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Individual Samples from Quail Harboring Diverse Bacterial populations and different serotypes of *E. coli*

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Abstract

The present study aimed to rule out the potential of multiple pathogens strains coexisting in a single sample and whether single colony could represent all microorganism present in the sample. A total of 20 samples (cloacal and tracheal) were collected from 10 apparently healthy quails in EL-Beheira governorate, Egypt. The samples were individually cultured on EMB agar. Eight out of 10 tracheal samples and 9 out of 10 cloacal samples were positive with green metallic sheen colonies. Fifteen colonies were individually collected from each plate of 8 tracheal samples and 20 colonies from each plate of 9 cloacal samples. As a result, 300 colonies were collected, with each colony being treated as a single sample. All 300 colonies were tested by Gram staining, motility test, biochemical tests, sugar fermentation and enzymatic reaction. The findings demonstrated that mixed infections were more common than single infections. *Escherichia coli* was the most frequently isolated organism (54.6%) followed by *Enterobacter* spp (22.3%), *Klebsiella* spp (11.6%), *Citrobacter* spp (9%), and *Serratia liquefaciens* (2.3%). Serological identification of *E. coli* (n=164) showed diversity of the serotypes among the samples derived from the same plate. Sixteen different serogroups in all the isolates were identified. Moreover, antimicrobial susceptibility of *E. coli* serotypes showed variable patterns with high sensitivity to gentamycin, doxycycline, ciprofloxacin, amikacin, tetracycline, and ampicillin indicating that these antimicrobials could be of great promise in combating *E. coli* infection in quails. These findings imply that diverse bacterial populations could be present in a single sample, making analysis of single colony from a bacterial culture plate insufficient to represent the actual population of pathogens. Further, quails could possibly play a significant role in harboring and spread of a variety of pathogens from the same or different strains.

Keywords: Quails; *E. coli*; Mixed infection; Serotypes; Single colony

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1. Introduction

Poultry is an important industry that contributes to meeting the world's growing demand for protein sources (Hussain et al. 2015). Quail meat and eggs are considered a viable source of protein in developing countries like Egypt (Khalifa et al. 2016) and now, is widely recognized as an important alternative species that offers a variety of benefits over others. Further, for their rapid growth and fast reproductive cycle as it can lay 250 eggs a year (Salehi and Ghanbarpour, 2010; Farghaly et al., 2017; Abd El-Ghany, 2019). Quail can be on market at 5 weeks of age with weight of about 215 g (Cheng et al. 2010). Quail meat and eggs have high value due to their high oleic acid, essential amino acids, minerals, Fe and Zn; so they could help lowering the risk of cardiovascular diseases, prevent diabetes, promote brain function, and prevent anemia (Cheng et al. 2010; Priti and Satish, 2014; Ali and Abd El-Aziz, 2019). Quails showed resistance against several diseases, minimizing costs on antimicrobials and thus economic for rearing (Priti and Satish, 2014; Khalifa et al. 2016).

Quails play an important role in harboring and dissemination of many pathogens of public health significance and act as mechanical transporting to humans and animals (El-Attor et al. 1997; Hamad et al. 2012). Quail market suffered from septicemia and high mortality caused by *E. coli* (Salehi and Ghanbarpour, 2010). *E. coli*, one of the most important members of the Enterobacteriaceae, is a normal inhabitant in the intestine of animals, birds, and humans (Cabral, 2010; Knutson, 2020). The Enterobacteriaceae is a large family of Gram-negative bacteria including organisms with a wide range of disease-causing potential (Quinn et al. 1994). *E. coli* is classified to non-pathogenic and pathogenic groups (Wen and Zhang, 2015). The non-pathogenic *E. coli* may be occasionally implicated in opportunistic infections and pathogenic *E. coli* includes enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAEC), and enterohemorrhagic *E. coli* (EHEC) (Manges and Johnson, 2012; Wen and Zhang, 2015). The avian pathogenic *E. coli* (APEC) is sub-grouped from extraintestinal pathogenic *E. coli* (ExPEC) (Johnson et al. 2007; Allocati et al. 2013). APEC causes colibacillosis and is the main cause of mortality in poultry farms with significant economic losses in poultry industry worldwide (Kobayashi et al. 2011, Novikova and Bartenev, 2015; Alber et al. 2021). Further, APEC is considered zoonotic, for its ability to be transmitted to humans through direct contact or using poultry products (Kobayashi et al. 2011; Ronco et al. 2017; Stromberg et al. 2017). *E. coli* is considered as a significant causative agent of respiratory diseases of poultry (Murthy et al. 2008; Wang et al. 2010). *E. coli* may cause depression and severe diarrhea (Fadel et al. 2009; Alizade et al. 2017). Also, may cause colibacillosis and septicemia, which lead to generalized clinical signs of sickness, such as listlessness, weakness, loss of appetite, swollen-head syndrome, coli granuloma and sudden death of birds, localized inflammation as perihepatitis, airsacculitis, pericarditis, peritonitis, salpingitis, orchitis, osteomyelitis/synovitis and lymphocytic depletion of the bursa and thymus in quail farms (Nolan et al. 2020). *E. coli* could be considered as a causative agent of high mortality in quail farms (Arenas et al. 1999; Salehi and Ghanbarpour, 2010).

