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Screening of virulence-associated genes in avian pathogenic Escherichia coli isolates from clinical cases

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Abstract

Escherichia coli has more than 150 strains that inhabit a wide range of environments. Among them, avian pathogenic E. coli (APEC) strains are responsible for causing infections in birds of different ages, affecting multiple organs. These infections, collectively referred to as colibacillosis, result in high mortality rates in chicken and consequently impose severe economic losses on the global poultry industry. The pathogenicity of APEC strains is closely associated with specific virulence genes encoded within their genome. The objective of the study is to evaluate genes linked to APEC isolates from colibacillosis cases that have been reported from University Poultry and Duck farm, KVASU, Mannuthy. Here, 19 E. coli strains isolated from birds with colibacillosis were analysed for occurrence of six virulence associated genes. The virulence profiles showed that 100 % of the isolates were possessing the gene fimC, 57.89 per cent of isolates possessed iucD gene, and none of them were possessing papC, stx1, stx2 and st1genes. The study revealed that APEC isolates carry plasmid-associated virulence genes, highlighting the importance of continuous surveillance to control the transmission of APEC within poultry farms.

Keywords: *APEC, virulence genes, characterisation, colibacillosis*

Escherichia coli organisms survive in various hosts and environments due to their highly diversified genome. These bacteria have coevolved and colonised a broad range of hosts, and survive as commensal organisms or pathogens. Phylogenetic investigations discovered the *E. coli* that adopted a pathogenic lifecycle in avian hosts are noticeably overlapped with the phylogroup of *E. coli* infecting humans, which indicates a high zoonotic potential of avian pathogenic *E. coli* (APEC). Colibacillosis caused by APEC isolates is one of the main causes of economic losses in the poultry industry worldwide, as a result of increased rejection at the slaughterhouse, decreased egg yields and increased mortality (Barnes et al., 2003). APEC strains cause airsacculitis, oophoritis, peritonitis and septicaemia in chickens, turkeys and other avian species. The APEC are found in the intestinal microflora of healthy birds, and most of the diseases associated with them are secondary to environmental and host predisposing factors.

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This disease occurs only when the *E. coli* strains code for virulence factors that enable them to adhere and proliferate in the host organism. *E. coli* strains can obtain virulence genes through horizontal gene transfer, including prophages from lysogenic bacteriophage infections (Brussow et al., 2004). Virulence-associated genes are commonly located on large circular plasmids (50-200 kb) in pathogenic *E. coli*, particularly within ColV and IncF plasmids (Johnson et al., 2006; Mellata et al., 2010). The genes occur individually or polygenically with varying frequencies in clinical isolates. These genes can be used to distinguish APEC from environmental, commensal, intestinal, and other extraintestinal *E. coli*. There are many virulence-associated genes (*iss, tsh, iroN, ompT, iutA, fimC, fimH, cvaC, hlyF, iucD, papG*allel(II/III), and *papC*) that are more frequently reported in APEC isolates than non-pathogenic *E. coli*. A surveillance study on the presence of virulence-associated genes in APEC isolates can serve as a guideline for future investigation and aid in the formulation of intervention strategies.

Materials and methods

The present study was conducted in the Department of Poultry Science, College of Veterinary and Animal Sciences, Mannuthy, Kerala. Samples were collected from the dead and infected birds with colibacillosis from University Poultry and Duck Farm, Mannuthy, in a span of three months. The affected live birds showed clinical signs ranging from lameness, retarded growth, head swelling, respiratory distress and ruffled feathers. The dead birds on necropsy revealed lesions typical of colibacillosis, like pericarditis, perihepatitis, salpingitis and peritonitis. For isolation of *E. coli*, samples from the heart, lung, spleen, ovary and liver were collected.

The isolation and identification of *E. coli* was carried out as per the standard microbiological procedure. The samples from (heart, spleen, lung and liver) were inoculated in nutrient broth and incubated at 37°C for 24 hours following re-inoculation on MacConkey agar and again incubated at 37°C for 24 hours. The lactose-fermenting colonies were re-inoculated on Eosin Methylene Blue agar.

