

International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
ISSN Online: 2617-4707
NAAS Rating (2026): 5.29
IJABR 2026; 10(5): 456-460
www.biochemjournal.com
Received: 03-02-2026
Accepted: 05-03-2026

Pranoti Sharma
Department of Veterinary
Pathology, College of
Veterinary Science and A.H.,
Mhow, NDVSU Jabalpur,
Madhya Pradesh, India

Nidhi Shrivastava
Department of Veterinary
Pathology, College of
Veterinary Science and A.H.,
Mhow, NDVSU Jabalpur,
Madhya Pradesh, India

Supriya Shukla
Department of Veterinary
Pathology, College of
Veterinary Science and A.H.,
Mhow, NDVSU Jabalpur,
Madhya Pradesh, India

KK Jadav
Department of Veterinary
Pathology, College of
Veterinary Science and A.H.,
Mhow, NDVSU Jabalpur,
Madhya Pradesh, India

Rakhi Gangil
Department of Veterinary
Pathology, College of
Veterinary Science and A.H.,
Mhow, NDVSU Jabalpur,
Madhya Pradesh, India

Ravi Sikrodiya
Department of Veterinary
Pathology, College of
Veterinary Science and A.H.,
Mhow, NDVSU Jabalpur,
Madhya Pradesh, India

MS Trivedi
Department of Veterinary
Pathology, College of
Veterinary Science and A.H.,
Mhow, NDVSU Jabalpur,
Madhya Pradesh, India

Corresponding Author:
Pranoti Sharma
Department of Veterinary
Pathology, College of
Veterinary Science and A.H.,
Mhow, NDVSU Jabalpur,
Madhya Pradesh, India

Virulence gene profiling of avian pathogenic *Escherichia coli* serotypes of Kadaknath Chickens

Pranoti Sharma, Nidhi Shrivastava, Supriya Shukla, KK Jadav, Rakhi Gangil, Ravi Sikrodiya and MS Trivedi

DOI: <https://www.doi.org/10.33545/26174693.2026.v10.i5f.8411>

Abstract

Avian pathogenic *Escherichia coli* (APEC) is a major etiological agent of colibacillosis in poultry, resulting in substantial economic losses worldwide. The present study was undertaken to investigate the prevalence of virulence associated genes (VAGs) among dominant APEC serotypes isolated from Kadaknath chickens in the Mhow region. A total of 18 available *E. coli* isolates recovered and presumptively identified from 150 examined carcasses were subjected to molecular characterization of certain virulence associated genes including *iss* (increased serum survival), *tsh* (temperature sensitive hemagglutinin), *papC* (P fimbriae), *iucC* (aerobactin synthesis) and *sitA* (iron transport), using polymerase chain reaction (PCR). All five genes were detected in isolates belonging to serogroups O8, O78, O7, and O84, whereas isolates of serogroup O115 lacked the *tsh* gene. The findings of this study provide important insights into the distribution of key virulence determinants among prevalent APEC serotypes in Kadaknath chickens. These virulence markers may serve as valuable tools for the identification, surveillance and development of targeted control strategies against APEC infections in poultry.

Keywords: APEC, colibacillosis, Kadaknath, virulence genes, serotyping, *iss*, *tsh*, *papC*, *iucC*, *sitA*

Introduction

Colibacillosis caused by avian pathogenic *Escherichia coli* (APEC), is one of the most economically significant bacterial diseases affecting poultry worldwide. APEC strains are characterized by the presence of virulence associated genes (VAGs) that enable colonization, immune evasion and systemic infection in avian hosts. The virulence associated genes are typically found in large circular plasmids (50-200 kb) of pathogenic *E. coli* such as ColV and IncF plasmids (Johnson *et al.*, 2006) [5].

