The occurrence of the multidrug resistance (MDR) and the prevalence of virulence genes and QACs resistance genes in *E. coli* isolated from environmental and avian sources

Mohamed E. Enany¹, Abdelazeem M. Algammal¹*, Soad A. Nasef², Sara A. M. Abo-Eillil², May Bin-Jumah³, Ayman E. Taha⁴* and Ahmed A. Allam⁵

**Abstract**

Colibacillosis is a major disease affecting poultry leads to high morbidity and mortality which causing tremendous economic losses worldwide. These economic disparities are amplified among low and middle-income where sanitation and hygiene are challenged by the increasing demand for quality sources of animal protein. With a view to investigating the prevalence of virulence genes and QACs resistance genes as well as monitoring the antibiogram of *E. coli* strains, a total of 368 specimens were collected from diseased broiler chickens (n = 226) and environmental sources (n = 142) at large-scale poultry farms in Ismailia Governorate, Egypt. The bacteriological examination proved that *E. coli* prevalence was 26.76% and 50.44% in the farm environment and diseased broilers, respectively. In tandem, the isolated *E. coli* strains were serogrouped, determining the most common serotypes were O78, O1:H7, O91:H21 and O126. Isolates were tested for antimicrobial susceptibility against 12 antibiotics, screened for 4 virulence genes (*iss, papC, eaeA, and cfaI*), and screened for 3 QACs resistance genes (*qacEΔ1, qacA/B, and qacC/D*). All the tested strains were positive for *iss* and *papC* genes, only 20.3% of the tested strains were positive for *eaeA* gene, moreover, the examined strains were negative to *CfaI* gene. Furthermore, all the tested strains were positive for *qacED1*, *qacA/B*, and *qacC/D* genes. In conclusion; virulence genes (*iss, papC*) as well as QACs resistance genes are common in avian Pathogenic *E. coli* and environmental strains and are mainly associated with multi-drug resistance phenomena.

**Keywords:** *E. coli*, Chickens, Virulence genes, QACs resistant genes

**Introduction**

The rise of poultry production and industrial breeds of chicken, such as meat broilers, have been instituted as a method to promote gender equity, economic stability, and food security within many low and middle-income countries (LMICs). Despite the benefits, large-scale poultry production facilities within LMICs are often confronted with a tradeoff between animal welfare and addressing a growing economic demand, leading to high amounts of sub-therapeutic antibiotics for growth promotion and prophylaxis as well as disinfectant agents (Joint 2008; Udomsantisuk et al. 2011). High use of antibiotics and disinfectants could promote further antibiotic resistance (ARB) and disinfectant resistance (DR) (Eid et al. 2016).

One primary target for antibiotic and disinfect use is colibacillosis, which remains one of the major drivers of poultry morbidity and mortality, leading to severe losses (Barnes et al. 2008). These biosecurity risks can be further intensified as some *E. coli* avian diseases can be zoonotically transmitted via a trophic transmission (González-Zorn et al. 2005) or occupational exposure (Bisi-Johnson et al. 2011). Knowledge of *E. coli* serology...
can inform how to best treat diseases based upon typing categorizations. For instance, *E. coli* serotypes can cause intestinal illness with digestive signs, while other serotypes referred to as Avian Pathogenic *E. coli* belong to ExPEC that cause various symptoms in chicken either systemic or localized including: omphalitis, respiratory colibacillosis and colisepticemia (Rodriguez-Siek et al. 2005; Mellata 2013). The plasticity of *E. coli* pathogenicity is a result of an extensive range of virulence factors that are regulated and encoded by virulence determinant genes such as (iss, papC, eaeA, and CFAI) (De Carli et al. 2015; Eid et al. 2019). Studies have found links of co-selection for bacterial resistance from disinfectant and antibiotic use. Often, selection for resistance to antibiotics can inadvertently lead to drug resistance by movable genetic components (Noguchi et al. 2005; Chuanchuen et al. 2007). Additionally, these multimodal pathways for resistance can also promote increased pathogenicity in other species of bacteria through horizontal gene transfer. The quaternary ammonium compounds could be a major cause of the antibacterial cross-resistance development (Buffet-Bataillon et al. 2012). Various disinfectant resistant genes were recorded in multidrug-resistant pathogenic bacterial species (Zhang et al. 2015) including; qacA/B, qacC/D, qacE and qacG genes (Correa et al. 2008). Often, in low-to-middle scale animal operations often apply high amounts of disinfectants and subtherapeutic antibiotics to mitigate are chemical agents that used to kill microorganisms on inanimate instruments by various mechanisms as well as a wide spectrum of activity and potency (Fraise et al. 2013). Quaternary ammonium compounds are less toxic, non-irritating substances that are widely used to disinfect poultry farms environment (Bore et al. 2007). Many reports revealed a molecular relationship between qac genes and antibiotic resistance in certain pathogenic bacteria (Sidhu et al. 2001).

