

International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
ISSN Online: 2617-4707
NAAS Rating (2026): 5.29
IJABR 2026; 10(5): 51-57
www.biochemjournal.com
Received: 02-03-2026
Accepted: 04-04-2026

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Prevalence and serological characterization of avian pathogenic *E. coli* in Kadaknath chicken: A comprehensive study

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DOI: <https://www.doi.org/10.33545/26174693.2026.v10.i5a.8310>

Abstract

Kadaknath chicken, an indigenous breed of Madhya Pradesh, India, represents a valuable breed renowned for its unique nutritional profile and disease resistance. This comprehensive study investigated the prevalence of avian pathogenic *Escherichia coli* (APEC) infection and characterized the serological diversity among isolates obtained from Kadaknath birds in and around Mhow region. A total of 150 Kadaknath carcasses were subjected to detailed necropsy examination, revealing multisystemic pathological manifestations. Representative tissue samples exhibiting gross lesions were collected aseptically for bacteriological analysis. Comprehensive cultural, morphological and biochemical evaluations confirmed 37 *Escherichia coli* isolates, indicating an overall APEC prevalence of 24.66%. Serotyping of these isolates demonstrated substantial serological heterogeneity, identifying 15 distinct serogroups. Among these, serogroup O8 was the most prevalent (21.4%), followed by O78 (14.28%), O84 (10.71%), O7 (10.71%) and O115 (7.1%). The detection of both commonly reported and less documented serogroups highlights the complex epidemiology of avian pathogenic *Escherichia coli* in Kadaknath chickens and underscores the need for region specific and breed focused surveillance strategies to inform targeted prevention and control measures.

Keywords: Kadaknath, APEC, *Escherichia coli*, serogroups, serotypes

Introduction

The Indian poultry industry has experienced exponential growth in recent decades, emerging as one of the fastest expanding agricultural sectors contributing significantly to rural economy and nutritional security. Within this context, indigenous poultry breeds have garnered increasing attention for their unique genetic characteristics, adaptability to local environmental conditions and potential for sustainable production systems. Among these native breeds, the Kadaknath chicken of Madhya Pradesh stands out as an exceptional genetic resource with distinctive attributes that differentiate it from commercial poultry strains. The Kadaknath breed possesses a unique nutritional composition that has generated considerable scientific and commercial interest. This breed offers superior nutritional value compared to conventional poultry varieties, characterized by elevated protein content, reduced fat levels and enrichment with essential nutrients including iron and omega-3 fatty acids (Kumar and Dwivedi, 2025) ^[13]. These biochemical attributes position Kadaknath meat as a high value poultry product preferred in health-conscious consumer segments, thereby potentially increasing its market price and contributing to improved livelihood prospects for rural producers. Furthermore, the Kadaknath breed exhibits inherent resistance to various infectious diseases, demonstrating robust survivability under adverse environmental conditions characterized by high temperatures and humidity (Ramamamy *et al*, 2010) ^[16]. Despite these advantageous characteristics, infectious diseases continue to pose substantial constraints to the development and expansion of Kadaknath farming enterprises. Avian colibacillosis caused by avian pathogenic *Escherichia coli* (APEC) is a critical challenge represents one of the most economically devastating bacterial infections affecting poultry production worldwide. The disease exerts its economic impact through multiple pathways, including elevated morbidity and mortality rates, reduced growth performance, decreased egg production, condemnation of carcasses at processing facilities and substantial

costs associated with therapeutic interventions and preventive measures (Kathayat *et al.*, 2021) ^[9]. The pathological manifestations of APEC infection are diverse and complex, reflecting the organism's capacity to colonize multiple anatomical sites and cause systemic disease. Initial infection frequently occurs through the respiratory tract, followed by bacterial dissemination that can result in septicaemia and subsequent localization in various organs and tissues. The resulting clinical syndrome may encompass airsacculitis, pericarditis, perihepatitis, peritonitis, salpingitis, synovitis, osteomyelitis, omphalitis and cellulitis, either individually or in complex combinations (Kim *et al.*, 2020) ^[10].

Colibacillosis prevalence rates varying considerably across different geographical regions and production systems. In the Indian subcontinent, several studies have documented the widespread occurrence of this disease, with Boro *et al.* (2017) ^[3] reporting a 17.16% prevalence in bird carcasses from both organized and unorganized farms around Guwahati city, Assam. The disease has shown particular impact on indigenous breeds, as Chakravarti *et al.* (2018) ^[5] observed 15.51% mortality in Kadaknath flocks with an overall incidence of 3.13%.