Poultry flocks are frequently reared under intensive conditions, with huge doses of antimicrobials used to prevent and treat diseases as well as promote growth (Salehi and Bonab, 2006; Lillehoj et al. 2018). Antimicrobial-resistant (AMR) poultry pathogens can cause treatment failure resulting in financial losses (WHO, 2014; Nhung et al. 2017). Appropriate knowledge about the disease is necessary for effective control and prevention programs in quail industry. This could be achieved through correct diagnosis of the causative pathogens due to the ability of some bacterial species and strains to mask the already present ones (Abd El-Ghany, 2019; Baaijens et al. 2020). Thus, mixed bacterial culture interferes with the identification of microorganisms due to the existence of different species of organisms (Hesseltine, 1992; Kato et al. 2008). Therefore, pure bacterial culture is necessary for correct identification (Holt et al. 2013). Pure culture containing a single species of an organism is usually obtained from mixed culture by picking up an individual colony (Nikita et al. 2012; Bertero et al. 2017; Rommes et al. 2021). All macroscopic distinct colonies in a pure culture must be isolated for accurate identification of the causative pathogen to able to give the appropriate treatment and avoid escape from medication (Vignuzzi et al. 2006; Baaijens et al. 2020; Rommes et al. 2021). Here, the primary goal was to determine whether a single bacterial colony could represent the overall bacterial population in an individual sample. Further, to investigate the frequency of detection of mixed bacterial isolates or serotypes of the same bacteria in a single sample.

2. Material and methods

2.1 Samples

A total of 20 (10 tracheal and 10 cloacal) swabs were individually collected from 10 apparently healthy quails from retail in EL-Beheira governorate, Egypt. Each sample was placed in a sterile test tube containing MacConkey broth (Oxoid, USA). Then they were labeled and transferred immediately to the laboratory in an ice box under complete aseptic conditions (Mailafia et al. 2017).

2.2 Isolation and identification of Enterobacteriaceae

The collected swabs in MacConkey broth were incubated at 37ºC for 12 hrs. From the broth tubes, 100 µL were cultured by spreading on Eosin Methylene blue agar (EMB) (Oxoid, USA) by using sterile spreader to obtain individual colonies, which were then incubated at 37° C for 24 hrs. Different individual colonies were picked up for bacteriological examination. The

presumptive Enterobacteriaceae colonies were identified based on colonial and microscopic characteristics using Gram's staining, motility test, biochemical tests including oxidase, catalase, triple sugar iron (TSI), hydrogen sulfide (H_2S), indole, methyl red (MR), Voges-Proskauer (VP), citrate utilization, urease, nitrate reduction, fermentation of sugars and enzymatic reactions (Ornithine decarboxylase (ODC), L- lysine decarboxylase (LDC), Arginine decarboxylase (ADH) and β- galactosidase (ONPG)) (MacFaddin, 2000; Quinn et al. 2011).

2.3 Antimicrobial susceptibility Testing

Antimicrobial susceptibility was performed by the disc diffusion method (Kirby-Bauer) (Mary and Usha, 2013) for all isolates. Antimicrobial discs (Oxoid Limited, Basingstoke, Hampshire and UK) were as follows: nalidixic acid (NA) 30 ug, clindamycin (CL) 10 ug, doxycycline (DO) 30 ug, cefotaxim (CF) 30 ug, gentamicin (G) 10 ug, norocillin (NO) 25 ug, tetracycline (T) 30 ug, ampicillin (AM)10 ug, ciprofloxacin (CP) 5 ug, amikacin (AK) 30 ug, cephalothin (CN) 30 ug, erythromycin (E) 15 ug, penicillin G (P) 10 IU, sulphamethoxazol (SXT) 25 ug. The Clinical Laboratory Standards Institute (CLSI) guidelines were used to interpret the results (CLSI, 2017).

2.4 Serogrouping of *E. coli*

The isolates were serologically identified according to Kok et al., (1996) by using rapid diagnostic *E. coli* antisera sets (DENKA SEIKEN Co., Japan) for diagnosis of the Enteropathogenic types.

Set1: O-antisera

Polyvalent antisera 1: O_1, O_4, O_{26}, O_{86a}, O_{111}, O_{119}, O_{127a} and O_{128}.
Polyvalent antisera 2: O_{44}, O_{55}, O_{113}, O_{125}, O_{126}, O_{146} and O_{166}.
Polyvalent antisera 3: O_{18}, O_{76}, O_{114}, O_{142}, O_{151}, O_{157} and O_{158}.
Polyvalent antisera 4: O_2, O_6, O_7, O_{27}, O_{78}, O_{148}, O_{159} and O_{168}.
Polyvalent antisera 5: O_{20}, O_{25}, O_{63}, O_{91}, O_{153}, O_{163} and O_{167}.
Polyvalent antisera 6: O_8, O_{15}, O_{17}, O_{102}, O_{115}, O_{141}, O_{169} and O_{171}.
Polyvalent antisera 7: O_{28ac}, O_{112ac}, O_{117}, O_{124}, O_{136} and O_{144}.
Polyvalent antisera 8: O_{29}, O_{121}, O_{143}, O_{152} and O_{164}.