Avian pathogenic *Escherichia coli* (APEC) employs a diverse array of virulence mechanisms that enable survival, colonization and systemic dissemination within the avian host these factors are VAGs, Key virulence factors include serum resistance (*iss*), adhesins (*papC*, *tsh*) and iron acquisition systems (*iucC*, *sitA*). Serum resistance is a critical determinant of pathogenicity, primarily mediated by the *iss* gene, which encodes an outer membrane lipoprotein that protects bacteria against complement mediated lysis and enhances bacterial persistence in host serum (Mellata *et al.* 2003) [9]. Adhesins such as P fimbriae, encoded by the *papC* gene, facilitate bacterial attachment to epithelial cells and are particularly associated with systemic infections as highlighted by Dziva and Stevens (2008) and Kathayat *et al.* (2021) [6]. The ability of APEC to colonize the respiratory tract is further enhanced by temperature-sensitive hemagglutinin (*tsh*), a multifunctional autotransporter protein exhibiting both adhesin and protease activities (Kwon *et al.* 2008) [8]. Studies by Dozois *et al.* (2000) [2] and Kobayashi *et al.* (2010) [7] have shown that *tsh* contributes to early-stage infection, mucosal colonization and lesion development in poultry. Siderophores such as aerobactin, encoded by the *iuc ABCD/iut A* locus and salmochelin, encoded by the *iroBCDEN* genes, play a crucial role in iron sequestration, thereby enhancing bacterial growth and virulence Caza *et al.* (2008) [1].

Virulence factors serve as molecular markers help in diagnosing and managing APEC infections. The detection of at least five VAGs is considered a reliable criterion for identifying APEC strains with enhanced virulence potential.

Kadakhnath, an indigenous Indian poultry breed, has gained increasing commercial relevance; however, data on the prevalence and molecular epidemiology of avian pathogenic *Escherichia coli* (APEC) in this breed remain scarce. The present study was designed to investigate the distribution of key VAGs among APEC serotypes obtained from Kadakhnath chickens. By profiling these genetic determinants, the study aims to elucidate the pathogenic potential and molecular characteristics of circulating strains. Such insights are essential for understanding disease dynamics and informing targeted control strategies in this native breed.

Materials and Methods

Genomic DNA extraction was performed using APEC isolates representing the prevalent serotypes identified in a prior study conducted in the department (Sharma *et al.*, 2026) [12]. Pure *E. coli* cultures were grown overnight in BHI broth at 37°C. Bacterial cells were pelleted by centrifugation (10,000 × g, 3 min) and genomic DNA was extracted using the QIAprep Spin Miniprep Kit (Qiagen) according to the manufacturer's protocol. DNA purity and concentration were assessed spectrophotometrically (A260/A280).

Virulence genes detection using PCR

Five VAGs viz *iss*, *tsh*, *papC*, *iucC* and *sitA* were targeted using published primer sequences and expected amplicon sizes are shown in Table 1.

Table 1: Primers used for virulence gene detection

Gene	Primer Sequence (5'-3')	Product (bp)	Reference
<i>iss</i>	F: GTGGCGAAAACCTAGTAAAACAGC / R: CGCCTCGGGGTGGATAA	762	Tivendale <i>et al.</i> (2004) [15]
<i>iucC</i>	F: CGCCGTGGCTGGGGTAAG / R: CAGCCGGTTCACCAAGTATCACTG	541	Skyberg <i>et al.</i> (2003) [13]
<i>tsh</i>	F: GGTGGTGCCTGGAGTGG / R: AGTCCAGCGTGATAGTGG	642	Tivendale <i>et al.</i> (2004) [15]
<i>papC</i>	F: GTGGCAGTATGAGTAATGACCGTTA / R: ATATCCTTTCTGCAGGGATGCAATA	205	Johnson and Stell (2000) [4]
<i>sitA</i>	F: AGGGGGCACAACCTGATTCTCG / R: TACCGGGCCGTTTTCTGTGC	608	Rodriguez-Siek <i>et al.</i> (2005) [10]

PCR was performed in 25 µL reactions containing 12.5 µL Emerald Amp 2× PCR Master Mix, 0.5 µL each primer (10 µM), 2 µL template DNA, and nuclease-free water. Cycling conditions were presented in Table 2. Amplicons were

resolved by electrophoresis on 1.5% agarose gels stained with ethidium bromide and visualized under UV transillumination.

