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Incidence of *Escherichia coli* in squabs and characterization of its virulent and antibiotic resistant genes

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Abstract
In the present study, 109 *E. coli* isolates (24.22%) were recovered from 450 samples collected from125 squabs (25 apparently healthy, 50 diseased and 50 recently dead) 1-4 week old. Seven serogroups were identified among isolates, the most prevalent serogroups were O78, O2 and O128 with a prevalence of 21.15%, 17.3% and 15.38%, respectively. E. *coli* serotypes showed higher sensitivity to norfloxacin (92.3%) cefotaxime (90.33%), cefoxitin (88.47%), nalidxic acid (88.47%) and neomycin (86%). High level of resistant was observed with tetracycline, doxycycline, chloramphenicol and sulphamethaxole (100%, 94.23, 84.62 and 76.92%, respectively). The screening of *E. coli* isolates by PCR for virulence gene profile revealed the presence of *iss, tsh* and *fim*C in (100% for each), *irp*2in (42.8%), *pap*C in (71,4%), and *vat* in (57.1%) of isolates. In addition, The antibiotic resistance genes profile revealed the presence of tetA in (100%) and cat1and sul1 in (42.8%for each) of isolates. Experimental study was conducted to evaluate pathogenicity of isolated virulent field strain of *E. coli* (O78 highly virulent and resistance to most of antibiotics) and assess the efficacy of norfloxacin in controlling the adverse effects of collibacillosis in experimentally infected pigeon. Rapid resolution of clinical signs, significant reduction in mortalities, gross pathological lesions, and the low rate of re-isolate *E. coli* from the infected-treated pigeon indicates the effectiveness of norfloxacin for treatment of this organism.
Conclusion: our findings provide valuable inputs for evaluating public health hazards associated with bacterial contamination.

Keywords: *Squabs, E. coli, antibiotic resistant genes, virulence genes.*

Introduction
Pigeons had been associated with human society as a source of food and as cage birds from a long time **(Dutta *et al.,* 2013).** The presence of pigeon feces in urban environments may contribute to the spread of infectious agents as they could harbor various microorganisms (**Tanaka *et al*., 2005**). These might include human pathogens such as diarrhea genic *Escherichia coli* strains, which are able to survive under adverse environmental conditions for extended periods of time if excreted in feces, thus creating potential for human exposure and infection **(Pedersen *et al*., 2006).**
E. coli organisms exist in nature as a number of strains that range from the most innocent to the most deadly. Some pathogenic strains of *E. coli* in the intestines may cause disease by their production of potent toxins that are absorbed through the intestinal wall into the blood stream, from which their far-reaching effects in many tissues. Other dangerous strains of *E. coli* are able to breach the intestinal wall, enter the blood stream where they multiply called ("septicemia") and are distributed to a variety of tissues to produce signs of illness in some joints, brain and ovarian infections, etc. in pigeons are caused by these tissue-invasive strains of *E. coli* which can produce dead-in-shell embryos, sudden death in youngsters or old birds. The pigeons may acquire infection from contaminated environment, feed and wa-

ter or from other carrier birds. **(Gordon, 2010).** Many researchers isolated and serotyped different *E. coli* strains from diseased pigeons, there are distinct sereotypical differences between the facultative *E. coli* and the ones that invade the tissue and cause infection**, (Farghaly and Mahmoud, 2011 and Khu-dair, 2012).** The zoonotic risk associated with EPEC infection in pigeons was first documented by **Silva *et al.* (2009)** in Brazil. Enter invasive *E. coli,* Shiga toxin producing *E. coli,* enteropathogenic *E. coli,* and enter toxigenic *E. coli* and enteroaggregative *E. coli* (EAEC), were detected from pigeons (**Sanches *et al*., 2017 and Vasconcelos *et al*., 2017**) .

APEC is considered a primary or secondary pathogen of poultry. Strains which carry virulence genes (adhesin, invasins, toxins, resistance to host serum, iron acquisition systems, temperature-sensitive hemagglutinin, andK1 capsule) have all been shown to contribute to APEC pathogenesis **(Dziva and Stevens, 2008)** and could induce colibacillosis without previous immune suppression factors; stress or concurrent infections (**Collingwood, 2014).** Some human and avian extra intestinal pathogenic *E. coli* (Ex PEC) has similar phylogenic backgrounds and shares similar virulence genes possessing zoonotic risk **(Manges and Johnson, 2012).** The *fimC* and *papC* are fimbria related virulence genes. The *fimC* play an important role in the adhesions of bacteria to the respiratory tract of the host and *papC* for adhesions to the internal organs of the host. The *iucD* and *irp2* are iron-acquisition system virulence factors which play a role by removal of iron from the host cells and enhance the multiplication and growth of the bacteria. The *tsh* is a heamolysin virulence factor which causes destruction of the erythrocytes and *iss* gene plays an important role in the protection of bacteria against the bactericidal activity of the host. The APEC strains have the potential of zoonotic risk **(Ewers *et al*., 2004).**