Set2: H-antisera

H, H_4, H_6, H_7, H_{11}, H_{18} and H_{21}.

3. Results

3.1 Identification of the isolated colonies

Eight out of 10 tracheal plates and 9 out of 10 cloacal plates were found to be positive on EMB agar. From these positive samples, 300 colonies were collected (15 colonies collected from each plate for 8 tracheal samples and 20 colonies collected from each plate for 9 cloacal sample). Gram stain, motility test, biochemical tests and enzymatic reaction results are shown in **Tables 1** and **2**. Isolates were identified to five different bacteria: *E. coli*, *Enterobacter* spp., *Citrobacter* spp., *Klebsiella* spp. and *Serratia* spp. at percentage of 54.6%, 22.3%, 9%, 11.6% and 2.3%, respectively. As shown in the **Table 3** and **Figure 1**, *E. coli* had the highest prevalence among the isolates in both cloacal and tracheal samples, followed by *Enterobacter* spp. in cloacal samples; while *Klebsiella* spp. in tracheal samples.

3.2 Distribution of various bacteria in individual cloacal and tracheal samples

The results revealed the prevalence of mixed bacteria being present in the same sample. Each sample contained different isolates of Enterobacteriaceae, as shown in the **Tables 4** and **5**. Twenty colonies per plate per quail collected from cloaca revealed diverse population within the same plate derived from each quail, as shown in the **Table 4**. Fifteen colonies per plate per quail collected from the trachea showed diverse population within the same plate derived from each quail, as shown in the **Table 5**.

3.3 Serogrouping of *E. coli* isolates recovered from quail samples

The isolated *E. coli* were serotyped using polyvalent and monovalent *E. coli* antisera to determine the *E. coli* serotype. *E. coli* isolates from the same sample were not necessary of the same serotype; 7 different serotypes of *E. coli* were detected in the same sample as showed in the **Tables 6** and **7**. Further, some serotypes were detected more in cloacal than tracheal samples as showed in the **Tables 6** and **7**. Furthermore, the predominant serotypes were O_{78} (24.3%; ETEC), O_{91} (14.6%; EHEC), O_{128} (13.4%; ETEC), O_{146} (9.1%; EPEC), O_2 (6.7%; EPEC), O_{26} (6%; EHEC), O_{121} and O_1 (5.4%; EPEC) and other serotypes 14.6% as shown in the **Table 8** and **Figure 2**.

3.4 Antimicrobial susceptibility of *E. coli* serotypes

The antimicrobial susceptibility of isolated *E. coli* serotypes was tested. The most common isolated serotypes in both cloacal and tracheal samples in quails were used. The susceptibility of isolated *E. coli* serotypes to different antimicrobial agents was variable. *E. coli* serotypes were highly resistant to erythromycin, cephalothin, clindamycin, sulphamethoxazol and nalidixic acid, sensitive to gentamicin, doxycycline, ciprofloxacin, amikacin, tetracycline and ampicillin and moderate to penicillin G, cefotaxim and norocillin as shown in the **Table 9**. Multiple antimicrobial resistance was also detected for all the serotypes as shown in the **Table 10**. Fourteen different patterns of multiple drug resistance were observed for *E. coli* serotypes from quails as shown in the **Table 10**.

Table 1. Morphological and biochemical identification of individually isolated colonies.

Biochemical Tests	**Bacterial Isolates**				
	E. coli	***Enterobacter* spp.**	***Citrobacter* spp.**	***Klebsiella* spp.**	***Serratia* spp.**
Gram stain	-ve	-ve	-ve	-ve	**-ve**
Motility	+ve	+ve	+ve	-ve	**+ve**
Oxidase	-ve	-ve	-ve	-ve	**-ve**
Catalase	+ve	+ve	+ve	+ve	**+ve**
TSI	A\A	A\A	A\A	A\A	**A\A**
H_2S	-ve	-ve	+ve	-ve	**-ve**
Indole	+ve	-ve	-ve	-ve	**-ve**
MR	+ve	-ve	+ve	+ve	**-ve**
VP	-ve	+ve	-ve	+ve	**+ve**
Citrate utilization	-ve	+ve	+ve	+ve	**+ve**
Urease	-ve	+ve	+ve	+ve	**+ve**
Gelatin hydrolysis	-ve	-ve	-ve	-ve	**+ve**
Nitrate reduction	+ve	+ve	+ve	+ve	**+ve**
Fermentation of sugars (lactose, sucrose, mannitol, mannose, arabinose, xylose and glucose)	+ve	+ve	+ve	+ve	**+ve**

A= acid, -ve= negative, +ve= positive.

Table 2. Enzymatic reactions of bacterial isolates.

Enzymatic tests	**Bacterial Isolates**				
	E. coli	***Enterobacter spp.***	***Citrobacter spp.***	***Klebsiella spp.***	***Serratia spp.***
Ornithine decarboxylase (ODC)	V	+ve	-ve	-ve	**+ve**
L- lysine decarboxylase (LDC)	V	+ve	-ve	+ve	**-ve**
Arginine decarboxylase (ADH)	V	-ve	V	-ve	**-ve**
β- galactosidase (ONPG)	+ve	+ve	+ve	+ve	**+ve**

V= Variable, -ve= negative, +ve= positive.