The epidemiological complexity of APEC infections stems from the remarkable genetic and serological diversity exhibited by pathogenic strains. Serotyping, based on the characterization of O somatic antigens present on the bacterial cell surface, has served as a fundamental tool for epidemiological surveillance and strain identification. While specific serogroups, including O1, O2, O8, O15, O18, O35, O36, O78, O88, O109, O111 and O115 have been frequently associated with colibacillosis (Younis *et al.*, 2017) ^[21], the distribution patterns of these serogroups demonstrate considerable geographical and temporal variation. Koutsianos *et al.* (2022) ^[12] reported that serogroups O78 and O2 were the most prevalent among flocks with increased mortality in both pullets and layers. Same study reported the 1.7% prevalence of O8 serogroup in pullets with normal mortality rate. Serotype distribution patterns may vary geographically and breed wise and therefore regional surveillance of O serogroups in indigenous breeds like Kadaknath, where limited epidemiological data exist, such surveillance assumes even greater significance for developing breed-specific and region-specific control measures.

The present investigation was undertaken to determine the prevalence of *E. coli* infection among Kadaknath birds in the Mhow region through systematic bacteriological examination of carcasses exhibiting pathological lesions and to identify the predominant APEC serogroups circulating in the local Kadaknath population through comprehensive serotyping of bacterial isolates to address the knowledge gap regarding APEC prevalence in Kadaknath chicken populations.

Materials and Methods

This study was carried out in the Department of Veterinary Pathology, College of Veterinary Sciences and Animal Husbandry, Mhow (Madhya Pradesh), a constituent college of Nanaji Deshmukh Veterinary Science University, Jabalpur. The study encompassed a comprehensive examination of 150 Kadaknath carcasses irrespective of birds age and sex collected from organized Kadaknath farms located in and around Mhow, Madhya Pradesh. Each carcass

underwent detailed necropsy examination in the Department of Veterinary Pathology, College of Veterinary Science and Animal Husbandry, Mhow, NDVSU. Tissue samples from representative organs showing gross pathological alterations viz. heart, liver, lungs, intestine and spleen were collected for bacteriological investigation.

Bacteriological investigation

The samples collected were inoculated in Brain heart infusion broth (BHI) (Hi-Media), incubated at 37 °C overnight under aerobic conditions, following incubation cultures demonstrating turbidity, indicative of bacterial proliferation, were subjected to further isolation procedures using selective and differential medias. Turbid broth streaked on MacConkey agar (Hi-Media) and incubated at 37 °C for overnight to obtain lactose fermenting colonies from each sample. Pink colonies were further streaked on Eosin methylene blue agar (EMB) (Hi-Media) to observe characteristic metallic sheen to confirm and obtain pure cultures of *E. coli* (Buxton and Fraser, 1977). Pure colonies were subjected to morphological characterization involved Gram staining of bacterial smears prepared from pure cultures and employed the biochemical methods using standard Indole production, Methyl Red reaction, Voges-Proskauer reaction, and Citrate utilization (IMViC) tests outlined by Barrow and Feltham (1993) ^[2]. The characteristic IMViC pattern for *E. coli* (+ + --) was used for definitive identification. Confirmed isolates were streaked on nutrient agar plates. Single pure colonies from nutrient agar plates were then transferred to nutrient agar slants and stored at 4 °C for further identification. The isolates were sub cultured on fresh nutrient agar slant every fortnightly.

Serological characterization:

Serotyping of confirmed *E. coli* isolates was performed at the National Salmonella and Escherichia Centre, Central Research Institute, Kasauli, a reference laboratory equipped with comprehensive typing sera representing the spectrum of known *E. coli* O-antigens. Serotyping involved agglutination reactions between bacterial suspensions and specific antisera, allowing identification of the O-somatic antigen and assignment to specific serogroups.

Statistical Analysis

The prevalence percentage was calculated using the formula:

$$\text{Prevalence (\%)} = \frac{\text{Total number of positive cases}}{\text{Total number of carcasses examined during study period}} \times 100$$

Results

Gross pathological observation

The necropsy examination revealed diverse pathological alterations affecting multiple organ systems, reflecting the systemic nature of APEC infections (Table 1). External examination identified periorbital swelling and sinusitis in 9.33% of carcasses, while pasty vent, indicative of enteritis, was observed in 4.66% of examined birds.