Antimicrobial resistant *E. coli* strains pose a serious problem for public health, since these strains could be passed to humans via the food chain or by direct contact with infected birds. In addition, resistant *E. coli* may act as transporters for antimicrobial resistant genes to other pathogens **(Akond, 2009)**. Resistance to tetracycline is mediated through efflux pump system which encoded by tetracycline resistance group of genes (tetA, tetB, tetC, tetD, tetE and tetG) **(Shin *et al*., 2015).** Phenicols resistance encoding genes are (cat1, cat2, cat3, cmlA and cmlB) and genes responsible for sulphonamide resistance are (sul 1, sul 2 and sul 3) **(Sharma *et al*., 2016).** Pigeons may play a role in disseminating multidrug-resistant *Escherichia coli* in the environment, by contaminating drinking water supplies, or spreading antibiotic resistant strains in farm environments (**Checkabab *et al*., 2013**). Antimicrobial-resistance *E. coli* occurred in diseased pigeons in various results as described by (**Ibrahim, 2007 and Dutta *et al.,* 2013).** The *E. coli* strains in particular had acquired resistance against the most commonly used antimicrobial in pigeons **(Kimpe *et al.,* 2002).** Antimicrobial resistances to amikacin, ampicillin, trimethoprim-sulfamethoxazol, estreptomycin, tetracycline neomycin erythromycin and doxycycline were detected in of *E. coli* isolates from pigeon **(Radimersky *et al*., 2010 Hassan and Bakeet, 2014)**.

In the light of the above, the current study was designed to survey the incidence of *E. coli* pathogens in apparently healthy, diseased and freshly dead squabs, their dissemination, virulence genes and antibiotic resistance profiles and to assess the risk for human consumers as well as in the choice of drugs and treatment.

Materials and Methods

Sample Collection:

A total of 450 samples were collected from 125 squabs (1-4weeks old) from different pigeon houses at Dakahalia province. Fifty samples (cloacal and muscles,25 for each) were collected from 25 apparently healthy squabs, 200 samples were aseptically and separately collected from heart blood, liver, lung, intestine and muscles of 50 diseased birds and 200 samples from the same organs of 50 recently dead birds.

Clinical, post mortem and sensory examination:

All pigeons were examined clinically, then sacrificed and immersed in a disinfectant before being autopsied. Gross pathological changes were recorded, summarized and presented with results for both recently dead and clinically diseased pigeons. While, apparently healthy squabs sensory evaluated after slaughter for general acceptance according to (**Land and Shepherd, 1989).**

Isolation and identification of *E. coli*:
Each sample was inoculated separately into buffer peptone water(25 gm from each tissue sample homogenated in 225 ml peptone water) and incubated at 37ºC for 18-24 hrs under aerobic condition. Then, samples were cultured on 5% sheep blood and MacConkey agar (Merck, Germany) media and incubated at 37°C for 24 h. Colonies with the typical color and appearance of *E. coli* were picked and streaked again on blood agar plates and re-streaked on EMB agar (Merck, Germany). All plates were further incubated for 24 h at 37°C. The green metallic sheen colonies were considered as *E. coli*. The confirmation of the suspected isolates was performed by biochemical tests, including conventional lactose and glucose fermentation (using TSI medium), urease, indol, methyl red, Voges Proskauer, citrate and lysine decarboxylase **(Quinn *et al*., 2011).** Congo red media was used for differentiation between pathogenic and non-pathogenic bacteria. Pink colored colonies were considered as pathogenic .

Serological identification of *E. coli*:
The somatic (O) antigen was determined by slide agglutination test according to **Edwards and Ewing, (1972)**. Serotyping of the 52 isolated *E. coli* strains was carried out at The Serology Unit in Animal Health Research Institute Dokki, Cairo, Egypt.

Antimicrobial sensitivity testing
A total of 52 pathogenic *E. coli* were tested by disc diffusion method using Muller-Hinton agar using 13 antibiotic disc belongs to seven different antimicrobial classes includ-ing sulfamethoxazole (100 µg/disk), levofloxacin (5 µg/disk), chloramphenicol (30 µg/disk), norfloxacine (10 µg/disk),), tetracycline (30 µg/disk), streptomycin (10 µg/disk), cefoxitin (30 µg/disk), neomycin (30 µg/disk), doxycy-cline (30 µg/disk), cefepime (30 µg/disk), cefotaxime (30 µg/disk), penicillin (10 µg/disk), amoxicillin (10 µg/disk), and nalidixic acid (30 µg/disk). Interpretation of the results was done following Clinical and Laboratory Standards Institute Guidelines **(CLSI, 2013)**.

Detecting virulence and antimicrobial resistance genes by PCR:
Seven strains were examined for the presence of the 6 virulence genes (fimc, *tsh*, *iss*, *papC, irp-2* and vat) and 3 antibiotic resistance genes (tet A, cat1 and sul1) using PCR in PCR unit in Animal Health Research Institute Dokki, Cairo, Egypt.

DNA extraction: The DNA was extracted with DAN extraction kits (QIAamp DNA Mini Kit Qiagen Germany, Cat. No. 51304)**.**

Primer selection (Sigma): The selected primers were shown in table (1)**.**

PCR amplification: DNA was amplified in a total of 50 µl of the following reaction mixture: 25µl Dream Taq TM Green Master Mix (2X), 1µl of each primers, 3µl template DNA and completed to 50 µl by water, nuclease-free. PCR cycling program was performed in thermal cycler (BIO-RAD S-1000 thermal cycler USA) as in cycling protocol.