Table 3. Prevalence of *Escherichia coli* and other Enterobacteriaceae in cloacal and tracheal samples from quails.

Bacterial Isolates	Cloacal (%)	Tracheal (%)	Total (%)
E. coli	102 (56.6)	62 (51.6)	**164 (54.6)**
Enterobacter aerogenes	23 (12.7)	14 (11.6)	**37 (12.3)**
Enterobacter agglomerans	10 (5.5)	8 (6.6)	**18 (6)**
Enterobacter cloacae	12 (6.6)	0	**12 (4)**
Total *Enterobacter spp.*	45 (25)	22 (18.3)	**67 (22.3)**
Citrobacter freudii	12 (6.6)	8 (6.6)	**20 (6.6)**
Citrobacter diversus	5 (2.7)	2 (1.6)	**7 (2.3)**
Total *Citrobacter spp.*	17 (9.4)	10 (8.3)	**27 (9)**
Klebsiella pneumoniae	8 (4.4)	20 (16.6)	**28 (9.3)**
Klebsiella oxytoca	4 (2.2)	3 (2.5)	**7 (2.3)**
Total *Klebsiella spp.*	12 (6.6)	23 (19.1)	**35 (11.6)**
Serratia liquefaciens	4 (2.2)	3 (2.5)	**7 (2.3)**
Total	180	120	**300**

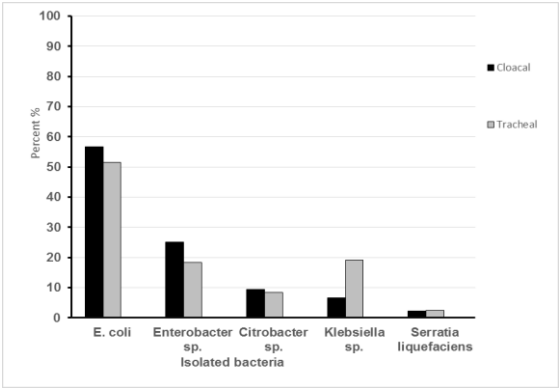

Figure 1. Percentage of the different bacterial isolates in individual tracheal and cloacal samples from quails

Table 4. Distribution of various bacteria in individual cloacal sample from quails.

Bacterial isolates	Quail ID.									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
E. coli	0	13	12	10	11	13	11	11	11	**10**
Enterobacter aerogenes	0	2	4	2	2	2	2	4	2	**3**
Enterobacter agglomerans	0	1	0	2	1	1	1	1	2	**1**
Enterobacter cloacae	0	1	1	3	1	1	1	1	1	**2**
Citrobacter freudii	0	1	0	2	1	2	2	0	2	**2**
Citrobacter diversus	0	1	0	0	1	0	1	1	1	**0**
Klebsiella pneumonia	0	1	0	1	2	0	1	1	1	**1**
Klebsiella oxytoca	0	0	1	0	1	1	1	0	0	**0**
Serratia liquefaciens	0	0	2	0	0	0	0	1	0	**1**

Number of positive colonies per plate per cloacal sample, Quail no 1 was negative with no colony growth on EMB agar.

Table 5. Distribution of various bacteria in individual tracheal sample from quails.

Bacterial isolates	Quail ID.									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
E. coli	0	6	7	8	8	9	7	8	0	**9**
Enterobacter aerogenes	0	2	3	2	1	1	2	1	0	**2**
Enterobacter agglomerans	0	1	2	1	1	0	1	2	0	**0**
Enterobacter cloacae	0	0	0	0	0	0	0	0	0	**0**
Citrobacter freudii	0	2	0	1	0	1	1	2	0	**1**
Citrobacter diversus	0	0	0	0	1	1	0	0	0	**0**
Klebsiella pneumonia	0	3	1	3	3	2	3	2	0	**3**
Klebsiella oxytoca	0	0	1	0	1	1	0	0	0	**0**
Serratia liquefaciens	0	1	1	0	0	0	1	0	0	**0**

Number of positive colonies per plate per tracheal sample, Quail no 1 and 9 were negative with no colony growth on EMB agar.

Table 6. The different serogroups of *E. coli* in individual cloacal samples.

***E. coli* isolates**	**Quail ID**								
	2	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
O_{78}	5	4	3	1	3	4	1	2	3
O_{91}	3	1	2	2	1	2	2	1	1
O_{128}	2	2	1	1	3	1	2	2	1
O_{146}	1	1	1	1	1	0	1	0	0
O_{2}	1	1	0	2	1	1	0	1	0
O_{26}	0	1	0	1	1	1	2	0	1
O_{121}	0	1	1	1	1	0	1	1	0
O_{1}	1	0	1	0	2	0	0	0	0
O_{55}	0	0	0	1	0	1	1	1	0
O_{153}	0	0	1	0	0	1	0	1	1
O_{158}	0	1	0	0	0	0	1	0	0
O_{124}	0	0	0	1	0	0	0	0	1
O_{119}	0	0	0	0	0	0	0	1	1
O_{159}	0	0	0	0	0	0	0	1	0

E. coli isolates	Quail ID								
	2	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
O_{44}	0	0	0	0	0	0	0	0	1
O_{127}	0	0	0	0	0	0	0	0	0
Total	13	12	10	11	13	11	11	11	10

Table 7. The different serogroups of *E. coli* in individual tracheal samples.

