Thesis Review

Virulence and antimicrobial resistance genes associated with the *in-vivo* pathogenicity of avian pathogenic *E. coli* isolates

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Abstract

In the current study, ten avian pathogenic *E. coli* (APEC) isolates of the most predominant APEC serogroups isolated from broiler chickens in Egypt were screened for their virulence and antimicrobial resistance genes pattern using PCR. Five selected virulence gene patterns were further investigated for their *in-vivo* pathogenicity test. Results showed a 100% prevalence of the β-lactams and tetracyclines resistance genes. However, aminoglycoside and quinolone resistance genes were not detected. Also, 80% of the tested isolates harbored *mcr*-1 gene, colistin resistance gene. *In-vivo* pathogenic strains consistently harbored the virulence gene pattern of *fimH, fimA, papC, iutA*, and *tsh*. Additionally, the *tsh* gene was consistently detected with lethal APEC isolates in day-old chicks. These results highlighted the high prevalence of antimicrobial and virulence genes in APEC that potentially represent a public health concern. In this study, the virulence genes *fimH*, *fimA*, *papC*, *iutA*, and *tsh* were the most common virulence gene patterns associated with pathogenicity in day-old chicks.

Keywords: *E. coli*, broiler chickens, colistin resistance, virulence, pathogenicity

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Background

Colibacillosis in poultry causes either localized or systemic infections. The avian pathogenic *E. coli* (APEC) pathogenicity is generally enhanced or initiated by environmental factors, viral infections, mycoplasma infections, and immunosuppression **(Ewers et al., 2003)**. However, based on the virulence genes detection, a pentaplex panel (APEC harboring *hlyF*, *iutA*, *iroN*, *iss*, and *ompT*) was proposed to distinguish between APEC and avian fecal *E. coli* isolates **(Johnson et al., 2008)**. Another study proposed that highly pathogenic *E. coli* strains harbored at least 8 to 13 virulence genes while intermediate pathogenic strains harbored at least 5 to 8 virulence genes **(Wang et al., 2015)**.

Several studies showed a high prevalence of *E. coli* among poultry farms in Egypt, especially in broiler chickens **(Abd-ElTawab et al., 2015)**. Although variable numbers and combination patterns of virulence-associated genes were detected, these patterns association was not linked to the isolated APEC's *in-vivo* pathogenicity. Extended-spectrum beta-lactamases (ESBL) producing *Enterobacteriaceae* are increasing in Egypt. This increase was attributed to the large-scale use of antimicrobials in animals **(El-Shazly et al., 2017)**. Recently, the plasmid-mediated colistin resistance gene (*mcr*-1) was firstly reported in a clinical human isolate in Egypt **(Elnahriry et al., 2016),** following the first reports in animals in Algeria **(Olaitan et al., 2016)** and Egypt **(Khalifa et al., 2016)**. The increasing prevalence of *mcr*-1 in animal isolates further indicates the misuse of antimicrobials in livestock with an increased risk of zoonotic transmission of resistant bacteria to humans **(Zhao et al., 2018)**.

The current study was designed to determine the distribution of virulence and resistance genes in APEC isolates and investigate the *in-vivo* pathogenicity of

selected APEC isolates with variable virulence gene patterns.

Materials and Methods

APEC isolates

In this study, ten APEC strains isolated from broiler chickens in El-Fayoum and Beni-Suef Governorates in Egypt were included. The isolates have been morphologically, biochemically, and serologically identified, and their antimicrobial susceptibility profiles were determined **(Ali et al., 2019)**.

Detection of the virulence and resistance encoding genes in E. coli isolates

Bacterial DNA was extracted from selected colonies using the QIAamp DNA mini kit (Qiagen) according to the manufacturer's instructions. Virulence and antimicrobial resistance genes detection were performed as previously described **(Dozois et al., 2000, Kaczmarek et al., 2012, Ali et al., 2019)**.