Cycling protocol (Thermo scientific):
FimC gene: initial denaturation at 94°C for 5 minutes then 30 cycles consisting of (denatureation at 94°C for 60 seconds, annealing at 59°C for 60 seconds and extension at 72°C for 60 seconds) followed by final extension at 72°C for 5 minutes.

Multiplex PCR of (iss, irp2 and tsh) and (vat and papC) virulence genes :
PCR cycling program was performed as following: initial denaturation at 94°C for 3 minutes then 25 cycles consisting of (denaturation at 94°C for 30 seconds, annealing at 58°C for 30 seconds and extension at 68°C for 3 minute) followed by final extension at 72°C for 10 minutes.

Multiplex PCR of various antimicrobial resistance genes: The PCR steps consisted of 5 min intial denaturation at 95C, 30 cycles of 1 min denaturation at 95C, 1 min of primer binding at 51 and 1 min of extension at 72C, followed by final extension at 72C for 7 min.

Detection of PCR products: Five µl of each amplicon were loaded on 1.5% agarose gel containing 1 µl of ethidium bromide/ gel. A 100 bp DNA ladder was used as a molecular weight standard (Thermo scientific Company, Cat.No.SM0243, US). The samples were electrophoresed at 90 V for 90 minutes on a mini horizontal electrophoresis unit (BIO-RAD, USA); the gel was visualized under UV trans illuminator (Spectroylyne Model TR-312 A) and photographed.

Table (1). Primer sets for detection of target virulence genes from avian pathogenic Escherichia coli (APEC) isolates

GENES	Oligonucleotide sequence (5`3`)	Amplicon size (pb)	References
fimC	5` GGGTAGAAAATGCCGATGGTG 3` 5` CGTCATTTTGGGGGTAAGTGC 3`	(497 bp)	**(Janßen *et al.*, 2001)**
Iss	5` ATCACATAGGATTCTGCCG 3` 5` CAGCGGAGTATAGATGCCA 3`	(309 bp)	**(Ewers *et al.*, 2005)**
irp2	5` AAGGATTCGCTGTTACCGGAC 3` 5` AACTCCTGATACAGGTGGC 3`	(413 bp)	**(Ewers *et al.*, 2005)**
Tsh	5` ACTATTCTCTGCAGGAAGTC 3` 5` CTTCCGATGTTCTGAACGT 3`	(824 b p)	**(Ewers *et al.*, 2005)**
Vat	5`- TCCTGGGACATAATGGTCAG-3 5`- GTGTCAGAACGGAATTGT-3`	(981-bp)	**(Ewers *et al.*, 2005)**
Pap C	5`- TGATATCACGCAGTCAGTAGC-3` 5`- CCGGCCATATTCACATAA -3`	(501pb)	**(Ewers *et al.*, 2005)**
TetA	F GCTACATCCTGCTTGCCTTC R GGCAGGCAGAGCAAGTAGAG	(280 bp)	**(Kanokwan *et al.*, 2009)**
cat1	F CCACCGTTGATATATCCCAA R CATTCTGCCGACATGGAA	(587bp)	**(Kanokwan *et al.*, 2009)**
sul1	F CCGATGAGATCAGACGTA R CCCAGATCCTTTACAGGA	(505 pb)	**(Kanokwan *et al.*, 2009)**

Experimental trials:

Pigeon: Fifty (50) clinically healthy pigeons (four weeks old) were obtained from commercial lofts were used for the experimental infection .Five of them examined bacteriologically to free from *Escherichia coli* infection .The remaining 45 pigeons classified into three groups (15 birds /group).

Experimental design:

Group A (GA): non infected non medicated (negative control).

Group B (GB): were inoculated with *E. coli (*O78 originally isolated from diseased pigeon with afield case of coli septicemia*)* orally (3×10^8 CFU/0.25 ml) (positive control).

Group C (GC): were inoculated with *E. coli* orally (3×10^8 CFU/0.25 ml) and medicated with Norfloxacin in drinking water at dose 10 mg/kg b.wt after two days post infection three successive days (8h/day).

Evaluation parameters*:*

Morbidity and mortality rates: The birds in the challenged group were observed daily after the challenge for any symptoms and deaths all the period of experiment (3 weeks). Dead birds were necropsied immediately after detection of their death and macroscopical lesion scores were reregistered. Also, on weekly basis, at 7, 14, 21 days post experimental infection two birds of surviving pigeon from each group were sacrificed and necropsied.

Bacteriological investigation: For bacterial re -isolation swabs from lungs, heart, livers, intestine and muscles were collected from two sacrificed birds in each group at the 1st, 2ndand3rd weeks after the challenge. Reisolation was done as recommended **by Sambrook *et al.* (1989**).

Results

Clinical signs, Postmortem lesions and sensory evaluation:

The clinical signs of the diseased birds with collibacillosis were depression, respiratory distress, reduced food consumption, loss of weigh, weakness, ruffling of feathers, yellowish diarrhea. While, the post mortem examination for dead and diseased birds showed Congestion of liver, spleen, kidneys and lungs, pericarditis, perihepatitis and air sacculitis. Congestion and hemorrhage in the intestinal mucosa and in some cases showed catarrhal enteritis (fig. 1). All apparently healthy squabs were accepted sensory.