E. coli isolates	Quail ID							
	2	**3**	**4**	**5**	**6**	**7**	**8**	**10**
O_{78}	2	2	1	2	2	2	2	1
O_{91}	1	1	1	1	1	1	1	2
O_{128}	0	1	2	1	1	0	1	1
O_{146}	2	0	1	1	2	1	1	1
O_2	0	1	1	1	0	1	0	0
O_{26}	0	1	0	0	1	0	1	0
O_{121}	0	1	0	1	0	1	0	0
O_1	1	0	0	0	1	0	2	1
O_{55}	0	0	0	0	0	1	0	1
O_{153}	0	0	1	0	0	0	0	0
O_{158}	0	0	1	0	1	0	0	0
O_{124}	0	0	0	1	0	0	0	0
O_{119}	0	0	0	0	0	0	0	0
O_{159}	0	0	0	0	0	0	0	1
O_{44}	0	0	0	0	0	0	0	0
O_{127}	0	0	0	0	0	0	0	1
Total	6	7	8	8	9	7	8	9

Table 8. Different Pathotypes of *E. coli* isolated from individual tracheal and cloacal samples from quails.

Pathotypes	**Percentage**	**Serotype**	**No. of isolates (%)**
ETEC	38.4	O_{78}	**40 (24.3)**
		O_{128}	**22 (13.4)**
		O_{127}	**1 (0.6)**
EPEC	34.1	O_{146}	**15 (9.1)**
		O_2	**11 (6.7)**
		O_{121}	**9 (5.4)**
		O_1	**9 (5.4)**
		O_{153}	**5 (3)**
		O_{158}	**4 (2.4)**
		O_{119}	**2 (1.2)**
		O_{44}	**1 (0.6)**
EHEC	24.3	O_{91}	**24 (14.6)**
		O_{26}	**10 (6)**
		O_{55}	**6 (3.6)**
EIEC	3	O_{124}	**3 (1.8)**
		O_{159}	**2 (1.2)**

ETEC= Enterotoxigenic *Escherichia coli*, EPEC= Enteropathogenic *Escherichia coli*, EHEC= Enterohaemorrhagic *Escherichia coli* and EIEC= Enteroinvasive *Escherichia coli*.

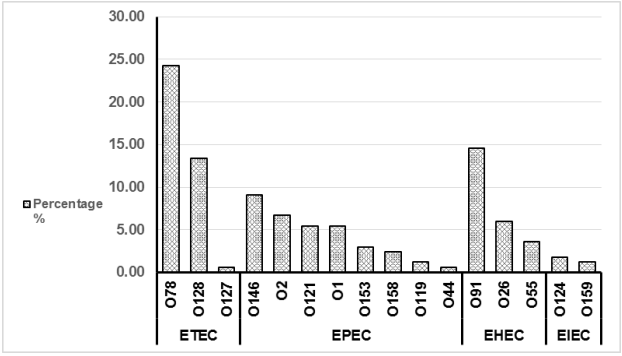

Figure 2. The prevalence of *E. coli* serogroups in tracheal and cloacal samples from quails

Table 9. Antimicrobial susceptibility of *E. coli* serotypes isolated from quails.

Antibiotic	**O_{78}**	**O_{91}**	**O_{128}**	**O_{146}, O_2**	**O_{26}, O_{121}, O_1**	**O_{55},O_{153},O_{158},O_{124},O_{119},O_{159},O_{44}, O_{127}**
Erythromycin	100	100	100	100	100	100
Cephalothin	100	95.7	100	92	100	100
Nalidixic acid	97.4	78.3	100	80	92.9	100
Clindamycin	84.2	65.2	81	76	78.6	91.7
Sulphamethoxazol	76.3	60.9	76.2	72	67.9	79.2
Penicillin G	65.8	47.8	66.7	60	50	62.5
Norocillin	47.4	39.1	47.6	52	50	50
Cefotaxim	42.1	39.1	42.9	36	42.9	41.7
Ampicillin	34.2	34.8	30.4	32	39.3	34.5
Tetracycline	23.7	26.1	28.6	32	35.7	33.3
Amikacin	10.5	21.7	23.8	20	21.4	20.8
Ciprofloxacin	10.5	17.4	9.5	12	14.3	12.5
Doxycycline	7.9	8.7	4.8	8	10.7	8.3
Gentamicin	2.6	4.3	4.8	4	7.1	4.2

Numbers represent percentages of resistance to each antimicrobial.

Table 10. Antimicrobial resistance patterns of *E. coli* serotypes isolated from individual tracheal and cloacal samples from quails.