Internal examination revealed more extensive and varied pathological changes. The hepatic system was most frequently affected, with hepatomegaly (Fig 1) observed in 22.66% of carcasses. Affected livers displayed congestion, enlargement, and focal necrotic areas, suggesting systemic bacterial dissemination with hepatic localization. The

gastrointestinal tract showed pathological alterations in 20% of cases, manifesting as thickened and congested intestinal serosa, mucoid to watery intestinal contents, hemorrhagic enteritis in some cases and distended ceca containing blood and caseous cores.

Splenomegaly (Fig 2) was documented in 14.66% of examined birds, reflecting the involvement of lymphoid organs in the infectious process. Respiratory system involvement was evidenced by pulmonary congestion in 13.33% of cases, occasionally accompanied by frothy exudate in the thoracic cavity (Fig 3), suggesting aerosacculitis. Cardiovascular manifestations included pericardial thickening in 6% of carcasses with occasional myocardial hemorrhages.

Reproductive system pathology was observed in female birds, including congested ovarian follicles (3.33%) and egg peritonitis (2%) characterized by malformed eggs and flocculent material in the abdominal cavity. Less frequently encountered lesions included pinpoint hemorrhages on the proventricular mucosa and visceral gout (1.33%).

Table 1: Various gross lesions observed in necropsied carcasses and their respective percentage to overall mortality

S. No.	Gross lesions recorded	Percentage of overall mortality	No. of birds
1	Swollen and congested liver	22.66%	34
2	Intestinal lesions	20%	30
3	Splenomegaly	14.66%	22
4	Pulmonary congestion/consolidation	13.33%	20
5	Periorbital swelling and sinusitis	9.33%	14
6	Thickened pericardium	6%	9
7	Congestion in ovarian follicles	3.33%	5
8	Egg peritonitis	2%	3
9	Uric acid deposition	1.33%	2
10	Pinpoint haemorrhages in proventriculus	1.33%	2

Bacteriological Findings

From 82 birds exhibiting the most apparent lesions, tissue samples were collected for bacteriological examination. MacConkey agar yielded lactose-fermenting pink colonies (Fig 4) from which 55 isolates were further investigated. Culture on EMB agar revealed that 37 isolates produced the

characteristic metallic sheen with purple-black coloration (Fig 5), tentatively identifying them as *E. coli*. Gram staining confirmed all 37 isolates as Gram-negative rods (Fig 6), consistent with *E. coli* morphology. Biochemical characterization using the IMViC test battery revealed that all isolates were positive for Indole production and Methyl Red reaction while negative for Voges-Proskauer and Citrate utilization tests, yielding the characteristic ++-- pattern diagnostic of *E. coli*.



Fig 1: Photograph showing enlarged liver with focal necrotic area on right lobe of Kadaknath chicken



Fig 2: Photograph showing enlarged and congested spleen of Kadaknath chicken

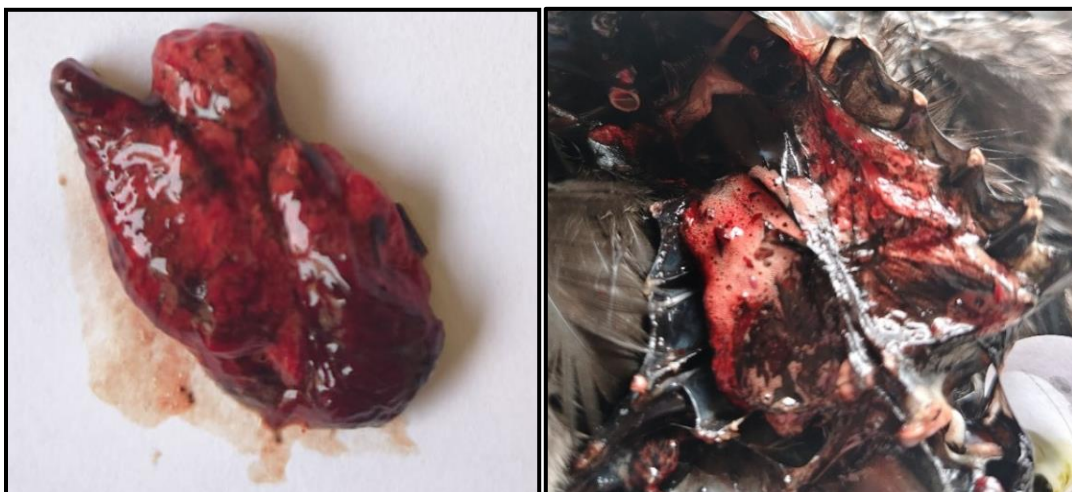


Fig 3: Photograph showing (A) congested and oedematous lungs (B) frothy exudate in thoracic cavity