Prevalence of *E. coli* within the Examined Birds:

Out of 450 examined samples, *E. coli* was identified in 24.22% (109/450) of the total examined samples based on morphological and biochemical characteristics. 27 strains (54%) were isolated from cloacal swabs and muscles of apparently healthy birds, 28 strains (14%) from diseased and 54 strains (27%) from freshly dead birds. Congo red media was used for differentiation between pathogenic and non pathogenic *E. coli*. 52 strains out of 109 strains were found Pathogenic which were observed as pink colonies on Congo red media (47.7%) (table 2). The recovery rates of the *pathogenic E. coli* from dif-ferent Squabs samples were 15%, 13.75%, 12.5%, 10%, 8.3%, 9.2% and 8% form liver, spleen, heart, lung, intestine, muscles and cloacal swabs, respectively (Table 3). *E. coli* isolates were serotyped into 7 serotypes including O78, O2, O128, O111, O114, O27, O125 the most prev-alent serotypes were O78, O2 and O128 with an incidence of 21.5%, 17.3%, and 15.38%, respectively (table 4).

Antimicrobial resistance profiles of *E. coli* isolates:

The antimicrobial susceptibility pattern of (52) pathogenic *E. coli* strains isolated from cloacal swabs and internal organs of the examined infected pigeons revealed variable results insusceptibility and zones of inhibition to the different antimicrobial drugs which commonly used in pigeon treatments. *E. coli* isolates demonstrated high rates of resistance to Tetracycline, Doxycycline, Chloramphenicol, and Sulfamethoxazole (100%, 94.23%, 84.62% and 76.92%) followed by Penicillin and Amoxicillin (71.15% and 65.38%) Conversely, Norfloxacin, Levofloxacin, Nalidixic acid and Cefotaxime showed the lowest resistance rate against *E. coli* isolates (7.69%, 9.62%, 11.53% and 13.46%, respectively) Table (5).

Virulence and antibiotic resistance genes

In this study, the presence of 6 virulence genes, including *iss, tsh, fimc vat, papc,* and *irp2* were verified by multiplex PCR analysis. Among the virulence genes detected, fimc*, iss*, and tsh were the most prevalent genes (100% each), followed by *papc* (71.4%), vat (57.1%), and *irp2* (42.8%). Also multiplex PCR was used for detection of anti-biotic resistance genes including tetA*, cat1* and *sul1*. The recovery rate of tet A was 100% while *cat1* and *sul1* was (42.8% both) table (6).

Results of experimental infection:

Clinical signs:

No clinical signs were observed in the uninfected-untreated group (GA). The clinical symptoms appeared within 48 hours after inoculation of *E. coli*. The clinical symptoms observed were; Reduced birds activity, rise in temperature, in-appetite, dullness, dysentery, shock and depression. Within 24 hours following initiation of treatment norfloxacin reduced the clinical signs which were present in infected birds prior to treatment and the signs continued to improve in the next days post-medication**.** Infected Birds treated with norfloxacin improved clinically during and after the 3-days treatment period and clinical signs disappeared within 5 to 7 days following treatment.

Post mortem:

Postmortem lesions of the dead and scarificed pigeons showed inflammation of liver, heart, spleen, lungs and intestine. The severity of postmortem lesions was severe in group B (GB) than group C (GC). There were no lesions appeared in group A (GA) (negative control). Seven days following initiation of norfloxacin treatment lesions virtually disappear**.**

Re-isolation of *E. coli* from organs:

All pigeons in uninfected-untreated group (GA) were negative for isolation of the challenge bacteria (Table7). *E. coli* could be recovered from tissues in some birds which infected and treated with norfloxacin in the drinking water (GC) with re-isolation rate of 6.6% to 13.4%. While infected-untreated group (GB) had a higher frequency of *E. coli* re-isolation that ranged from 20% to 53.3% from different organs than those from treated birds. These results demonstrated that norfloxacin reduced the number of birds from which *E. coli* was isolated.

Table (2). Prevalence of *E. coli* in examined samples collected from squabs

Squabs	Number of examined sample	No. of *Escherichia coli* positive samples	Prevalence (%*)	No. of *E. coli* positive Congo red	Prevalence (%**)
Healthy	50	27	54	4	14.8
Diseased	200	28	14	14	50
Recently dead	200	54	27	34	62.96
Total	450	109	24.22	52	47.7

(%*) No of *E. coli* positive samples/No. of examined samples.
(%**) No. of *E. coli* positive congo red / No. of *Escherichia coli* positive samples.

Table (3). Recovery rate of positive pathogenic *E. coli* from examined samples.

Organs	No of examined samples	No of positive	%
Liver	80	12	15
Spleen	80	11	13.75
Heart	80	10	12.5
Lung	60	6	10
Intestine	60	5	8.3
muscles	65	6	9.2
cloacal	25	2	8

N.B.: 65 muscles samples, 25 from apparently healthy (2 pathogenic isolates) and 40 from diseased and freshly dead (4 pathogenic isolates).