Antimicrobial agents	***E. coli* Serotypes**																**Total**
	O_{78}	**O_{91}**	**O_{128}**	**O_{146}**	**O_{2}**	**O_{26}**	**O_{121}**	**O_{1}**	**O_{55}**	**O_{153}**	**O_{158}**	**O_{124}**	**O_{119}**	**O_{159}**	**O_{44}**	**O_{127}**	
E, CN, NA, CL, SXT, P, NO, CF, AM, T, AK, CP, DO, G	1	1	1	1	-	1	1	-	1	-	-	-	-	-	-	-	**7**
E, CN, NA, CL, SXT, P, NO, CF, AM, T, AK, CP, DO	2	1	-	-	1	-	-	1	-	1	-	-	-	-	-	-	**6**
E, CN, NA, CL, SXT, P, NO, CF, AM, T, AK, CP	2	2	1	1	-	-	1	-	-	-	1	-	-	-	-	-	**7**
E, CN, NA, CL, SXT, P, NO, CF, AM, T, AK	-	1	3	1	1	1	1	-	2	-	-	-	-	-	-	-	**10**
E, CN, NA, CL, SXT, P, NO, CF, AM, T	5	1	1	2	1	2	-	2	-	1	-	1	1	-	-	-	**17**
E, CN, NA, CL, SXT, P, NO, CF, AM	4	2	1	-	-	-	1	-	1	-	-	-	-	-	-	-	**9**
E, CN, NA, CL, SXT, P, NO, CF	3	1	2	-	1	1	-	-	-	1	-	-	-	-	-	-	**9**
E, CN, NA, CL, SXT, P, NO	2	-	1	3	1	1	1	-	-	-	-	-	1	1	-	-	**11**
E, CN, NA, CL, SXT, P	7	2	4	2	-	-	-	-	-	1	1	-	-	-	1	-	**18**
E, CN, NA, CL, SXT	4	3	2	-	3	2	1	2	1	-	-	1	-	1	-	1	**21**
E, CN, NA, CL	3	1	1	2	-	-	2	1	1	-	1	1	-	-	-	-	**12**
E, CN, NA	5	3	5	1	-	1	1	2	-	1	1	-	-	-	-	-	**19**
E, CN	2	4	-	2	1	1	-	1	-	-	-	-	-	-	-	-	**10**
E	-	2	-	-	2	-	-	-	-	-	-	-	-	-	-	-	**3**
Total	40	24	22	15	11	10	9	9	6	5	4	3	2	2	1	1	**164**

E; Erythromycin, CN; Cephalothin, NA; Nalidixic acid, CL; Clindamycin, SXT; Sulphamethoxazol, P; Penicillin-G, NO; Norocillin, CF; Cefotaxim, AM; Ampicillin, AK; Amikacin, T; Tetracycline, CP; Ciprofloxacin, DO; Doxycycline, G; Gentamicin.

4. Discussion

The present study aimed to determine whether collecting a single colony for isolation and identification of the causative organism could be representative for the population present in the sample through studying the prevalence of Enterobacteriaceae in cloacal and tracheal samples. Thus, 20 samples (cloacal and tracheal) were collected from 10 seemingly healthy quails in EL-Beheira governorate, Egypt and cultivated on EMB agar. Next, 15 colonies were recovered from each tracheal sample (8 out of 10) and 20 colonies from each cloacal sample (9 out of 10). As a result, 300 individual colonies were collected, each of which represents an independent sample. The results revealed the isolation of various bacterial species as a mixed culture from the same tracheal and cloacal swabs from quails. The isolated strains were *E. coli*, *Enterobacter* spp., *Citrobacter* spp., *Klebsiella* spp.and *Serratia* spp. with percentages of 54.6%, 22.3%, 9%, 11.6% and 2.3%, respectively. *E. coli* was the most common Enterobacteriaceae (in both cloacal and tracheal swabs) followed by *Enterobacter spp*. in cloacal swabs and *Klebsiella* spp. in tracheal swabs. Overall, these findings imply that the isolation and identification of a pathogen from a single colony collected is insufficient to represent the actual population in case of a possible mixed infection.

According to the obtained results, significant difference was found among the isolates from a single quail. Mixed infections were noticed in both tracheal and cloacal samples, individual colonies per plate per tracheal and cloacal sample showed diversity among bacterial species and serotypes. The studies performed by Kuipers et al. (2000); Farzi et al. (2014) revealed the presence of mixed infections and diverse strains in a single host. In addition, Kim et al. (2004) showed that a sample may harbor different strains. Further, Haraszthy et al. (2007) who picked up 200 bacterial colonies from dorsal tongue surface of 13 adults and identified 32 different species. However, the above-mentioned reports did not use individual colonies from the same plate; rather, they used a sample representing the host in general or analyzed the samples collectively and not individually. Farzi et al. (2014) picked up 5-6 single colonies from each individual patient and showed diversity among the *H. pylori* strains and found that 23% of patients showed single type infection and 77% showed mixed infection. However, they analyzed for the prevalence of different genotypes or quasispecies of *H. pylori* only. While Rommes et al. (2021) emphasized the necessity of isolation of all distinct colonies in pure culture to obtain correct identification. Moreover, MacFadden, (1985) common methods depended on collecting individual colony from a sample. Also, Ferrari and Signoroni, (2014) picked up individual colony from sample for examination but for evaluation of laboratory automation.

