250 examined broiler chickens with an incidence of (40%). These findings agreed to some extent in the isolation percentages of *E.coli* as described previously in Jordon by **Ibrahim et al. (2019) and** Indonesia by **Hardiati et al. (2021)** who mentioned that *E. coli* was isolated with (53.4%) from chickens in Jordan andwith (55.6%) from broiler farms in Indonesia. Previous studies recorded higher percentages of *E. coli* isolation than this study such as **Lebert et al. (2018) and Al Azad et al. (2019)** who isolated *E.coli* with (99%) from chicken flocks in Ontario, and with (100%) from broiler in Bangladesh respectively. Meanwhile a study conducted by **Adam et al. (2018)**, recorded a lower *E. coli* isolation (21.6%) than the current study. The serotyping in this study revealed the detection of 12 *E. coli* serogroups (O1, O6, O55, O86, O91, O124, O125, O128, O144, O158, O159 and O166) (**Table 2**). These results agreed with previous studies performed by **Ibrahim et al. (2019) and Amer et al. (2015)** who recoded O1 from chickens in Jordan, and O86, O91, O125 and O158 from broiler chickens in Egypt respectively. *E. coli* that isolated from broiler chickens showed antimicrobial resistance which ranged from 25 % - 95% respectively. The isolated *E. coli* showed resistance to amoxicillin, ampicillin-sulbactam, tetracycline, ciprofloxacin, doxycycline and erythromycin (**table 4**) and these findings were in near similarity with **Adam et al. (2018) who** recorded resistance of *E.coli* isolates against amoxicillin, ampicillin, erythromycin, tetracycline, streptomycin and doxycycline. However previous researcher such as **Hardiati et al. (2021)** showed resistance of *E. coli* isolates to ampicillin (100%), erythromycin (92%), tetracycline (88%) and ciprofloxacin (88%), **and Talebiyan et al. (2014)** recorded resistance to erythromycin (71.70%), doxycycline (16.98%), ciprofloxacin (7.55%).

In addition, the findings of this study revealed the presence of several strains of *Salmonella* in several species of migratory birds in two different geographical locations in Egypt. Previous studies conducted by **Shalaby et al. (2012)** and **Islam[a] et al. (2021)** indicates the presence of *Salmonella* in migratory birds in Egypt and Bangladesh with an incidence of (28.26%) and (21.21%) respectively. Meanwhile **Elsohaby et al. (2021) and Grigar et al. (2017)** isolated *Salmonella* from migratory birds in Saudi Arabia and Texas with percentages of (2.4%) and (0.5%) respectively and their findings were lower than the current study.

In the current study *S. typhimurium* and *S. kentucky* were isolated from Northern pintail and Mallard ducks, *S. infantis and S. enteritidis* were isolated from Northern pintail and Common pochard. Meanwhile a study conducted by **Wei et al. (2020)** isolated *S. enterica* strains (*S. typhimurium*, *S. berta*, and *S. virchow*) from Mallard duck, Northern pintail, Spot-billed duck, Intermediate egret, Eastern great egret and Eurasian wigeon migratory birds in from South Korea. Our results differ from **Grigar *e*t al. (2017)** who isolated *S. Thompson* and *S. braenderup* from water fowl in Texas, **and Antilles et al. (2015)** who recorded that all of examined free living birds in north eastern Spain were negative for *Salmonella*.

A higher incidence of *E. coli* isolation was found compared to *Salmonella* in migratory birds mainly in Common teal in both locations of this study. A study performed in Pakistan by **Mohsin et al. (2017)** isolated ESBL- *E. coli* from mallard duck, red headed pochard, common pochard, shoveler duck, Eurasian wigeon, Eurasian coot and rosy starling with an incidence of (17.3%) which was lower than recorded in this study. However another reports performed by **Islam[b] et al. (2021) Shobrak and Abo-Amer (2014)** isolated *E. coli* from migratory birds with percentages of (93.94%) and (94%) respectively and these incidences were higher than this study. The isolation of *E.coli* from mallard ducks in this study (18.4%) was lower from a study conducted in Germany by **Ewers et al. (2009)** who isolated *E. coli* from wild mallard ducks with (82.4%), however it was higher than **Seleem et al. (2021)** who reported *E.coli* (O86) from migratory ducks and (O125) quails in Egypt with (0.57%).

Overall, with regard to the results of antimicrobial susceptibility in this study; *Salmonella* and *E. coli* isolated from migratory birds showed higher antimicrobial resistance which ranged from 60% - 100% and 66.7 % - 96.7% respectively. Majority of the isolated Salmonella and *E.coli* showed multidrug resistance. These findings were in agreement with **Mohsin et al. (2017) Seleem et al. (2021) Islam[b]et al. (2021) and Yuan et al. (2021)** who reported multidrug resistance of *E. coli* isolated from migratory birds in Pakistan, Egypt, Bangladesh and China respectively.

CONCLUSION

Our study shows that *Salmonella* and *E. coli* were isolated from broiler chickens with an incidence of (14%) and (40%) respectively, and in100 migratory birds (obtained from live bird market) with an incidence of (10%) and (30%) respectively. This calls for continuous monitoring of antimicrobial resistance in Egypt and highlights the needs for future genetic studies including whole genome sequencing to understand the genetic relatedness between isolates retrieved from domestic and migratory birds.

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