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Original Research

Molecular Detection of Certain Virulence Genes of Avian Pathogenic *Escherichia coli* (APEC) in Chicken

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Abstract

In the present study, 86 avian pathogenic E. coli isolates from chicken were subjected to multiplex PCR to detect the presence of virulence genes stx1, stx2, eaeA, and hlyA. Forty-seven isolates harbored one or more virulence genes, stx1, stx2, and eaeA while gene hlyA was not detected in any of the samples. Of these, 15 (17.44%) isolates harbored stx1gene, 11 (12.79%) isolates carried stx2 gene, 18 (20.93%) isolates were positive for both stx1 and stx2 genes and 3 (3.49%) isolates carried eaeA gene.

Keywords: APEC, chicken, E. coli, Virulence Genes

Introduction

Avian pathogenic *Escherichia coli* (APEC) are the etiological agents of colibacillosis in poultry. *E. coli* are present in the normal microflora of the intestinal tract and other host mucosal surfaces and in the bird's environment, and only certain strains possessing specific virulence attributes are able to cause disease. APEC strains are major pathogens responsible for morbidity and mortality in chicken and are mostly associated with extraintestinal infections, including systemic and localized infections.

A variety of virulence factors of *E. coli* have been implicated in promoting extraintestinal diseases in avian species. Of these, APEC-producing Shiga toxins (*stx*1 and *stx*2) are detected in infected chicken and in various poultry products (Salehi *et al.*, 2007). The present paper describes the molecular detection and presence of certain virulence genes (*stx, stx2, eae*A, and *hly*A) in *E. coli* isolates by multiplex PCR in commercial chicken.

Material and Methods

The materials for the present study were collected from 525 dead birds. During necropsy, sterile swabs were collected for bacteriological studies from different organs showing the lesions. Isolation and identification of *E. coli* were done as suggested by Edwards and Ewing (1972). For molecular detection of virulence genes (*stx*1, *stx*2, *eae*A, and *hly*A) of *E. coli*, DNA templates were prepared by boiling and snap chilling about 2-3 colonies of an overnight grown culture of *E. coli* on EMB agar plates. Multiplex PCR was carried out and the primers used were described previously by Paton and Paton (1998).

A total of 86 isolates were identified as *E. coli* based on morphological and biochemical properties. Of these 47 isolates (54.65%) were found positive for one or more virulence genes by multiplex PCR. The *E. coli* isolates harbored 3 (*stx*1, *stx*2 and *eae*A) out of the 4 genes investigated and none of the isolates were positive for *hlyA* gene. Multiplex PCR assay yielded amplified products of 180 bp, 255bp, and 384bp that were specific for *stx*1, *stx*2, and *eae*A genes respectively in positive isolates (Fig.1). Out of 86, *E. coli* isolates 15 (17.44%) isolates harbored *stx*1gene, 11 (12.79%) isolates carried *stx*2 gene, 18 (20.93%) isolates were positive for both *stx*1 and *stx*2 genes and 3 (3.49%) isolates carried *eae*A gene. Salehi *et al.* (2007) also used multiplex PCR for the detection of stx1, *stx*2, *eae*A and *hly*A virulence genes of *E. coli* in poultry.

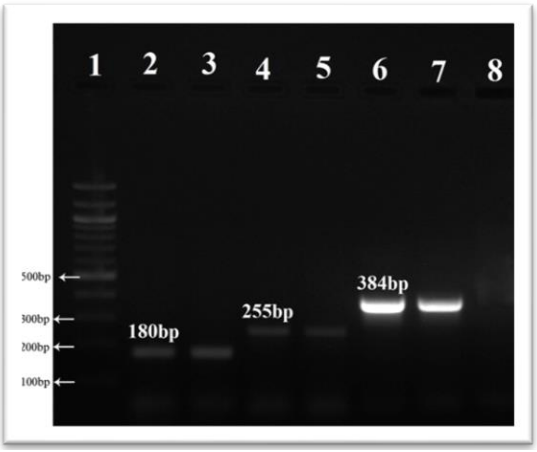

Fig 1. Agar gel electrophoresis for amplification of *stx*1, *stx*2 and *eae*A genes in APEC isolates
(Lane 1: 100bp DNA ladder, **Lane 2 &3**: Products of amplification of *stx*1 gene; **Lane 4 & 5**: Products of amplification of *stx*2 gene, **Lane 6 & 7**: Products of amplification of *eae* gene; **Lane 8**: Negative control)