Pathogenicity of characterized APEC in day-old chicks

Chicken experiments were conducted according to Animal Research Ethics Guidelines at the Faculty of Veterinary Medicine, Beni-Suef University, Egypt. For the *in-vivo* pathogenicity test, five *E. coli* serotypes were selected based on virulence genes pattern and zoonotic importance (strains ID's: EC82-O125, EC6-O119, EC73-O126, EC91-O86a, and EC54-O78). The pathogenicity was conducted in 1-day old chicks (5 chicks/group) by inoculation of 0.5 ml containing 10^8 CFU/ml of the APEC isolate subcutaneously. Birds were observed for clinical signs and mortality until four days post-inoculation. The strains were classified as pathogenic when at least one chick died **(Schouler et al., 2012)**.

Results and Discussion

In the current study, antimicrobial resistance and virulence genes were screened in 10 *E. coli* isolates. As expected, amoxicillin, ampicillin, oxytetracycline, and streptomycin-resistant *E. coli* represented 80-90% of tested isolates **(Younis et al., 2017)** with the detection of the β-lactams and other corresponding resistance genes associated with phenotypic resistance profiles. In contrast to phenotypic characterization, two genes , the aminoglycoside and the quinolones resistance genes (*aadA* & *qnrA*, respectively, were not detected in any examined isolates. This contradiction is probably because the phenotypic resistance to these two antimicrobial categories might be mediated by other genes of resistance (e.g., *strA* and *strB* for aminoglycoside and gyrA for quinolones resistance **(Figure 1) (Xie et al., 2014)**.

Colistin is one of the drugs of choice for treating MDR Gram-negative bacteria in humans **(Lv et al., 2018)**. In this study, 10% of isolates were resistant to colistin. On the other hand, 8 out of 10 tested *E. coli* isolated were positive for *mcr*-1 PCR, including two phenotypically resistant, four intermediately susceptible, and two susceptible isolates to colistin. The emergence of *mcr*-1 associated with colistin resistance in *E. coli* is increasing in Egypt and worldwide, resulting in a potential source of resistant bacteria to the human **(Lima Barbieri et al., 2017)**. Detection of virulence gene markers associated with APEC isolates' lethality was proposed as a diagnostic tool for identifying APEC **(Dozois et al., 2003)**.

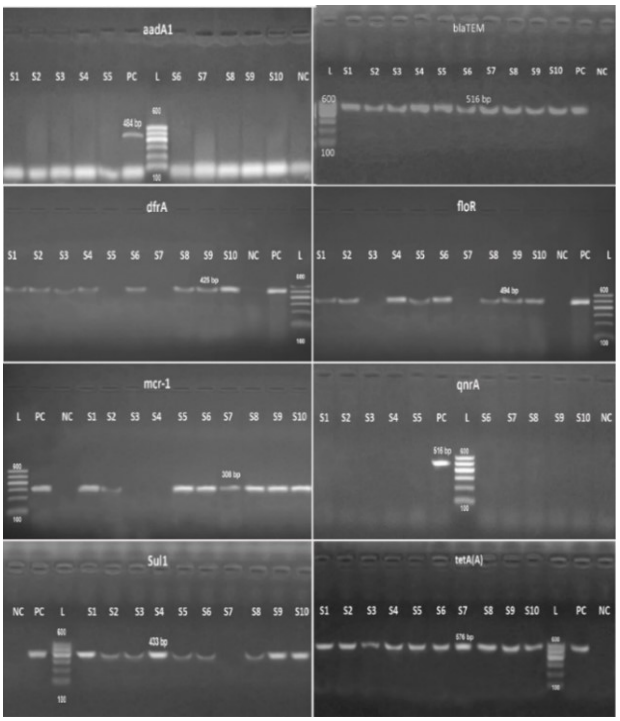

Figure 1. Antibiotic resistance genes detected in the selected *E. coli* isolates by gene-specific PCR. L: ladder, PC: positive control, NC: negative control, S1-S10: samples

The gene constellation of *fimH*, *fimA*, *papC*, *iutA*, and *tsh* was the most prevalent virulence gene pattern of the isolated APEC **(Figure 2)**.