There are few data on the prevalence of Enterobacteriaceae in tracheal and cloacal swabs in quails. However, this study agreed with previous studies about the general isolation of bacteria from quail, revealing that *E. coli* was the most frequently isolated organism (Thenmozhi et al. 2010; Hamad et al. 2012; Alizade et al. 2017). In this study, *E. coli* was isolated from both cloacal and tracheal samples with 54.6% prevalence that agrees with Ihab, (2007) who found 51% of isolates from different internal organ to be *E. coli*. Also, agrees with Ibrahim, (2019) who isolated *E. coli* (28%) from different organs. Our study showed the prevalence of *E. coli* in cloacal swabs as 56.6%. This further agrees with Abdul Wahid, (2019) who reported a high prevalence of *E. coli* (83%) in cloacal samples of Japanese quails. In addition, Fadel et al. (2009) isolated *E. coli* at high rates from quails suffering from depression and severe diarrhea from cloacal swabs (80%). Further, Dipineto et al. (2014) reported the prevalence of *E. coli* (21.4%) from cloaca of quail during migratory season. This indicates that *E. coli* could be easily recovered from cloacal swabs of quails. In our study, tracheal swabs showed 51.6% prevalence of *E. coli*. Thenmozhi et al. (2010) found that *E. coli* were isolated (56.14%) from trachea of Japanese quails showing respiratory diseases symptoms that agrees with the current study. Corynebacterium spp. and *E. coli* were shown to be the most common bacteria in the internal organs of quails, according to Hamad et al. (2012). The findings of the present study showed that the prevalence of *E. coli* in cloacal swabs was higher followed by Enterobacter spp., Citrobacter spp., Klebsiella spp. and Serratia spp. While tracheal swabs showed the higher prevalence of *E. coli* followed by Klebsiella spp., Enterobacter spp., Citrobacter spp. and Serratia spp. A study performed by El-Demerdash, (2013) showed a high prevalence of *E. coli* followed by Salmonella spp, *Proteus mirabilis*, Pseudomonas spp., Klebsiella spp., Citrobacter spp., *Proteus vulgaris* and Arizona spp. Only *E. coli* and Salmonella were recovered from cloacal swabs in live birds, and no pathogens were recovered from tracheal swabs, according to Farghaly et al. (2017). On the contrary, Salmonella, *E. coli*, *S. aureus*, and *Pasteurella haemolytica* were recovered from freshly dead birds. A study was performed by Boroomand, (2018) to investigate the role of some members of Enterobacteriaceae for early mortality in Japanese quail chicks. They found that the isolated bacteria were *E. coli* (44%), *K. pneumonia* (8%), *Salmonella serovar ruzizi* (5%), *Salmonella serovar typhimurium* (3%), *Enterobacter cloacae* (4%), *Enterobacter aerogenes* (4%), *Proteus vulgaris* (5%) and *Proteus mirabilis* (5%) agreeing with a high *E. coli* prevalence. Collectively, this indicates that *E. coli* is the most isolated bacteria from either cloacal or tracheal samples from quails and the possibility of their involvement in a possible associated disease. Further, Klebsiella spp. were isolated at a higher percentage from the trachea indicating a possible interaction with *E. coli* in respiratory problems in quails.

Regarding the serotyping of 164 identified *E. coli* strains recovered from quails to detect whether the isolated *E. coli* from the same sample (tracheal or cloacal) belong to the same serotype or not. Sixteen serogroups were detected, with O78, O91, O128 and O146 the most detected in cloaca and trachea. These results disagree with those obtained by Gita et al. (2000) and Roy et al. (2006). Gita et al. (2000) isolated 18 serogroups of *E. coli* from quail namely O6, O8, O9, O12, O18, O20, O32, O36, O33, O54, O103, O106, O109, O111, O132, O153, O157 and O171. Roy et al. (2006) isolated *E. coli* serogroups such as O4, O9, O38, O42, O88 and 4 untypeable isolates from diseased Japanese quail, dead-in-shell embryos, fluff samples, footbath and drinking water samples in a hatchery. Further, in this study, the most prevalent serovars in tracheal and cloacal samples were O78 (24.3%), O91 (14.6%) and O128 (13.4%). Moreover, diversity of serotypes in the same sample was noticed. Roy et al. (2006) found that most *E. coli* isolates from infected Japanese quails (54.5%) belonged to serogroup O9, and the same serotype was also prevalent in the hatchery environment. Moreover, Fadel et al. (2009) reported that *E. coli* isolates from cloaca of quails were serotyped as O26, O118, O86, O119, O115, O158 and O164. Similarly, Awadallah et al. (2013) showed that *E. coli* was serogrouped into O127, O128 and O26 from cloaca of quails. Furthermore, Dipineto et al. (2014) revealed the presence of *E. coli* serotypes O128 and O26 in one quail and three quails, respectively. Farghaly et al. (2017) reported that only O55 was recovered from life bird's cloacal swabs and *E. coli* was not isolated from tracheal swabs of life quails. On the other hand, O125, O20, O44, O127 and O78 were recovered from dead quail with presence of O78 only in liver and heart disagrees with the findings of the current study. Ibrahim, (2019) demonstrated that 5 serotypes were isolated from Japanese quails reared in Sharkia Governorate, namely O2, O20, O35, O78 and O127. *E. coli* O127 and O2 were the most predominant serotype (28.56%, 24.99%, respectively). However, *E. coli* O78 was identified at 21.42%, which nearly agrees with the present study. However, all the above-mentioned studies did not indicate whether these isolates were derived from the same plate or sample or even the same bird.