Results and Discussion

Out of 47, *E. coli* isolates that possessed virulence genes, STEC (Shiga-like toxin-producing *E. coli*) associated genes (stx1and *stx*2) were found in 44 (51.16%) isolates, and all these *stx* positive isolates were negative for *eae* gene. Infection with STEC in chicken requires flagella but not intimin (encoded by *eae*), the surface adhesion responsible for attachment of the organism to the epithelial cells in mammals (Best *et al.*, 2005). Similarly, Parreira and Gyles (2002) found *stx* genes in 53% of APEC isolates, and all the *stx*- positive isolates were found negative

for the *eae* and *hly*A genes. A higher percentage (83.33%) of the *E. coli* isolates of chicken carried *stx* genes in a study conducted by Salehi *et al.* (2007) in Iran and all these *stx*- positive isolates were negative for *eae* and *hly* genes.

STEC is a group of bacteria that produce one or more Shiga toxins and are of concern in humans. Shiga toxin-producing *E. coli* strains responsible for hemolytic uremic syndrome, hemorrhagic colitis, diarrhea, and renal failure in children are zoonotic water and food-borne pathogens (Coombes *et al.,* 2008). The pathogenicity of STEC is mediated by Shiga toxins encoded by *stx*1 and *stx*2 genes. The Stxs are AB_5 toxins that halt protein synthesis in the host cell, and both epithelial and endothelial cells intoxicated with Stx may undergo apoptotic cell death after intoxication (Tesh, 2010). There is a difference of opinion about the role of STEC of poultry in human infections. Paton and Paton (1998) opined that the STEC strains found in the gastrointestinal tract of domestic animals may have a low degree of virulence in humans and these strains are less likely to produce putative accessory virulence factors such as intimin (encoded by *eae*A) and enterohaemolysin (encoded by *hly*A). Salehi *et al.* (2007) stated that the *stx* isolates that were not found positive to *eae* may not be able to pose a risk to public health. Hence, further studies are required to know the role of STEC isolates of poultry as a potential health hazard for humans.

Enteropathogenic *E. coli* (EPEC) associated *eae*A gene was found only in three (3.49%) *E. coli* isolates and none of the *stx* positive isolates possessed *eae*A. The presence of *eae* gene in *E. coli* isolates of avian origin was noticed earlier by Kilic *et al.,* 2007 but they found a higher level of prevalence of *eae*A gene. The *eae*A gene encodes a protein named intimin, a bacterial adhesion molecule that is responsible for the intimate attachment of *E. coli* to the enterocytes causing attaching and effacing (A/E) lesions in the intestinal mucosa (Agin and Wolf, 1997). The genes encoding the proteins responsible for A/E lesions map to a chromosomal "pathogenicity island" termed the "locus of enterocyte effacement" (LEE). The *eae* gene is essential for the virulence of EPEC strains and the presence of *eae* gene, along with the absence of *stx,* is sufficient to define EPEC, because possession of this sequence correlates with the existence of the locus of enterocyte effacement pathogenicity island (Nataro and Kaper, 1998). Animals and birds may constitute a natural reservoir of attaching and effacing *E. coli* strains and some of these are known as human pathogens (Salehi *et al.*, 2007).

Conclusion

In conclusion, the present paper describes the molecular detection and presence of *stx*1, *stx*2 and *eae*A virulence genes of APEC in commercial chicken.

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Contribution of Authors

The authors contributed equally.

Conflict of Interests

There is no conflict of interest.

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