This study was aimed to investigate the prevalence of virulence genes (iss, papC, eaeA, and CFAI) and QACs resistance genes (qacEΔ1, QacA/B, and QacC/D) in *E. coli* strains originated from diseased broiler chicken and farm environment as well as monitoring of the antimicrobial susceptibility of the isolated strains.

**Materials and methods**

**Sampling**

A total of 368 specimens were collected aseptically from large scale farms [142 environmental samples: feeders (n = 32), drinkers (n = 32), walls (n = 36) and floors (n = 42) and 226 samples from diseased broiler chickens: heart (n = 70), liver (n = 82), lung (n = 12), yolk (n = 30), spleen (n = 16) and air sac (n = 16)] at Ismailia Governorate, Egypt. Average broiler chicken age was 7 weeks and the average weight was 1.8 kg. Handling of birds was performed according to the Animal Ethics Review Committee of Suez Canal University, Egypt. Samples were collected in the period from November 2016 until August 2017. The collected samples were prepared for bacteriological examination.

**Isolation and identification of *E. coli***

The collected specimens were inoculated in peptone water and then incubated at 37 °C for 24 h. A loopful from the incubated broth was streaked onto MacConkey’s agar and EMB plates and then incubated at 37 °C for 24 h. Suspected colonies were identified by microscopic examination, cultural characters as well as biochemical reactions as described by Quinn et al. (2011).

**Serotyping of *E. coli* strains**

The isolates of *E. coli* were subjected to serotyping where somatic (O) antigen was investigated by slide agglutination test as described by Edwards and William (1972). Flagellar (H) antigen stereotyping was performed as described by Davies and Wray (1997).

**Antimicrobial susceptibility testing**

*Escherichia coli* strains were tested against 12 antimicrobial agents (ampicillin, amoxicillin/clavulanic acid, erythromycin, gentamicin, neomycin, tetracycline, doxycycline, levofloxacin, norfloxacin, trimethoprim/sulphamethoxazole, sulphamethoxazole, and colistin sulphate) according to the methods described by NCCLS (2015) using disc diffusion technique. The susceptibility was determined according to the size of the inhibition zone. Multidrug resistance (MDR) was categorized for resistance to two or more unique antibiotic classes.

**PCR detection of virulence and disinfectant resistance genes of *E. coli***

*Escherichia coli* serotypes (n = 113; 23 environmental strains and 90 strains of avian origin) were tested for the detection of 4 virulence genes (iss, papC, eaeA, and cfaI) and 3 QACs resistance genes (qacEΔ1, QacA/B, and QacC/D) by using PCR. The DNA extraction was performed as described by the instructions of QIAamp DNA mini kit. The reaction volume includes (6 μl of the extracted DNA, 12.5 μl of Emerald Amp GT PCR master mix (2 × premix) and 1 μl of each primer forward and reverse, PCR grade water 4.5 μl). Positive control strains were kindly given by Animal Health Research Institute, Dokki, Egypt. Primers used in PCR were illustrated in Table 1. PCR Protocol: initial denaturation at 94 °C for 5 min; denaturation at 94 °C for 30 s; annealing at 54 °C for 30 s for iss gene, at 51 °C for 30 s for eaeA, at 58 °C for 40 s for papC, at 50 °C for 40 s for cfaI, 58 °C for 40 s for
qacEΔ1 gene, at 53 °C for 40 s for qacA/B and at 53 °C for 30 s for qacC/D; extension at 72 °C for 30 s in papC and cfaI qacEΔ1 and qacA/B; cycles repeated for 35 times. Finally, the PCR products were separated by using electrophoresis and then photographed.

Statistical analyses
The data frequencies were analyzed by the nonparametric test (Chi square) with the aid of SAS (2004) software to test the null hypothesis of different treatment groups. The level of significance was P < 0.05.