In the present study, different strains existed within the same sample. So, strains with high relative frequency could mask other strains already present and at low relative frequency (Baaijens et al. 2020). Because of medical treatment and host immune response occur at the strain level not species level, low frequency bacterial strains are considered problematic due to possible escape from medication (Bull et al. 2005; Vignuzzi et al. 2006; Baaijens et al. 2020). Bertels et al. (2017) emphasized that the large divergence between bacterial strains affects the evolution of the entire populations. *E. coli* strains are closely related, although they contain variation in number of repetitive extragenic palindromic (REP) sequence (Bertels and Rainey, 2011). Bertels et al. (2017) described the evolution of REP with a quasispecies model, the quasispecies model describes the mutation selection balance of a set of similar sequences that evolve on a fitness landscape (Bull et al. 2005; Bertels et al. 2017). The sequences with high fitness leave many offspring, sequences with low fitness leave few offspring. In one bacterial lineage, a certain REP population may prosper whereas in another, it declines (Bertels and Rainey. 2011; Bertels et al. 2017). Lynch et al. (2016) showed that there was a direct correlation between mutation rates and duplication rates, if the mutation rates is high, it leads to higher duplication rates and vice versa. *E. coli* showed higher mutation rates, a certain strain may show significant mutation rates change (Wielgoss et al. 2013; Lynch et al. 2016).

In the present study, all the identified *E. coli* serotypes exhibited marked resistance to several antimicrobials; erythromycin, cephalothin, clindamycin, sulphamethoxazol and nalidixic acid and it is considered a major public health risk. However, the sensitivity pattern indicated that *E. coli* isolates were highly sensitive to gentamycin, doxycycline, ciprofloxacin, amikacin, tetracycline and ampicillin. The resistance profiles of the identified *E. coli* serotypes from quails and 14 different patterns of multiple drug resistance were observed. The present findings were nearly identical to those previously reported in quails that exhibited multiple antimicrobial resistance. Gita et al. (2001) observed multiple drug resistance in *E. coli* isolates from Japanese quail and most *E. coli* were resistant to ampicillin, chloramphenicol and tetracycline. Roy et al. (2006) found that all *E. coli* isolates exhibited resistance to multiple drugs; against ampicillin/cloxacillin, chloramphenicol, tetracycline, and cotrimoxazole.

In contrast, highest sensitivity was observed against nitrofurantoin. All *E. coli* isolates from Boroomand, (2018) study showed high resistance to enrofloxacin, doxycycline and oxytetracycline and they observed the highest sensitivity to ceftriaxone, these studies do not agree with the present study. Our study agreed with Salehi and Ghanbarpour, (2010) who investigated the prevalence of antimicrobial resistance in pathogenic *E. coli* isolates from Japanese quail, more than 70% of the isolates were resistant to the tested antimicrobials and 19 different patterns of multiple drug resistance were detected. *E. coli* isolates from quails were extremely sensitive to ciprofloxacin, tobramycin, amikacin, enerofloxacin, and exhibited resistance to erythromycin, according to El-Demerdash et al. (2013). Roy et al. (2013) showed the sensitivity of *E. coli* isolates to enterofloxain, gentamicin and chloramphenicol. Youssef and Mansour, (2014) observed a multi-drug resistance pattern and strong susceptibility to enrofloxacin, nitrofuranation, and ofloxacine. *E. coli* isolates from Abdul Wahid et al. (2019) study were highly resistance to cephazolin, erythromycin and chloramphenicol. The present study partially agreed with Ibrahim, (2019) who found that *E. coli* isolates were resistant to ampicillin, ceftiofur, penicillin and nalidixic acid, and were very sensitive to gentamycin, neomycin, enrofloxacin, and erythromycin. Therefore, from the present study and other previous investigations in quails, *E. coli* isolates showed multiple drug resistance and exhibited diverse patterns of resistance to a vital group of antimicrobials as a result of possible overuse or misuse. This suggests that quails could act as reservoir for antimicrobial-

resistant bacteria. Thus, a spotlight on antimicrobial use in poultry production must be explored to minimize the prevalence of mixed infections and, as a result, the dissemination of antimicrobial resistance genes across bacterial populations in quails.

We conclude from this study that one colony collected from a sample for analysis is insufficient to represent the overall population of bacteria contained in the sample. Further, one colony in a plate should be considered and treated as an individual sample. Bacterial species and strains with high frequency could mask the low frequency strains and other bacterial species already present in the same sample, so it may lead to incorrect diagnosis and thus incorrect treatment that can result in the evolution of drug resistant bacteria or bacteria from the minor population. Thus, quails could play a significant role in transmission of many diseases despite appearing clinically healthy. It was found to harbor a wide range of bacterial species, with *E. coli* infection being the most frequent as well as various serogroups within the same sample. A high level of multidrug resistance to commonly used antimicrobials in Egyptian poultry farming was noticed in identified *E. coli* isolates. However, gentamycin, doxycycline, ciprofloxacin, amikacin, tetracycline, and ampicillin are observed to be good candidates for reducing *E. coli* infection in quails.

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