Table (4). Serotyping of *E. coli* isolates and its percentage

Serogro up	O78	O2	O128	O111	O114	O27	O125	UNTYPED
Number	11	9	8	6	6	5	5	2
%	21.15	17.3	15.38	11.53	11.53	9.62	9.62	3.85

Table (5). Results of antimicrobial sensitivity test for *E. coli*

Antimicrobial agent	Antimicrobial class	Resistant		Sensitive	
		No	%	No.	%
Amoxicillin	β-lactams	34	65.38	18	43.62
Penicillin	β-lactams	37	71.15	15	28.85
Cefoxitin	Cephalosporins	8	15.38	44	84.62
Cefotaxime	Cephalosporins	7	13.46	45	86.54
Sulfamethoxazole	Sulfonamides	40	76.92	12	23.08
Neomycin	Aminoglycosides	10	19.23	42	80.77
Streptomycin	Aminoglycosides	12	23.07	40	76.92
Tetracycline	Tetracycline	52	100	0	0
Doxycycline	Tetracycline	49	94.23	3	5.77
Chloramphenicol	Phenicols	44	84.62	8	15.38
Norfloxacin	Quinolones	4	7.69	48	92.31
Levofloxacin	Quinolones	5	9.62	47	90.38
Nalidixic acid	Quinolones	6	11.53	46	88.46

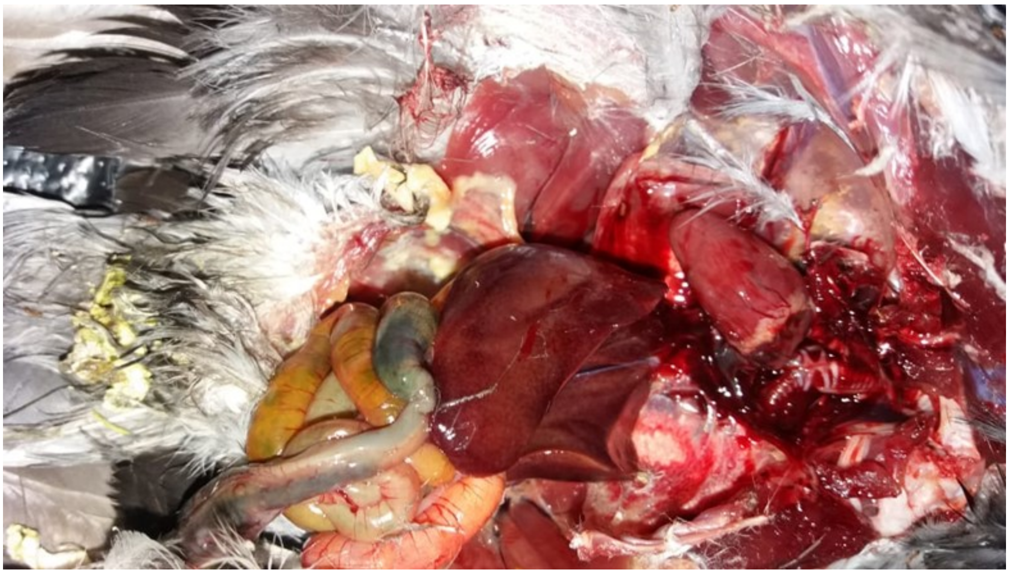

Fig. (1): Squab (3 week) suffering collibacillosis showing congested liver and enteritis (field case)

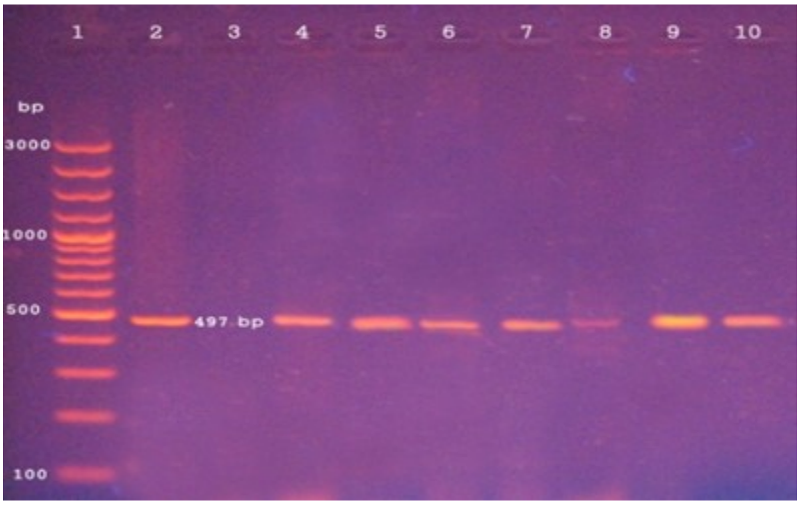

Fig. (2): Agarose gel electrophoresis of *E. coli* DNA product (*fim*C gene). Lane 1: 100 bp DNA ladder "Marker" Lane (2): control positive. Lane (3): control negative . Lane (4-10): *E. coli* field isolates.

