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Virulent genes detection in avian pathogenic *E. coli* instigating respiratory infections of commercial chicken

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Abstract
This study was conducted to investigate the concurrent presence of *Escherichia coli (E. coli)* bacteria in chicken suffering from the respiratory disease. Avian pathogenic *E. coli* (APEC) were detected based on isolation of bacteria, it's cultural and colony characteristics and confirmed by polymerase chain reaction. Sixty isolates were confirmed as *E. coli* based on bacteriological isolation and molecular detection. Further, PCR assay was conducted for four virulent genes i.e. *iss, vat, papC,* and *tsh* and 54(90%) were positive on PCR for one or more virulent genes. Out of 60 isolates, 48 (80%) were found positive for *iss* gene, 43 (71.6%) positive for *vat* gene, 31 (51.6%) positive for *tsh* gene and 18 (30%) positive for *papC* gene. Two or more virulent genes were found in 46 (76.6%) isolates.

Keywords: *E. coli*, poultry, virulent genes, respiratory disease, PCR

1. Introduction
Avian pathogenic *Escherichia coli* (APEC) was causing colibacillosis in poultry and it leads to high morbidity and mortality in birds worldwide. It is considered to be causing severe economic losses to the poultry industry. APEC were always associated with extraintestinal infections in which respiratory system was principally affected. In the intestinal tract and other mucosal surfaces of bird's environment *E. coli* was present as inhabitant in the normal microflora but only some strains cause disease which were having specific virulent factors.
Many virulent factors helps in promoting the extraintestinal diseases in poultry which includes iron acquisition systems (yersiniabactin and aerobactin), adhesins (F1-, AC/I-, P-, and F17-fimbriae, fimbrial adhesins and curli fibers), hemolysins (temperature- sensitive hemagglutinin and hemolysin E), antibactericidal factors (protein for increased serum survival, outer membrane protein A, K1-capsule, lipopolysaccharide, and colicin production), and toxins (flagella toxin, cyto-/verotoxin, vacuolating auto transporter toxin and heat stable toxin) [1]. The frequency and distribution of virulent factors in APEC isolates were reported worldwide [2, 3].
Therefore, this study was conducted to identify *E. coli* isolates to detect certain virulent genes in chicken suffering from respiratory disease conditions.

2. Materials and Methods
The 65 samples of bronchial swabs and bronchial tissues were collected in sterilized vials. On MacConkey agar plates samples were streaked and observed pink color colonies. These colonies were selected and again sub cultured on Eosin Methylene Blue (EMB) agar plates.
Organisms are identified by the colony characteristics, biochemical tests and gram's staining method. From overnight grown EMB agar plates about 2-3 colonies of *E. coli* were taken and suspended in a microfuge tube (1.5 ml) containing of 100 µl of autoclaved milli Q water. The microfuge tube was kept in boiling water bath and heated for 10 min, then it was transferred immediately onto ice and waited for 20 min. Then tube was centrifuged at 4ºC at a speed of 8000 rpm (Eppendorf) for 5 min and the supernatant was collected which was used for molecular studies as DNA template.
After extraction of bacterial DNA, PCR was performed to amplify genus specific16S rRNA of *E. coli* and virulent marker genes. The primers details for amplification of *E. coli* and its virulent gene used in this study were given in Table 1 and the thermal cycling conditions were presented in Table 2.
PCR assay was performed in a thermal cycler (Nexus Gradient, Eppendorf). In the thermal cycler The PCR products were stored at 4 °C until they were removed. The detection and separation of DNA molecules in PCR products was carried out by using Gel electrophoresis system.