Table 02: PCR cycling conditions for detection of virulence genes

Genes	Steps	Temperature °C	Time	Number of cycles
<i>Iss, Tsh</i>	Initial denaturation	94	3 min	1
	Denaturation	94	30s	
	Annealing	55	30s	26
	Extension	72	45s	
	Final extension	72	7	1
<i>Sit A</i>	Initial denaturation	94	3 min	1
	Denaturation	94	30s	
	Annealing	59	30s	30
	Extension	72	30s	
	Final extension	72	5 min	1
<i>IuuC</i>	Initial Denaturation	95	5 min	1
	Denaturation	95	1min	
	Annealing	55	30s	30
	Extension	72	1min	
	Final extension	72	5 min	1
<i>Pap C</i>	Initial Denaturation	95	5 min	1
	Denaturation	95	1min	
	Annealing	55	30s	30
	Extension	72	1min	
	Final extension	72	5 min	1

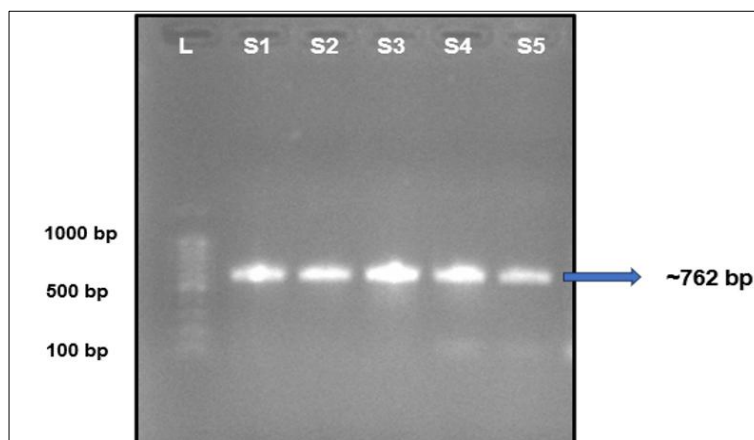
Results

A total of 18 isolates representing the predominant serogroups O8, O78, O84, O7 and O115 were identified in earlier study as the most prevalent based on their frequency of occurrence (Sharma *et al.*, 2026) [12] were subsequently selected as a representative subset for identification of VAGs. Gel electrophoresis following PCR amplification confirmed the expected product sizes for all five targeted VAGs *iss* (762 bp), *tsh* (642 bp), *papC* (205 bp), *sitA* (608 bp) and *iucC* (541 bp), as illustrated in Figures 1-5.

The prevalence and distribution of the five virulence genes across the 18 screened isolates are summarized in Table 3. Four of the five genes *iss*, *papC*, *iucC* and *sitA* were detected in all 18 isolates (100%), indicating their universal presence across all five serogroups. The *tsh* gene, encoding a temperature-sensitive hemagglutinin protease, was detected in 16 of the 18 isolates (88.8%). It was present in serogroups O8, O78, O84, and O7, but was notably absent in the O115 serogroup. Consequently, isolates belonging to serogroup O115 harboured four of the five screened VAGs.

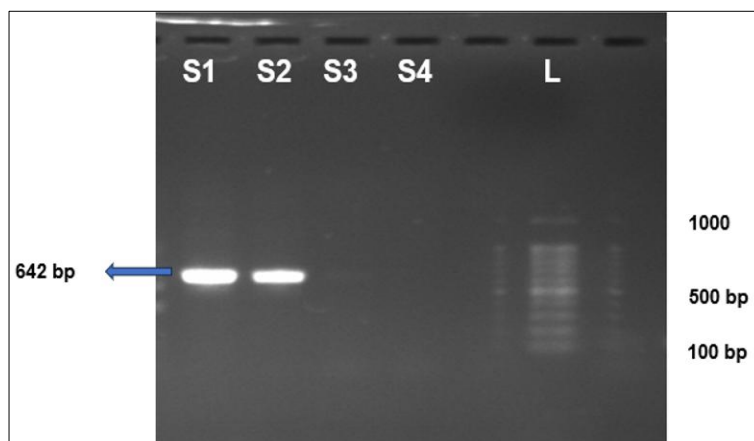
Table 3: Detection of virulence genes in prevalent APEC serotypes (n=18)

Gene	Function	Product (bp)	Positive (%)	Serotypes Detected
<i>iss</i>	Serum resistance	762	18 (100)	O8, O78, O84, O7, O115
<i>papC</i>	P fimbriae (adhesion)	205	18 (100)	O8, O78, O84, O7, O115
<i>iucC</i>	Aerobactin synthesis	541	18 (100)	O8, O78, O84, O7, O115
<i>sitA</i>	Iron transport	608	18 (100)	O8, O78, O84, O7, O115
<i>tsh</i>	Hemagglutinin/protease	642	16 (88.8)	O8, O78, O84, O7