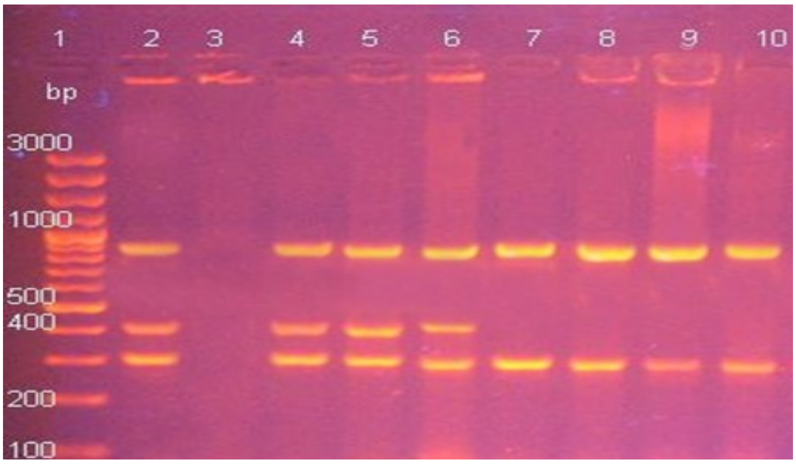

Fig. (3): Agarose gel electrophoresis of *E. coli* DNA product virulence genes (iss, irp2 and tsh genes) in a multiplex PCR. Lane (1): 100 bp DNA ladder. Lane (2): control positive. Lane (3): control negative Lane (4-10): *E. coli* field isolates.

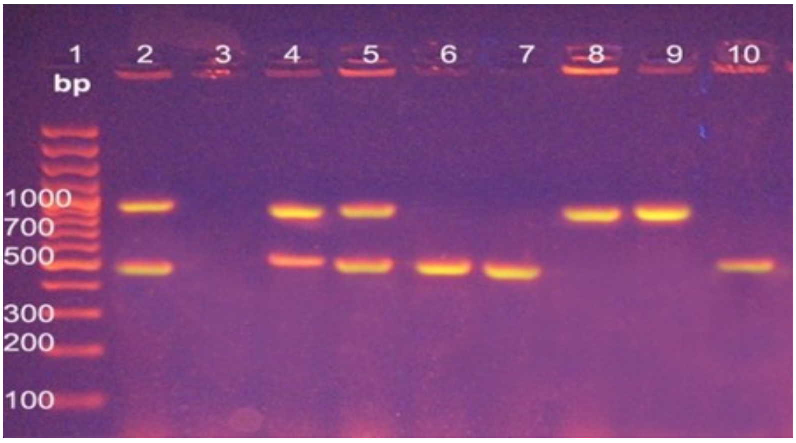

Fig. (4): Agarose gel electrophoresis of *E. coli* DNA product virulence genes (papc, and vat genes) in a multiplex PCR. Lane (1): 100 bp DNA ladder. Lane (2): control positive. Lane (3): control negative Lane (4-10): *E. coli* field isolates

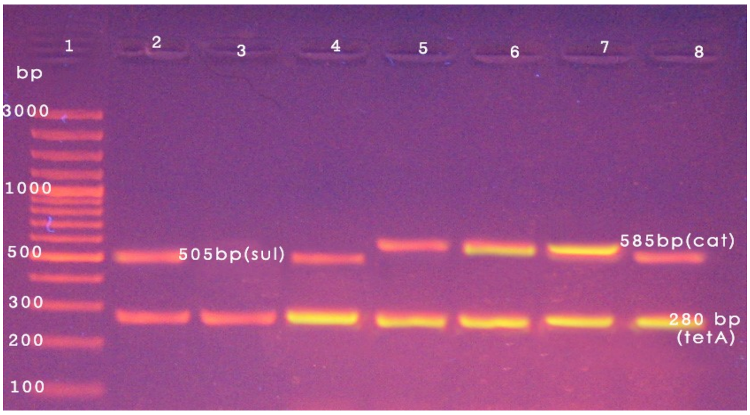

Fig. (5): Agarose gel electrophoresis of *E. coli* DNA product (antimicrobial resistance genes). Lane 1: 100 bp DNA ladder "Marker".Lane (2-8): *E. coli* field isolates.

Table (6). Recovery rate of virulence and antibiotic resistance genes in isolated *E .coli*

Samples number	Virulence genes						Antimicrobial resistance genes		
	Fim c	iss	tsh	Irp2	papc	vat	cat	Sul1	Tet A
1	+	+	+	+	+	+	-	+	+
2	+	+	+	+	+	+	-	-	+
3	+	+	+	+	+	-	-	+	+
4	+	+	+	-	+	-	+	-	+
5	+	+	+	-	-	+	+	-	+
6	+	+	+	-	-	+	+	-	+
7	+	+	+	-	+	-	-	+	+
Total	100%	100%	100%	42.8%	71.4%	57.1%	42.8%	42.8 %	100%

Table (7). Re-isolation of *E .coli* from the internal organs of pigeon experimentally infected with *E. coli*

Experimental groups	No .of examined pigeon	Number and percentage of positive samples with pathogenic *E. coli* isolation from internal organs				
		Liver	Heart	spleen	Lung	muscles
GA	15	0/15(0%)	0/15(0%)	0/15(0%)	0/15(0%)	0/15(0%)
GB	15	8/15 (53.3%)	7/15(46.67%)	6/15(40%)	4/15(26.7%)	3/15(20%)
GC	15	2/15(13.4%)	1/15(6.66%)	0/15(0%)	1/15(6.66%)	0/15(0%)

Discussion

Housing system of pigeons under Egyptian conditions give the chance for pigeons to come into close contact with wild and domesticated bird that enabling direct transfer of the infectious agents to take place especially when kept out to doors. Pigeon spreads of infectious agents through fecal contamination of drinking water sources, pastures and agricultural crops.