Table 1: The primers sequences used for amplification of *E. coli* and its virulent genes

S. No.	Genes	Primer sequences (5'→ 3')	Size	Reference
1.	16S rRNA	F:ATCAACCGAGATTCCCCCAGT	231 bp	Dong-bo *et al.* (2011) [4]
		R:TCACTATCGGTCAGTCAGGAG		
2.	*Iss*	F:CCCCAATTGGACAGAGAAAA	174 bp	Ewers *et al.* (2004) [1]
		R:ATCGATGGGCCTATTGTGAG		
3.	*papC*	F:AATAAAAACGTGGCGGACTG	204 bp	
		R:ACGCAGGTAAGCAGAATCGT		
4.	*Tsh*	F:TCTCAATGCGTCGTAACAGC	153 bp	
		R:CCTTCAGATGAACGTCAGCA		
5.	*Vat*	F:CACGCTACTGAATGCCTGAA	168 bp	
		R:TGGCAGGTTAATGGTGTGAA		

Table 2: Conditions for Thermal cycling detection of *E. coli* and its virulent genes

Steps	16S rRNA	*Iss* gene	*PapC, Tsh* and *Vat* gene	No. of cycles
Initial denaturation	5 min at 94 °C	5 min at 94 °C	5 min at 94 °C	1
Denaturation	30 sec at 94 °C	30 sec at 94 °C	30 sec at 94 °C	35
Annealing	90 sec at 56 °C	90 sec at 56 °C	90 sec at 56 °C	
Extension	30 sec at 72 °C	30 sec at 72 °C f30sec	30 sec at 72 °C	
Final extension	7 min at 72 °C	7 min at 72 °C	7 min 72 °C	1

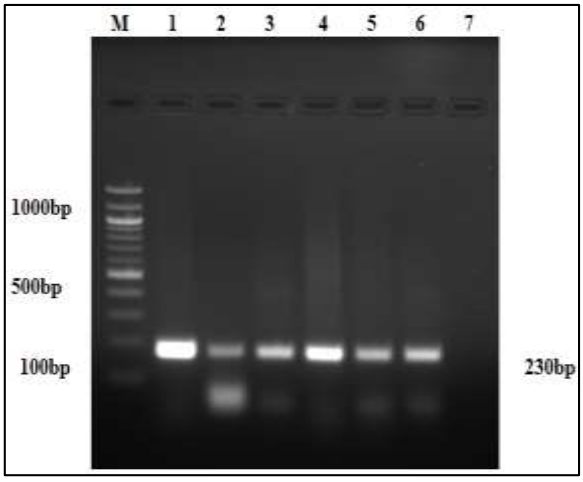
3. Results and Discussion

In this present study, *E. coli* occurred as a single infectious agent or as a concurrent infection with other infectious like New castle disease virus, Fowl Adeno virus, Infectious Bronchitis virus, *Mycoplasma gallisepticum*, *Mycoplasma sinoviae*, *A. paragallinarum* and *P. multocida* which cause respiratory disease.

Although *E. coli* was present in the normal microflora of the intestinal tract and other host mucosal surfaces, but some certain strains possessing specific virulent factors attribute to cause disease in chickens.

Extraintestinal pathogenic *E. coli* (ExPEC) group of avian pathogenic *E. coli* were responsible for morbidity and mortality in chicken. This ExPEC sub group were associated with extraintestinal infections which includes localized and systemic infections that result in a variety of diseases [5]. The most common form of disease is colibacillosis with an initial respiratory infection followed by generalized systemic infection.

The PCR assay was conducted by using the DNA extracted from *E. coli* isolates. PCR primers targeting 16S rRNA of *E. coli* amplified 230 bp amplicon that confirmed the presence of *E. coli* (Fig. 1). Among 65 samples, 16S rRNA was found in 60 samples in PCR assay and the results are in agreement with the results of Tonu *et al.* (2011) [6]; Islam *et al.* (2014) [7] and Matin *et al.* (2017) [8].