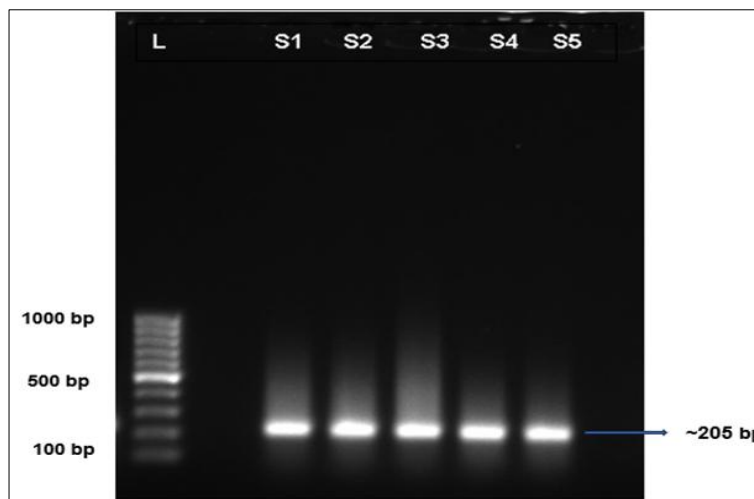


Lane L: 100 bp DNA ladder (Thermo scientific, 0.5 µg/ µl)

Lane S1-S5: APEC isolates

Fig 1: Agarose gel electrophoresis showing PCR amplified *Iss* gene (~762 bp) of APEC isolates

Lane L: 100 bp DNA ladder (Thermo scientific, 0.5 µg/ µl) Lane S1-S4: APEC isolates

Fig 2: Agarose gel electrophoresis showing PCR amplified *Tsh* gene (~642 bp) of APEC isolates

Lane L1 :100 bp DNA ladder (Thermo scientific, 0.5 µg/µl)

Lane S1-S5: APEC isolates

Fig 3: Agarose gel electrophoresis showing PCR amplified *Pap C* gene (~205 bp) of APEC isolates

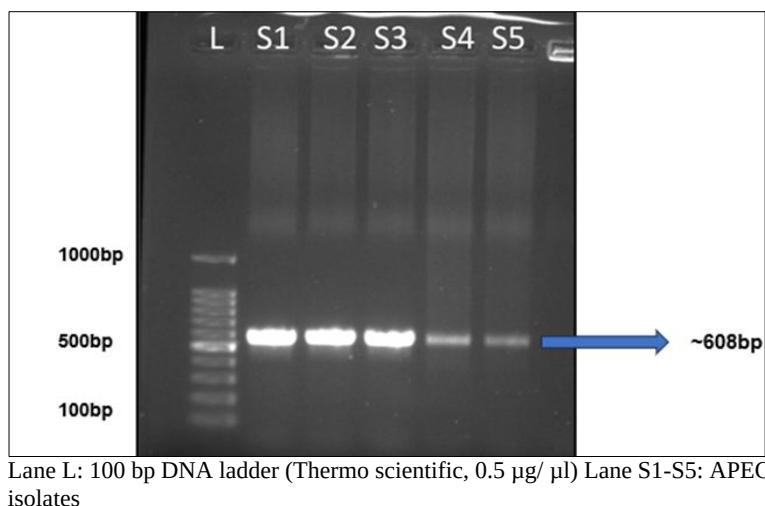


Fig 4: Agarose gel electrophoresis showing PCR amplified *Sit A* gene (~608 bp) of APEC isolates