Results
Prevalence of *E. coli* in diseased broiler chickens and farm environment
The bacteriological examination of 142 environmental samples revealed 38 *E. coli* strains (26.76%) including; feeders, drinkers, walls and floors samples with percentages of 37.5%, 31.25%, 11.11%, 28.57%, respectively. While 226 organ samples, revealed 114 *E. coli* strains with a prevalence of (50.44%). *E. coli* was isolated from internal organs (heart, liver, lung, yolk, spleen, air sac) with percentages of 42.86%, 60.98%, 33.33%, 20%, 50%, 100%, respectively. The total *E. coli* prevalence was (41.30%) as illustrated in Table 2.

Serotyping of the isolated *E. coli* strains
In this study, 38 *E. coli* strains originated from environmental samples were subjected to serological identification, 23 strains (60.5%) have belonged to the following 10 different serotypes: O78 (13.16%), O119:H4, O113:H4, O169, O91:H21, O142, O111:H2, O1:H7, O26:H11 and O128:H2 (5.26% for each), while 15 strains (39.5%) were untypable. In addition, 114 *E. coli* strains (originated from organs of diseased broilers) were subjected to serological identification, 90 strains (78.95%) have belonged to the following 12 different serotypes: O1:H7 (13.16%), O78 (13.16%), O126 (8.77%), O91:H21 (8.77%), O125:H21, O44:H18, O121:H7, O15:H2, O146:H21, O124, O20 and O128:H2 (4.39% for each), moreover 24 strains (21.05%) were untypable as described in Table 3.

Antimicrobial susceptibility of *E. coli* strains
As described in Table 4, the antimicrobial susceptibility testing of the isolated strains proved that, the tested strains were highly resistant (100%) to, ampicillin, erythromycin and tetracycline, followed by amoxicillin-clavulanic acid, norfloxacin, and sulphamethoxazole (80.92% for each), trimethoprim/sulphamethoxazole (75%) and gentamycin (50%). While (100%) of the tested strains were sensitive to colistin sulphate, followed by neomycin (87.5%). Meanwhile, the tested strains were intermediate sensitive to doxycycline (75%) and levofloxacin (62.5%).

PCR detection of virulence genes and QACs resistance genes
PCR was used for detection and amplification of 4 virulence genes (*iss, papC, eaeA* and *CFAI*) in the isolated strains as illustrated in Table 5, where (100%) of the tested strains were positive for *iss* gene at specific amplicon size 266 bp (Fig. 1a) and *papC* gene with specific amplicon size 501 bp (Fig. 1b). Only (20.3%) of the tested isolates were positive for *eaeA* gene with specified amplicon size 248 bp (Fig. 1c), the positive strains including; O1:H7 (n = 12), O78 (n = 5), O128:H2 (n = 2), O119:H4

| Primer | Target gene | Primer sequence (5′–3′) | Product (bp) | References |
|--------|-------------|--------------------------|--------------|------------|
| iss-1  | Iss         | F-ATGTTATTTCTGCGCCTCTTG  | 266          | Yaguchi et al. (2007) |
| iss-2  |             | R-CTATTGTGACGTTATACCC    |              |            |
| eaeA-1 ( intimin) | eaeA | F-ATGCTTTACGCAATATACCCA | 248          | Bisi-Johnson et al. (2011) |
| eaeA-2 |             | R-GCTCTTATGCTGTTGTTG     |              |            |
| papC-1 | papC        | F-TGATATCCACGGCTAGTGG    | 501          | Jin et al. (2008) |
| papC-2 |             | R-CCTGCTTACATCCGATATG    |              |            |
| CFAI-1 | CFAI        | F-GCTCTGACCAAAGTGTTGGA  | 364          | Ghosal et al. (2007) |
| CFAI-2 |             | R-TTACCCCGATGCAATGAAAT  |              |            |
| QacEΔ1-1 | qacEΔ1     | F-TAAACCCATACAAATCTGAGATAT | 362        | Chuanchuen et al. (2007) |
| QacEΔ1-2 |           | R-GTCTTATACCAATACATGTTG  |              |            |
| QacA/B-1 | qacA/B     | F-GACGAAAGTCTAGATGTTG   | 361          | Noguchi et al. (2005) |
| QacA/B-2 |           | R-CCAGTCCAATCATGCTGTA   |              |            |
| QacC/D-1 | qacC/D     | F-GCTCTGACCAAAGTGTTGGA  | 195          |            |
| QacC/D-2 |           | R-GACTACCGTTTGTAAAGACTAACT |          |            |
In addition, cfaI gene was absent in all examined strains as shown in (Fig. 1d). Concerning the detection of the QACs resistance genes, PCR was used for the detection and amplification of (qacAΔ1, qacA/B and qacC/D) genes in the isolated strains, all the tested strains (100%) were positive for qacAΔ1, qacA/B and qacC/D genes with specific ampli-