In the present study, 109 *E. coli* isolates (24.22%) were recovered from 450 samples collected from healthy, diseased and recently dead squabs. Compared with our findings, Nearly similar results were recorded by **Hassan and Bakeet, (2014**) who isolated *E. coli* from squabs with total recovery rate (19.7%), while much higher isolation rate of *E. coli* (60.67 %) has been reported by **(Dutta *et al.,* 2013).** The incidence of *E. coli* isolates according to the status of the examined squabs it give higher incidence in freshly dead squabs (27%) than diseased squabs (14%), the results in literatures obtained by **(Ibrahim, 2007 and Hassan *et al.,* 2008**) similar to our results.

The rate of isolation of *E. coli* from apparently healthy squabs was (54%). Higher recovery rates were recorded in a previous study carried out by **(Dey *et al*.,2013)** who isolated *E. coli* from apparently healthy pigeons with recovery rate (86.11%). However, lower isolation rate (36%) was reported by **Chika, 2018**. Noteworthy, these asymptomatic carriers represent a potential source of human colibacillosis (**Behravesh *et al*., 2014)** due to an increasing infection pressure in the environment **(Kabir, 2010**).

The characteristic of CR (congo red media) binding constitutes a moderately stable, reproducible, and easily distinguishable phenotypic marker. Pathogenic *E. coli* can be identified by their abil-ity to bind CR and produce brick red colonies **(Abhilasha and Gupta, 2001).** The results of the CR-binding assay indicate that 52 isolates of 109 (47.7%) were positive. A similar result recorded by (**Riaz *et al*., 2016**) who reported that 40% of avian *E. coli* iso-lates was CR positive.

In the current study, Pathogenic *E. coli* was isolated from both intestine and other extra-intestinal organs. The prevalence rates of pathogenic *E. coli* in the collected samples were 15%, 13.75%, 12.5%, 10%, 8.3% and 9.2% from the examined liver, spleen, heart, lung, intestine and muscles, respectively. The dissemination of *E. coli* into different organs such as liver, lungs and heart is going in agreement with the postmortem lesions including perihepatitis, pericarditis, enteritis and pneumonia indicating that *E. coli* led to septicemia and followed by death of the birds **(Kabir, 2010).**

In the present study, out of 25 examined muscles samples from apparently healthy squabs only 2 samples (8%) were contaminated with pathogenic *E. coli* which indicate bad sanitation during slaughtering and processing specially with intestinal content. In contrary to the present findings **(Abdou, 2017)** reported higher rate of isolation of *E. coli* (15%) from poultry meat.

A more specific identification approach is the serotyping analysis that aims at classifying pathogenic strains based on their surface antigens. Based on O, H, and K antigens. O Serogrouping is one of the basic diagnostic methods for the classification of pathogenic strains of *E. coli*. In the present study, 52of the obtained *E. coli* isolates recovered from squabs were serotyped by slide agglutination test. In this study, seven O serogroups were identified among the 52 APEC isolates could be typed, the most prevalent serogroups were O78, O2 and O128 with a prevalence of 21.15%, 17.3% and 15.38%, respectively. In many studies conducted in Egypt, nearly the same serotypes were detected in diseased pigeons with a predominance of O78 have been identified (**Ibrahim, 2007**). O2 and O78 are the most frequently isolated from colibacillosis in the many countries worldwide that proven their role as particularly adapted pathogens that allow involvement in extra intestinal infections **(Ewers *et al*., 2004).**

It is clear from the achieved results that, none of the antibiotic showed 100% effectiveness against *E. coli* meanwhile the *E. coli* serotypes showed higher sensitivity to norfloxacin (92.3%), levofloxacin (90.38%), nalidxic acid (88.47%) and cefotaxime (86.54%). High level of resistant was observed with tetracycline, doxycycline, chloramphenicol sulphamethaxole and penicillin (100%, 94.23%, 84.62, 76.92%, and 71.15%, respectively). These results go in parallel with those obtained by (**Chidamba and Korsten 2015 and Chika, 2018)** who declared that *E. coli*, isolated from pigeon showed marked sensitivity to norfloxacin, cefoxitin and nalidixic acid. Similar antibiotic resistance patterns with *E. coli* isolated from pigeons for Tetracycline, Doxycycline, sulphamethaxazole and chloramphenicol were reported by (**Dey *et al.,* 2013**, **Stenzel *et al.,* 2014** and **Zigo *et al*., 2017**). Pigeons may play a role in disseminating multidrug-resistant *Escherichia coli* in the environment, by contaminating drinking water supplies, or spreading antibiotic resistant strains in farm environments (**Checkabab *et al*., 2013)**.