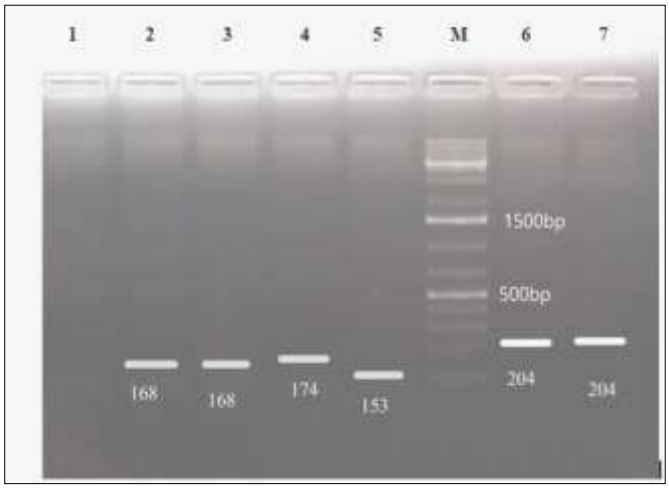
L M- Ladder 100 bp
L1-Positive control
L2 to 6-DNA samples
L7-Negative control

Fig 1: Gel electrophoresis showing PCR products of 16S rRNA in *E. coli* (230 bp)

In this current study we detected APEC with its virulent genes *viz. Vat, Iss*, *Tsh* and *papC* enables them to live in extra-intestinal area like respiratory system conveniently.

The four virulent genes (*Vat, Iss*, *Tsh* and *papC*) were detected by PCR assay using the DNA extracted from 60 samples. PCR assay confirmed 54(90%) isolates were positive for virulent genes. Among 60 samples, 48 (80%) were positive for the *iss* gene (174 bp), 43 (71.6) % for *vat* gene (168 bp), 31 (51.6%) for *tsh* gene (153) and 198 (30%) for *papC* gene (204 bp). Out of these 46 (76.6%) isolates were positive for two or more virulent genes.

Type I fimbriae helps in adhesion of APEC strains to epithelial cells of upper respiratory tract. P fimbriae are expressed in some strains of bacteria that colonize in the lower respiratory tract and viscera.



L1-Negative control
L 2 and 3-*Vat* gene
L 4-*Iss* gene
L 5-*Tsh* gene
L M- Ladder 100 bp
Lane 6 and 7-*PapC* gene

Fig 2: Gel electrophoresis showing PCR products of virulent genes (174 bp, 204 bp, 153 bp and 168 bp) in APEC

The *papC* operon in the bacterial chromosome encodes for adhesion factor P fimbria which prevents phagocytosis of the of bacteria in the host. The *iss* gene helps in survival of bacteria in the serum and it plays important role in production of severe colibacillosis in chicken. The temperature-sensitive haemagglutinin *(tsh)* is necessary for binding of bacteria to the host cell membrane hence it was important for adhesion. The *vat* gene responsible for vacuolating and transferring of cytotoxin which encodes carrier proteins.

The virulent genes *viz. Vat Iss, Tsh* and *papC,* of *E. coli* in poultry were detected by PCR by various authors in colibacillosis [2, 9, 10].

In this present study *iss* gene was found in 48 (80%) samples. Similarly, *iss* gene was found positive in 82.7% [1] and 73.8% [3] of isolates from chicken. In respiratory infections of chicken, Chaudhari *et al.* (2018) [10] found *iss* gene 90% *E. coli* isolates.

Out of 60 samples, 43 *E. coli* samples (71.6%) were found positive for *vat* gene and these results were in accordance with Roussan *et al*. (2014) [9] and Chaudhari *et al.* (2018) [10] results. These authors reported that 70% and 83.33% of isolates were positive for *vat* gene respectively in birds with respiratory diseases. In contrast, Ewers *et al.* (2004) [1] found only 48.7% of positive isolates for *vat* gene in colibacillosis of poultry.

In the present study, 31 *E. coli* samples (51.6%) were found positive for *tsh* gene. Similarly, few authors detected *tsh* gene positive in 53.3% [1], 55.7% [3], 50% [10] and 66% in their samples.

Few samples (30%) were positive for *pap C* gene. Similarly in previous studies 22.7% [1], 24.3% [3] and 33.33% [10] of APEC isolates were found positive for *pap C* gene by various authors in their studies. In contrast, Roussan *et al*. (2014) [9] noticed 82.10% of positive isolates for *pap* C gene in birds.