### Table 2 Total prevalence of E. coli isolated from all examined samples (feeder, drinker, wall, floor and organs of diseased broiler chickens)

| Sources                          | Type of samples | No. of examined samples | E. coli No. | %    | Chi square P value |
|----------------------------------|-----------------|-------------------------|-------------|------|-------------------|
| Environmental samples            | Feeder          | 32                      | 12          | 37.5 | 0.0792 NS         |
|                                  | Drinker         | 32                      | 10          | 31.25|                   |
|                                  | Wall            | 36                      | 4           | 11.11|                   |
|                                  | Floor           | 42                      | 12          | 28.57|                   |
| Total                            |                 | 142                     | 38          | 26.76|                   |
| Organs of diseased broiler chickens (ExPEC) | Heart | 70                      | 30          | 42.86| <0.0001*         |
|                                  | Liver           | 82                      | 50          | 60.98|                   |
|                                  | Lung            | 12                      | 4           | 33.33|                   |
|                                  | Yolk            | 30                      | 6           | 20   |                   |
|                                  | Spleen          | 16                      | 8           | 50   |                   |
|                                  | Air sac         | 16                      | 16          | 100  |                   |
| Total                            |                 | 226                     | 114         | 50.44| <0.0001*         |
| Total of all                     |                 | 368                     | 152         | 41.30|                   |

%The percentage was calculated according to the no. of each type of samples

Concerning the detection of the QACs resistance genes, PCR was used for the detection and amplification of (qacAΔ1, qacA/B and qacC/D) genes in the isolated strains, all the tested strains (100%) were positive for qacAΔ1, qacA/B and qacC/D genes with specific ampli-

### Table 3 Serotyping of E. coli strains isolated from environmental and diseased broiler chickens samples

| Serotypes | Environmental E. coli (n = 38) | Organs of diseased broiler chickens (ExPEC) (n = 114) |
|-----------|--------------------------------|----------------------------------------------------------|
| O119:H4   | 2                              | –                                                        |
| O113:H4   | 2                              | –                                                        |
| O78       | 5                              | 15                                                       |
| O169      | 2                              | –                                                        |
| O91:H21   | 2                              | 10                                                       |
| O142      | 2                              | –                                                        |
| O111:H2   | 2                              | –                                                        |
| O146:H2   | 2                              | –                                                        |
| O147      | 2                              | 15                                                       |
| O26:H11   | 2                              | –                                                        |
| O128:H2   | 2                              | 5                                                        |
| O126      | –                              | 10                                                       |
| O125:H21  | –                              | 5                                                        |
| O44:H18   | –                              | 5                                                        |
| O121:H7   | –                              | 5                                                        |
| O15:H2    | –                              | 5                                                        |
| O146:H21  | –                              | 5                                                        |
| O124      | –                              | 5                                                        |
| O20       | –                              | 5                                                        |
| Total     | 23/38                          | 90/114                                                   |
| Untyped   | 15/38                          | 24/114                                                   |

* Chi square (P < 0.0243)

### Discussion

Cross-resistance between antibiotics and QAC could occur by various mechanisms on the same resistance plasmid and or transposon (Hegstad et al. 2010). The presence of Quaternary Ammonium Compounds determinants on various mobile constituents helps in the transport of resistance to another microorganism (Gillings et al. 2009). The massive and improper application of antibiotics for long-term in poultry farms resulted in multidrug resistance in different bacterial pathogens (Singer and Hofacre 2006). In the present work, the bacteriological examination showed that the prevalence of E. coli in environmental specimens was (26.76%), while was (50.44%) in diseased broiler chickens. The total prevalence of E. coli was (41.30%) as illustrated in Table 2.