Hence, we tested the pathogenicity of five molecularly characterized predominant serogroups *E. coli* isolates with different virulence gene constellations. Three out of five isolates were found pathogenic in day-old chicks. The pathogenic strains consistently harbored the virulence gene pattern of *fimH, fimA, papC, iutA*, and *tsh,* which was the most common gene constellation detected. Additionally, the temperature-sensitive hemagglutinin (*tsh*) gene was the most important APEC virulence marker as its absence remarkably diminishes the *E. coli* isolates **(Figure 3).**

Studies have shown different virulence criteria associated with *tsh* gene, including the proteases, adhesins, cytotoxins, and cell invasion proteins activities. The strong association with internal organs colonization, septicemia, and lethality in day-old chicks **(Ngeleka et al., 2002)**, probably makes it a good target for pathotyping of APEC.

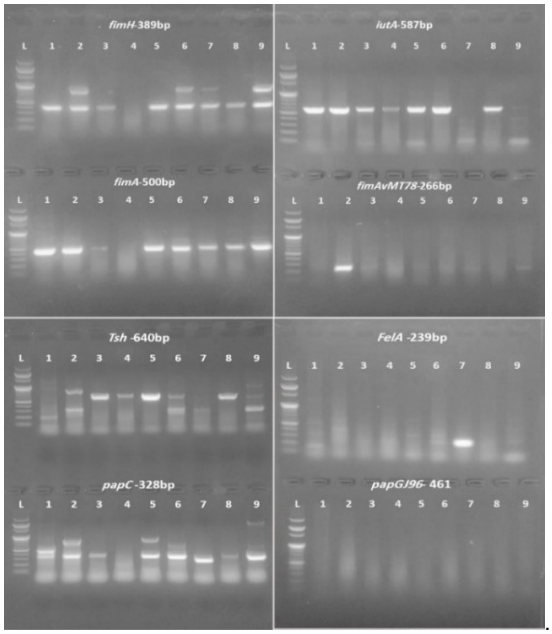

Figure 2. Virulence genes detection in the selected *E. coli* isolates by gene-specific PCR. L; ladder, 1-9, samples

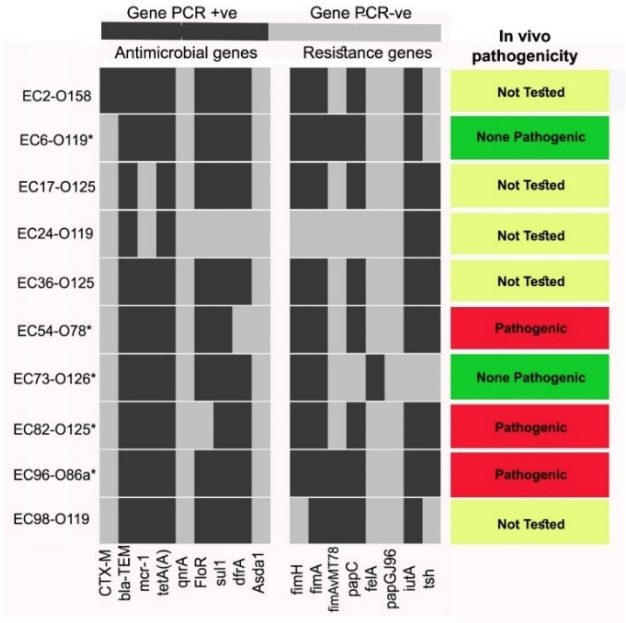

Figure 3. The *in-vitro* and *in-vivo* characterization of the isolated APEC strains. (*) denotes the APEC isolates tested for *in-vivo* pathogenicity in day-old chicks

Conclusions and recommendations

The current study demonstrated the prevalence of antibiotic resistance genes among isolated *E. coli* in broiler chickens in Egypt. An increased detection rate of the colistin resistance (*mcr*-1 gene) in *E. coli* associated with colibacillosis in broiler chickens was found, indicating a potential public health concern. Finally, the gene constellation of *fim*H, *fim*A, *pap*C, *iut*A, and *tsh* was the most prevalent virulence markers of APEC and could be used as fast pathotyping avian *E. coli* isolates.

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