Characterisation of isolates was carried out by morphological, biochemical and molecular methods. The colonies were identified as *E. coli* through colony morphology and grams staining, catalase test. Indole, Methyl Red, Voges-Proskauer, and Citrate (IMViC) test was performed for biochemical identification. 16SrRNA sequence-specific primer was used for molecular-level identification. DNA of the isolates was extracted using the QIAamp DNA Mini Kit (Qiagen, North-Rhine-Westphalia, Germany). Avian pathogenic *E. coli* (APEC) specific virulence genes ie., *papC* (fimbriae assembly usher protein), *iucD* (Aerobactin biosynthesis protein), *fimC* (Periplasmic chaperone for type 1 fimbriae) and human pathogenic *E. coli* virulence genes like *stx1, stx2* (Shiga toxin genes) and *st1* (Heat-stable enterotoxin gene) were used to confirm the virulence and zoonotic potential of isolates. Sequence details for the primers used are given in Table 1.

Results and discussion

Avian colibacillosis caused by APEC poses a major challenge to the poultry industry, not only due to its economic consequences but also because of its public health relevance. Several studies have highlighted colibacillosis as one of the most significant diseases affecting commercial chicken (Subedi et al., 2018; Mellata,

Table 1. Sequences and properties of primers used to identify APEC

Sl. No.	Name	Sequence (5'- 3')	Annealing temperature (° C)	Product size (bp)	Reference
1	*16S rRNA* F	GACCTCGGTTTAGTTCACAGA	55	585	Sobur et al., (2019)
	16S rRNA R	CACACGCTGACGCTGACCA			
2	*papC* F	TGATATCACGCAGTCAGTAGC	61	483	Janben et al., (2001)
	papC R	CCGGCCATATTCACATAA			
3	*iucD* F	ACAAAAAGTTCTATCGCTTCC	61	692	
	iucD R	CCTGATCCAGCTGATGCTC			
4	*fimC* F	GGGTAGAAAATGCCGATGGTG	65	496	
	fimC R	ACAAAAAGTTCTATCGCTTCC			
5	*stx1* F	ATAAATCGCCATTCGTTGACTAC	56	180	Paton and Paton (1998)
	stx1 R	AGAACGCCCACTGAGATCATC			
6	*stx2* F	GGCACTGTCTGAAACTGCTCC	61.6	255	
	stx2 R	TGCCCAGTTATCTGACATTCTG			
7	*st1* F	TTTATTTCTGTATTGTCTTT	45	255	
	st1 R	ATTACAACACAGTTCACAG			

2013). Worldwide, poultry producers continue to face substantial financial losses as a result of the severe impact of this disease on flock health and productivity.

In the present study, samples such as heart blood, liver and ovarian follicles were collected from the dead carcass and infected birds suffered from colibacillosis at University Poultry and Duck Farm, Mannuthy, in a span of three months. A total of 19 samples were found to be positive for *E. coli*. In the current study, colonies of all of the *E. coli* isolates showed a typical metallic sheen on EMB agar. The appearance of a metallic sheen on EMB can be attributed to its lactose-fermenting ability. They appeared as Gram-negative bacilli and catalase positive. Biochemical Characterisation of the isolate through IMViC test exhibited a ++-- reaction pattern, indicating positive results for Indole and Methyl Red tests, while showing negative results for Voges-Proskauer and Citrate utilisation tests. This characteristic IMViC profile is consistent with *E. coli*

All of the isolates were confirmed as *E. coli* by molecular method using the genus specific primers in the 16s rRNA sequence (Fig. 1).

Chui et al. (2010) reported that virulence genes can serve as reliable molecular markers for identifying APEC. In the present study, APEC strains were characterised through the detection of the virulence-associated genes *fimC*, *iucD*, and *papC* (Janben et al., 2001). The colonisation of *E. coli* isolates in host tissue is thought to be initiated by fimbrial adhesins. The presence of fimbrial adhesins are encoded by *fim* cluster genes like *fimH* and *fimC,* and are claimed to be responsible for the first step in the colonisation and has an important role in the pathogenesis of avian colibacillosis (Pusz et al., 2014 and Roussan et al., 2014). The presence of *iucD* promotes the survival of APEC, as the gene is associated with iron transport, which is essential for *E. coli* survival (Gao et al., 2012). The gene *papC* is responsible for the adhesion of *E. coli* to cells as it encodes a protein involved in the assembly of P fimbriae (Paixao et al., 2016 and Zakariazadeh et al., 2019). The genes *stx1*, *stx2*, and *st1* are generally not common in APEC strains. These genes are more typically associated with human-pathogenic *E. coli.* The presence of the genes in poultry isolates may reflect cross-species transmission and indicates its zoonotic potential. *Escherichia coli* strains can obtain these virulence genes through horizontal gene transfer including prophages from lysogenic bacteriophage infections (Brussow et al., 2004).