The pathogenesis of *E. coli* is related to the wide range of different virulence genes. In this study, the presence of 6 virulence genes, including *iss, tsh, fim c, vat, papc,* and *irp2*were verified by multiplex PCR analysis. Among the virulence genes detected, *iss, fimc* and tsh were the most prevalent genes (100% each), followed by *papc* (71.4%), vat (57%), and *irp2* (42.8%). The *iss* gene is an important gene present in APEC which is responsible for serum resistance **(Sampaio *et al*., 2010**). Several studies have indicated that *E. coli* can escape from the bactericidal actions of complement system due to serum resistance; this may lead to avian colisepticemia **(Parreira *et al*., 1998).** In this study, *iss* gene was detected in 100% of APEC isolated from squabs. This finding was similar with (**Kwon *et al*., 2008 and Ammar *et al*., 2015)** who detected *iss* gene in 100% of *E. coli* isolated from broiler chickens. Contrary to the present findings **Won *et al.* (2009)** reported low prevalence of *iss* gene(41.5%)in isolated *E. coli* from broilers. The temperature-sensitive haemagglutinin (*tsh*) gene help to development of lesions and fibrin precipitation in air sacs, increase colonization at this site and induce lesion and ulcers. The finding of present study (100%) was in accordance with **Arabi *et al.* (2013**) who detected *tsh* gene in (96.4%) of avian pathogenic genes. Contrary to the present findings (**Roussan *et al*., 2014 and Chaudhari *et al*., 2017)** reported low prevalence of *tsh* gene (55% and 50%) among virulent *E. coli.* The type1 fimbriae C (fimC) gene is an important gene having the role in the internal organ adhesion **(JanBen *et al*., 2001).** The high prevalence of fimc gene in the present study are similar with (**Knobl *et al*., 2001)** who detected fimC in (96%) of isolates from septicemiac cases. The *papc* gene provides the capacity to binding to internal organs and protects against hetero-phils **(Johnson, 1991*)***. In this study 71.4% of isolates was positive for papc. This finding was lower than the report of (**Ammar *et al*., 2015)** who detected *papc* gene in 82% of isolated *E. coli* strains. Contrary to the present findings (**Nyrah *et al.,* 2017** and **Subedi *et al*., 2018)** who reported low rate of isolates (33.72% and 11%) carrying *papc* gene in *E. coli*. *Vat* gene responsible for vacuolating and transferring of cytotoxic produced by pathogenic. In the current study 57% of isolates were positive for vat gene. This results was higher than prevalence previously reported by **Arabi *et al*., (2013) and Roussan *et al*. (2014)** who detecte d*vat* gene in 85.7% and 70% in APEC whereas (**Nyrah *et al.,* 2017)** reported lower prevalence (31.39%) of *vat* gene. Iron uptake-genes include iron-repressible high-molecular-weight proteins 2 (irp2) is one of the most the most prevalent iron uptake-genes among APEC strains. In this study (irp2) gene was detected with prevalence (42.8%) which is higher than the prevalence previously reported by (**Ibrahim, 2019)** who detected (irp2) gene in (34%) APEC. Whereas **Nyrah *et al.,* (2017)** detected irp2in (55.23%).

Many studies have been reported the presence of antibiotic resistance genes in APEC strains **(Ahmed and Shimamoto, 2013).** The resistance genes mediated by plasmid can make the resistance prevail among various bacteria that lead to acquiring resistance genes without difficulty and pro-duce MDR **(Liu *et al*., 2012).** Regarding the distribution of these antibiotic resistance genes among *E. coli* isolates, The most frequent resistance found was to tet A (100%) and (42.8%) for both sul1 and cat1.

In contrast to our results, a higher percentage of resistance to tetracycline, sulphonamides and chloramphenicol was found in this study compared to data reported by **Blanco-Pena *et al*., (2017)** who detected tetA, sul1and cat1 in (14.2%, 17.7% and 29.1%) of *E. coli* isolated from pigeon, respectively. The higher rates of antimicrobial resistance and MDR in strains could be due to environmental contamination with antibiotic residues in aviculture industries and/or selective pressure caused by the indiscriminate use of antimicrobial compounds as a result of poor monitoring by regulatory bodies **(Koga *et al*., 2015**).

Our experimental study was conducted to evaluate pathogenicity of isolated virulent field strain of *E. coli* (O78) and assess the efficacy of norfloxacin in controlling the adverse effects of collibacillosis in experimentally infected pigeon. The clinical symptoms and gross pathological lesions observed in this study were observed by (**Haq *et al*., 2015**) in pigeons experimentally infected with *E. coli*. Using of norfloxacin at dose level of 10 mg/kg b.wt/day for 3 successive days (8hs / daily).
In the drinking water for the treatment of *E. coli* infected pigeon resulted in rapid control of experimental colibacillosis as evidenced by rapid resolution of clinical signs, significant reduction in mortalities, gross pathological lesions, rate of *E. coli* re-isolation. The low rate of re-isolate *E. coli* from the infected-treated pigeon indicates the advantage in using norfloxacin for treatment of this organism. Our results are supported by the work of many authors who found that all new quinolone derivatives including norfloxacin, were very active against *E. coli* infection **(Haq *et al*., 2015 and Lakho *et al*., 2017**).

Conclusion

This study indicates that squabs are reservoir of virulent and multidrug resistant *E. coli.* These strains may be create a potential danger to human health through food chain, if proper hygienic measures are not undertaken during rearing, handling and processing of pigeon.

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