The PCR studies have been previously employed for the virulent genes detection of *E. coli* in poultry by various authors [11, 12]. The studies showed variation in the distribution and percentage of APEC isolates either possessing the genes singly or in combination or with absence of some of the genes. This might be due to prevalence of different serotypes/genotypes of *E. coli* in the study jurisdiction and also due to different type of samples used for isolation of *E. coli* from that of earlier studies.

4. Conclusions

Molecular identification of virulent genes affiliated with avian pathogenic *E. coli* found in this study showed that the respiratory disease may be caused by combined effect of more than one virulent genes which were attributed to pathogenicity of bacteria.

5. Acknowledgments

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6. References

1. Ewers C, Janssen T, Kiessling S, Philipp HC, Wieler LH. Molecular epidemiology of avian pathogenic *Escherichia coli* (APEC) isolated from colisepticemia in poultry. Veterinay Microbiology. 2004;104:91-101.
2. Samanta I, Jordar SN, Das PK, Sar TK. Comparative possession of shiga toxin, intimin, enterohaemolysin and major extended spectrum beta lactamase (ESBL) genes in *Escherichia coli* isolated from backyard and farmed poultry. Indian Journal of Veterinary Research. 2015;16:90-93.
3. Rocha AC, Rocha SL, Lima-Rosa CA, Souza GF, Moraes HL, Salle FO, *et al*. Genes associated with pathogenicity of avian *Escherichia coli* (APEC) isolated from respiratory cases of poultry. Pesquisa Veterinária Brasileira. 2008;28(3):183-186.
4. Dong-bo S, Rui WU, Xian-jing H, Shuang W, Yun-cheng L, HAN Xu, *et al*. Development of a multiplex PCR for diagnosis of *Staphylococcus aureus, Escherichia coli* and *Bacillus cereus* from cows with endometritis. Agricultural Sciences in China. 2011;10(10):1624-1629.
5. Gross WG. Diseases due to *Escherichia coli* in poultry in *Escherichia coli* in domestic animals and humans. Gyles C L (ed) Cab International edn, Wallingford, United Kingdom; c1998. p. 237-259.
6. Tonu NS, Sufian MA, Sarker S, Kamal MM, Rahman MH, Hossain MM. Pathological study on colibacillosis in chickens and detection of *Escherichia coli* by PCR. Bangladesh Journal of Veterinary Medicine. 2011;9(1):17-25
7. Islam. Prevalence of colibacillosis in chickens in Gazipur district. M S Thesis, Bangladesh Agricultural University, Mymensingh, Bangladesh; c2014. p. 148-153.
8. Matin Md, Islam Md, Khatun MM. Prevalence of colibacillosis in chickens in greater Mymensingh district of Bangladesh. Veterinary World. 2017;10(1):29-33.
9. Roussan DA, Zakaria H, Khawaldeh G, Shaheen I. Differentiation of avian pathogenic *Escherichia coli* strains from broiler chickens by multiplex polymerase chain reaction (PCR) and random amplified polymorphic (RAPD) DNA. Open Journal of Veterinary Medecine. 2014;4:211-219.
10. Chaudhari SV, Joshi BP, Desai DN, Bhanderi BB, Choudhary KR, Madhwal A. Isolation and characterisation of *E. coli* infection from the bronchial plug of broiler birds associated with respiratory diseases. Advances in Animal and Veterinary Sciences.

2017;5(8):334-341.
11. Dutta TK, Roychoudhury P, Bandyopadhyay S, Wani SA, Hussain I. Detection and characterization of shiga toxin producing *Escherichia coli* (STEC) & enteropathogenic *Escherichia coli* (EPEC) in poultry birds with diarrhoea. Indian Journal of Medical Research. 2011;133:541-545.
12. Hizlisoy H, Serhat AL, Onmaz NE, Yildirim Y, Gnulalan Z, Gumuşsoy KS. Antimicrobial resistance profiles and virulence factors of *Escherichia coli* O157 collected from a poultry processing plant. Turkish Journal of Veterinary and Animal Sciences. 2017;41:65-71.