In the current study, all of the isolates possessed *fimC* gene (Fig. 2) and 11 out of 19 isolates (57.89 %) were having *iucD* virulence gene (Fig. 3). No isolates in the study possessed *papC* gene. Similar trends have been

Fig. 1. Confirmation of *E. coli* isolates using the genus specific primers in the 16s rRNA sequence (Lane 1-19 representing the isolates with PCR product of 585 bp size, Lane 20 showing 100 bp ladder)

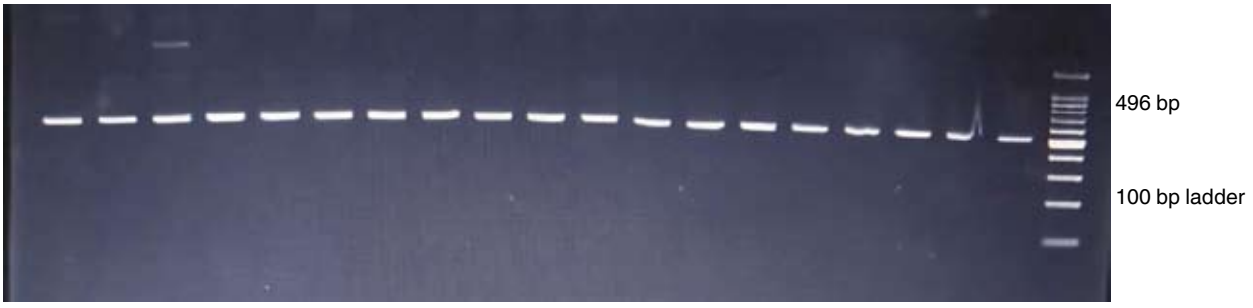

Fig. 2. Confirmation of presence of virulence gene *fimC* in *E. coli* isolates (Lane 1-19 representing the isolates with PCR product of 496 bp size, Lane 20 showing 100 bp ladder)

Fig. 3. Confirmation of presence of virulence gene *iucD* in *E. coli* isolates (Lane 1-19 representing the isolates with PCR product of 692 bp size, Lane 20 showing 100 bp ladder)

reported previously, with Janben et al., 2001) documenting prevalences of virulence genes *fimC* (92.7 %) and *iucD* (78.79 %) and Paixão et al., 2016 reported the presence of *fimC* (96.97%), *iucD* (82.7%) and *papC* (30 %) in APEC isolates. Consistently, higher detection rates of *fimC* relative to other virulence genes have been observed across different regions, ie., 95 % *fimC*, 71.65 % *iucD*, and 36.65 % *papC* in Japan (Kawano et al., 2006) and 94.87 % *fimC* with 8.69 % for both *iucD* in Italy (Camarda et al., 2007). Hamed et al. (2023) found that 100 out of 135 (74 %) screened isolates were positive for the presence of *fimC* gene and 74 isolates (54.8 %) showed the presence of *papC* gene, which is contrary to the current findings that we could not detect *papC* gene in any of the 19 isolates screened. Laopiem et al. (2025) also observed the presence of the virulence gene *iucD* in 16.4 per cent of screened *E. coli* isolates obtained from colibacillosis affected birds.

The current study could not detect *stx1*, *stx2* and *st1* genes in any of the isolates screened. Similarly to the present study, Singh et al. (2023), Janben et al. (2001), and Wani et al. (2004) could not detect the presence of shiga toxin genes, *stx1* and *stx2* in APEC isolates. But in contrast, Eid et al. (2016) detected *stx1* gene in all of the *E. coli* isolates from poultry screened for presence of virulence genes. Even though the virulence factors of human ExPEC strains were absent in the screened APEC, continued surveillance remains important to monitor potential gene transfer and the possibility of horizontal acquisition of virulence determinants under field conditions.

Conclusion

Molecular screening revealed the predominance of the *fimC* gene, highlighting its critical role in host colonisation and pathogenesis. In contrast, the *papC* gene was absent in all isolates, while the *iucD* gene associated with iron acquisition was detected in more than half of the isolates. Importantly, none of the isolates carried shiga toxin genes (*stx1*, *stx2*) and gene *st1*, suggesting that these APEC strains are unlikely to possess the virulence traits commonly linked to human pathogenic *E. coli*. Future research can be done to try and establish an *in-vivo* chicken infection model to determine the actual pathogenicity of the strains used in the present study for the correlation of the virulence gene profiles with pathogenicity in chicken.

Conflict of interest

The authors declare no conflict of interest related to this study.

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