The prevalence of E. coli from organs of diseased broiler chickens compared to those of the environmental sources. The highest prevalence was recorded in air sac (100%) followed by liver (60.98%), while the lowest prevalence was recorded in the yolk (20%). Higher prevalence of (84%) was obtained by Oboegbulem et al. (2009) who...
Table 4  Results of antimicrobial susceptibility testing of the isolated *E. coli* strains (n = 152)

| Antimicrobial disc       | No. of *E. coli* (n = 152) | Resistant | Intermediate | Sensitive |
|--------------------------|-----------------------------|-----------|--------------|----------|
|                          | No | %   | No | %   | No | %   |
| Ampicillin               | 152 | 100 | 0 | 0 | 0 | 0 |
| Amoxicillin/clavulanic acid | 123 | 80.92 | 29 | 19.08 | 0 | 0 |
| Erythromycin             | 152 | 100 | 0 | 0 | 0 | 0 |
| Gentamicin               | 76 | 50 | 0 | 0 | 76 | 50 |
| Neomycin                 | 0 | 0 | 0 | 0 | 152 | 100 |
| Tetracycline             | 152 | 100 | 0 | 0 | 0 | 0 |
| Doxycycline              | 38 | 25 | 114 | 75 | 0 | 0 |
| Levofoxacin              | 48 | 31.58 | 95 | 62.5 | 9 | 5.92 |
| Norfloxacine             | 123 | 80.92 | 19 | 12.5 | 10 | 6.58 |
| Trimethoprim/sulphamethoxazole | 114 | 75 | 0 | 0 | 38 | 25 |
| Sulphamethoxazole        | 123 | 80.92 | 0 | 0 | 29 | 19.08 |
| Colistin sulphate        | 0 | 0 | 0 | 0 | 152 | 100 |

Chi square P value < 0.0001*  < 0.0001*  < 0.0001*

% calculated according to No of tested *E. coli* strains

Table 5  Prevalence of virulence genes and QACs resistance genes between the isolated *E. coli* serotypes

| Sources                      | Type of sample | Serotypes          | No of tested serotypes | iss | papC | eaeA | cfaI | qacΔ1 | qacA/B | qacC/D |
|------------------------------|----------------|--------------------|------------------------|-----|------|------|------|-------|--------|--------|
| Environmental *E. coli*      | Feeder         | O119:H4            | 2                      | 2   | 2    | 2    | 0    | 2     | 2      | 2      |
|                              |                | O113:H4            | 2                      | 2   | 2    | 2    | 0    | 2     | 2      | 2      |
|                              |                | O142               | 2                      | 2   | 2    | 0    | 0    | 2     | 2      | 2      |
|                              | Floor          | O78                | 5                      | 5   | 5    | 5    | 0    | 5     | 5      | 5      |
|                              |                | O111:H2            | 2                      | 2   | 2    | 0    | 0    | 2     | 2      | 2      |
|                              |                | O1:H7              | 2                      | 2   | 2    | 2    | 0    | 2     | 2      | 2      |
|                              | Drinker        | O26:H11            | 2                      | 2   | 2    | 0    | 0    | 2     | 2      | 2      |
|                              |                | O169               | 2                      | 2   | 2    | 0    | 0    | 2     | 2      | 2      |
|                              |                | O91:H21            | 2                      | 2   | 2    | 0    | 0    | 2     | 2      | 2      |
|                              | Wall           | O128H2             | 2                      | 2   | 2    | 2    | 0    | 2     | 2      | 2      |
|                              | Heart          | O15:H2             | 5                      | 5   | 5    | 0    | 0    | 5     | 5      | 5      |
|                              |                | O78                | 7                      | 7   | 7    | 0    | 0    | 7     | 7      | 7      |
|                              |                | O91:H21            | 5                      | 5   | 5    | 0    | 0    | 5     | 5      | 5      |
|                              |                | O124               | 5                      | 5   | 5    | 0    | 0    | 5     | 5      | 5      |
|                              |                | O146               | 5                      | 5   | 5    | 0    | 0    | 5     | 5      | 5      |
|                              | Liver          | O126               | 10                     | 10  | 10   | 0    | 0    | 10    | 10     | 10     |
|                              |                | O44:H18            | 5                      | 5   | 5    | 0    | 0    | 5     | 5      | 5      |
|                              |                | O1:H7              | 15                     | 15  | 15   | 10   | 0    | 15    | 15     | 15     |
|                              |                | O125:H21           | 5                      | 5   | 5    | 0    | 0    | 5     | 5      | 5      |
|                              |                | O78                | 8                      | 8   | 8    | 0    | 0    | 8     | 8      | 8      |
|                              |                | O91:H21            | 5                      | 5   | 5    | 0    | 0    | 5     | 5      | 5      |
|                              | Spleen         | O121:H7            | 5                      | 5   | 5    | 0    | 0    | 5     | 5      | 5      |
|                              | Air sac        | O20                | 5                      | 5   | 5    | 0    | 0    | 5     | 5      | 5      |
|                              |                | O128H2             | 5                      | 5   | 5    | 0    | 0    | 5     | 5      | 5      |
| Total                       |                |                    | 113                    | 113 | 113 | 23   | 0    | 113   | 113    | 113    |
isolated this organism from commercial and backyard poultry farms and chicken markets. Multiple predisposing conditions could rise the susceptibility of poultry to colibacillosis, including; respiratory viruses, overcrowding, bad handling of birds and bad sanitation (Eid et al. 2016).