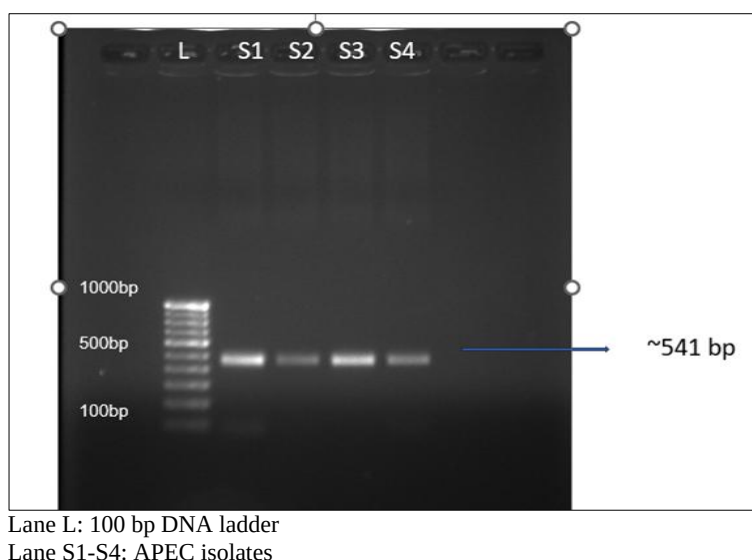


Fig 5: Agarose gel electrophoresis showing PCR amplified *IucC* gene (~541 bp) of APEC isolates

Discussion

The *iss* gene, encoding an outer membrane lipoprotein that confers serum resistance, was detected in 100% of isolates, consistent with its recognition as the most prevalent and reliable marker for APEC (Subedi *et al.* 2018) [14]. The ability to evade host serum bactericidal activity is critical for APEC survival in the bloodstream following translocation from the respiratory or gastrointestinal tract, and the ubiquitous carriage of *iss* in the present collection reinforces its pivotal role in systemic pathogenesis. Given its universal distribution, *iss* has been proposed as an indispensable component of any minimum gene panel intended for the molecular identification and classification of APEC (Ewers *et al.*, 2004) [3].

Molecular characterization demonstrated that all five targeted VAGs were present in serotypes O8, O78, O7 and O84 indicating high pathogenic potential. The universal prevalence of *papC*, *iucC*, and *sitA* observed in this study further substantiates the central role of adhesion and iron acquisition systems in APEC pathogenicity. The *papC* gene encodes an outer membrane protein essential for the assembly of P fimbriae, which mediate adhesion to uroepithelial cells and respiratory mucosa, facilitating colonization and initial tissue invasion. The *iucC* gene, a component of the aerobactin siderophore biosynthesis

cluster, enables APEC to chelate iron from the iron-limiting environment of host tissues, a prerequisite for sustained bacterial replication and the establishment of systemic infection (Dozois *et al.*, 2003) [2]. Similarly, *sitA*, encoding the substrate-binding component of a ferrous iron and manganese transport system, provides an additional, complementary iron acquisition strategy that enhances APEC fitness in the host (Sabri *et al.*, 2006) [11]. The co-occurrence of multiple iron uptake systems in the isolates from this study highlights the redundancy of iron acquisition mechanisms in APEC, a trait that confers a competitive survival advantage within the host microenvironment. The *tsh* gene, which encodes a temperature-sensitive hemagglutinin functioning as an autotransporter protease, was detected in all serogroups except O115 isolates. The *tsh*-encoded protein facilitates colonization of the respiratory mucosa by degrading mucus glycoproteins and immunoglobulins and its hemagglutinating activity has been proposed to promote initial attachment to host cells (Dozois *et al.*, 2000) [2]. The absence of *tsh* in O115 isolates is a notable observation and suggests potential variability in early colonization mechanisms among APEC serogroups, possibly reflecting differences in the plasmid content or genomic island configurations of O115 strains compared to the predominant serogroups. This is consistent with earlier

reports demonstrating that *tsh* is frequently carried on ColV-type plasmids and that its distribution is not uniformly conserved across all APEC lineages (Johnson *et al.*, 2006; Skyberg *et al.*, 2003)^[5, 13].

In conclusion, the findings of the present study confirm the universal carriage of *iss* and the near complete distribution of the remaining four VAGs in the predominant serogroups underscore the pathogenic capacity of circulating APEC strains. These data support the implementation of a standardized five-gene molecular panel for routine APEC surveillance and provide a baseline for future epidemiological and comparative genomic investigations in indigenous poultry breeds. Continuous monitoring of APEC virulence profiles and antimicrobial resistance patterns in Kadaknath and allied breeds will be essential for informing disease management strategies and safeguarding both poultry health and public health at the human animal interface. These findings provide a molecular basis for surveillance and control of colibacillosis in indigenous poultry breeds and highlight the need for continued epidemiological monitoring to track emerging serotypes and virulence profiles.