Regarding the serotyping of the isolated \textit{E. coli} strains, 38 \textit{E. coli} strains isolated from environmental samples were subjected to the serological identification, 23 strains (60.5%) have belonged to 10 serogroups and the most predominant serogroup was \textit{O78} (13.16%), while 15 \textit{E. coli} strains (39.5%) were untypable. In addition, 114 \textit{E. coli} strains originated from organs of diseased broilers were subjected to serological identification, 90 strains (78.95%) have belonged to 12 different serogroups and the most predominant serogroups were \textit{O1:H7} (13.16%), \textit{O78} (13.16%), \textit{O126} (8.77%), \textit{O91:H21} (8.77%), while 24 strains (21.05%) were untypable, as described in Table 3.

There is a significant difference in the prevalence of the isolated serotypes (P < 0.0243). These results disagree with those obtained by Yousef et al. (2015) who recorded that the most prevalent serotypes originated from different sources of poultry broiler farms were untypable \textit{E. coli} serovars; followed by \textit{O26}; then \textit{O2}, \textit{O124}, \textit{O125}, and \textit{O114}. Chart et al. (2000) proved that the Avian Pathogenic \textit{E. coli} are mainly included specific serotypes, especially serotypes \textit{O78}, \textit{O2} and \textit{O1}, followed by \textit{O55}, and \textit{O15}. The emergence of certain serotype and its responsibility for disease occurrence is mainly depending upon the health condition of chicken, the environmental conditions, handling and management procedures (Srinivasan et al. 2013).

In the present study, the isolated \textit{E. coli} strains were tested against 12 antimicrobial agents. The resistance and sensitivity of the isolated strains to different antimicrobial discs were differed significantly (P < 0.0001).
as described in Table 4. The tested strains showed multiple drug resistance and were highly resistant (100%) to ampicillin, erythromycin and tetracycline, followed by amoxicillin/clavulanic acid, norfloxacin, and sulphamethoxazole (80.92%), trimethoprim/sulphamethoxazole (75%) and gentamycin (50%). While (100%) of the tested strains were sensitive to colistin sulphate, followed by neomycin (87.5%). These results are nearly agreed with those obtained by Hashem et al. (2012) and Ola (2017).

Production of β-lactamase enzyme that breaks down the beta-lactam ring of penicillin is the major mechanism of antibiotic resistance in *E. coli*. Gene encoding β-lactamase enzyme could be carried on plasmid or on bacterial chromosome (Udomsantisuk et al. 2011), while aminoglycosides resistance is mainly taking place in pathogenic *E. coli* due to aminoglycoside modifying enzyme (Galimand et al. 2003) which is encoded on R-plasmids (González-Zorn et al. 2005). Sulfonamides, penicillins and tetracyclines, and are the most popular and the oldest antimicrobial agents that used heavy against bacterial infection so that a high level of drug resistance has emerged with the time (Joint 2008).