Conflict of Interest

The authors declare no conflict of interest.

References

1. Caza M, Lepine F, Milot S, Dozois CM. Specific roles of the iron BCDE genes in virulence of an avian pathogenic *Escherichia coli* O78 strain and in production of salmochelins. *Infection and Immunity*. 2008;76(8):3539-3549.
2. Dozois CM, Dho-Moulin M, Brée A, Fairbrother JM, Desautels C, Curtiss R. Relationship between the Tsh autotransporter and pathogenicity of avian *Escherichia coli* and localization and analysis of the Tsh genetic region. *Infection and Immunity*. 2000;68(7):4145-4154.
3. Ewers C, Janssen T, Kiessling S, Philipp HC, Wieler LH. Rapid detection of virulence-associated genes in avian pathogenic *Escherichia coli* by multiplex polymerase chain reaction. *Avian Diseases*. 2005;49:269-273.
4. Johnson JR, Stell AL. Extended virulence genotypes of *Escherichia coli* strains from patients with urosepsis in relation to phylogeny and host compromise. *Journal of Infectious Diseases*. 2000;181:261-272.
5. Johnson TJ, Johnson SJ, Nolan LK. Complete DNA sequence of a ColBM plasmid from avian pathogenic *Escherichia coli* suggests that it evolved from closely related ColV virulence plasmids. *Journal of Bacteriology*. 2006;188:5975-5983.
6. Kathayat D, Lokesh D, Ranjan R, Dhama K. Avian colibacillosis: Current understanding of pathogenomics, virulence factors, and control strategies. *Veterinary Quarterly*. 2021;41(1):64-92.
7. Kobayashi RK, Gaziri LC, Vidotto MC. Functional activities of the Tsh protein from avian pathogenic *Escherichia coli* (APEC) strains. *Journal of Veterinary Science*. 2010;11(4):315-319.
8. Kwon HM, Kim TE, Park MS, Lim TH, Kim JH, Lee HJ, *et al.* Prevalence of virulence-associated genes in avian pathogenic *Escherichia coli* isolates from chickens in Korea. *Poultry Science*. 2008;87(12):2492-2496.
9. Mellata M, Dho-Moulin M, Dozois CM, Curtiss R III, Lehoux B, Fairbrother JM. Role of virulence factors in resistance of avian pathogenic *Escherichia coli* to serum and in pathogenicity. *Infection and Immunity*. 2003;71(1):536-540.
10. Rodriguez-Siek KE, Giddings CW, Oetkott C, Johnson TJ, Nolan LK. Characterizing the APEC pathotype. *Veterinary Research*. 2005;36(3):241-256.
11. Sabri M, Leveillé S, Dozois CM. A Sit ABCD homologue from an avian pathogenic *Escherichia coli* strain mediates transport of iron and manganese and resistance to hydrogen peroxide. *Microbiology*. 2006;152:745-758.
12. Sharma P, Shrivastava N, Shukla S, Agrawal V, Gupta SK, Jadhav KK, *et al.* Prevalence and serological characterization of avian pathogenic *E. coli* in Kadaknath chicken: A comprehensive study. *International Journal of Advanced Biochemistry Research*. 2026;10(5):51-57.
13. Skyberg JA, Horne SM, Giddings CW, Wooley RE, Gibbs PS, Nolan LK. Characterizing avian *Escherichia coli* isolates with multiplex polymerase chain reaction. *Avian Diseases*. 2003;47:1441-1448.
14. Subedi M, Luitel H, Devkota B, Bhattarai RK, Phuyal S, Panthi P, *et al.* Antibiotic resistance pattern and virulence genes content in avian pathogenic *Escherichia coli* (APEC) from broiler chickens in Chitwan, Nepal. *BMC Veterinary Research*. 2018;14:113-118.
15. Tivendale KA, Allen JL, Ginns CA, Crabb BS, Browning GF. Association of *iss* and *iucA* but not *tsh*, with plasmid-mediated virulence of avian pathogenic *Escherichia coli*. *Infection and Immunity*. 2004;72:6554-6560.