Regarding the genetic detection of virulence genes, in the present study PCR was used for detection and amplification of *(iss, papC, eaeA, and cfaI)* genes, as illustrated in Table 5 (100%) of the tested strains were positive for *iss* gene at specific amplicon size 266 bp (Fig. 1a) and *papC* gene with specific amplicon size 501 bp (Fig. 1b). Only (20.3%) of the tested strains were positive for *eaeA* gene with specified amplicon size 248 bp (Fig. 1c), furthermore, all the examined strains were negative for *cfaI* gene as illustrated in (Fig. 1d). These results are agreed with those described by Dissanayake et al. (2014) who reported that 85.4% of Avian Pathogenic *E. coli* which originated from diseased birds suffering from colibacillosis in the USA were positive for *iss* gene, also this result is supported by Ewers et al. (2004) who mentioned that *iss* gene was detected in 82.7% of APEC strains originated.
from birds suffering from coli septicaemia in Germany. In contrary to the results of papC gene in this study Rodríguez-Siek et al. (2005) stated that the papC gene is commonly associated APEC with a percentage (44.1%).

Moreover, attaching and effacing is a term that used to clarify the lesion caused by E. coli in the host intestine, where (attaching) refers to the intimate adhesion to the cell membrane of intestinal cells, while (effacing) refers to the destruction of intestinal microvilli (Stordeur et al. 2000). In this study, the prevalence of eaeA gene was (20.3%), these results are agreed with Ola (2017) who reported that the incidence rate of eaeA gene in the tested E. coli strains was 15.79%. Also, these results nearly agreed with those obtained by (DebRoy and Madox 2001). In contrary to these findings, Ramadan et al. (2016) stated that all the tested E. coli strains derived from chicken viscera were carried the eae gene (100%).

Concerning the detection of the disinfectant resistance genes, PCR was used for detection and amplification of QACs resistance (qacAΔ1, qacA/B, qacC/D) genes in the isolated E. coli strains, as illustrated in Table 5 all the tested E. coli strains (100%) were positive for qacAΔ1, qacA/B and qacC/D genes with specific amplicon size 362 bp, 361 bp and 195 bp, respectively, as illustrated in (Fig. 2a–c). These results are supported by Amira (2016) who found that the distribution of qacEΔ1 was (93.1%).

The massive use of QACs in the farm environment could result in acquired QACs resistance in E. coli strains (Sidhu et al. 2002). Many QACs resistant genes are commonly associated with multidrug-resistant pathotypes especially qacC/D, qacA/B, and qacE (Zhang et al. 2015). The qacEΔ1 gene is common in enteric bacterial pathogens possessing Sulphonamide resistant determinants. Seventy percent of qacEΔ1+ve strains exhibit cross-resistance to Sulphamethoxazole, 60% of qacEΔ1+ve strains exhibit cross-resistance to Sulfamethoxazole-trimethoprim. Also, 40% of qacEΔ1+ve strains were highly resistant to Gentamicin (Kücken et al. 2000).

In conclusion, E. coli continues to be one of the most important pathogens in poultry and poultry farm environment, the most predominant E. coli serotypes affecting broiler chickens are O78, O1:H7, O91:H21 and O126. The QACs resistance genes are frequently distributed with the multidrug-resistance pathotypes which may be transmitted to humans by the consumption of chickens or any byproduct containing such strains. The high proportion of virulence genes (iss and papC) and the multidrug-resistance phenomena is prevalent in Avian Pathogenic E. coli and environmental strains. There is a directly proportional relationship between the presence of multidrug-resistance, disinfectant resistant genes, virulence genes and the severity of lesions associated with E. coli infection and complications.

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Authors’ contributions
MEE and AHA conceived and designed the experiments and performed the experiments. AMA, SAN, SAMA and MB analyzed the data. AMA, MEE, AET and AAA wrote and revised the paper. All authors read and approved the final manuscript.

Availability of data and materials
Not applicable.

Ethics approval and consent to participate
Handling of birds was performed according to the Animal Ethics Review Committee of Suez Canal University, Egypt.

Consent for publication
All authors gave their informed consent prior to their inclusion in the study.

Competing interests
The authors declare that they have no competing interests.

Author details
1 Bacteriology, Mycology and Immunology Department, Faculty of Veterinary Medicine, Suez Canal University, Ismailia 41522, Egypt. 2 Reference Laboratory for Veterinary Quality Control on Poultry Production, Animal Health Research Institute, Giza, Egypt. 3 Bioiology Department, College of Science, Princess Nourah Bint Abdulrahman University, P.O. Box 24428, Riyadh 11671, Saudi Arabia. 4 Department of Animal Husbandry and Animal Wealth Development, Faculty of Veterinary Medicine, Alexandria University, Edfina 22578, Egypt. 5 Department of Zoology, Faculty of Science, Beni-Suef University, Beni-Suef 6521, Egypt.

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