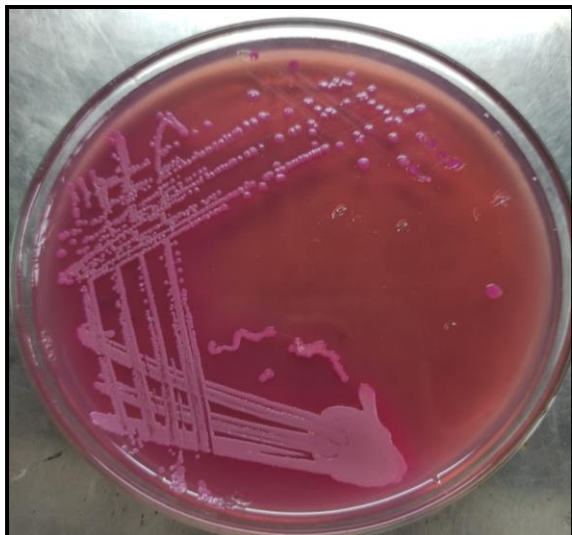


Fig 4: Lactose fermenting rose pink colonies of *E. coli* on MacConkey agar



Fig 5: Characteristic metallic sheen of *E. coli* on EMB

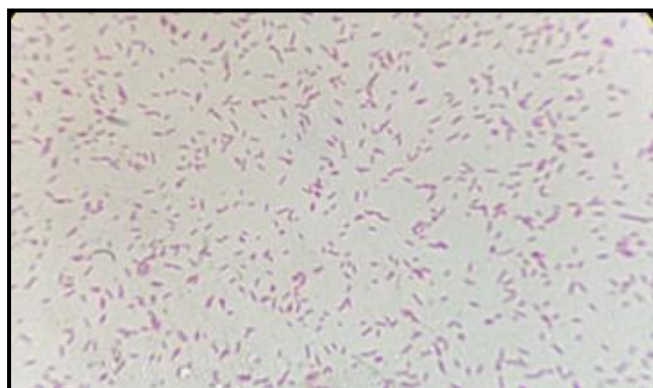


Fig 6: Gram negative pink, rod-shaped *E. coli* (Gram's X1000)

Prevalence of *E. coli* Infection

After bacterial isolation and identification on the basis of cultural, morphological and biochemical characteristics, *E. coli* was confirmed in 37 samples collected from Kadaknath birds during necropsy. Thus, on the basis of result obtained the overall prevalence of colibacillosis in Kadaknath chicken was found to be 24.66% out of 150 carcasses. Results revealed the presence of colibacillosis among all age group of Kadaknath chicken.

Serological Diversity of APEC Isolates

Serotyping of the 37 confirmed APEC isolates revealed remarkable serological heterogeneity. A total of 15 distinct O-serogroups were identified, while 9 isolates could not be typed and were classified as untypeable (UT). This diversity reflects the complex epidemiology of APEC infections and the multiple pathogenic strains circulating within the local Kadaknath population. Serogroup O8 emerged as the predominant type, representing 21.4%. This was followed by O78 at 14.28%, demonstrating substantial representation of this globally important APEC serogroup. Serogroups O7 and O84 each accounted for 10.71% each with O84 representing a less commonly reported serogroup in poultry. Serogroup O115, associated with high virulence in other studies, comprised 7.1%.

The other least frequent serogroups O11, O17, O18, O20, O22, O37, O49, O77, O104 and O149 each represented 2.85%. Thus, collectively broad serological diversity was present in the Kadaknath population.

Discussion

The gross pathological findings documented in this investigation align substantially with previous reports of colibacillosis in various poultry species while also revealing certain distinctive features potentially related to breed-specific responses or regional strain characteristics. Present research findings are in agreement to earlier research reported various disease conditions viz. coccidiosis, colibacillosis, enteritis, hepatomegaly, visceral gout, ascites in Kadaknath birds (Chakravarti *et al.*, 2018) [5]. Similarly, Gumasta *et al.* (2021) [8], observed catarrhal and haemorrhagic enteritis, pneumonic lungs and pin point haemorrhages in proventriculus of Kadaknath birds. The findings of the present study align with earlier reports describing gross macroscopic lesions associated with colibacillosis in birds. Boro *et al.* (2017) [3] documented colisepticaemia in indigenous birds, observed congestion of multiple organs along with enteritis, oophoritis, and omphalitis. Similarly, Wani *et al.* (2020) [19] reported congested, oedematous lungs and splenomegaly in colibacillosis affected chickens indicating colonization of *E. coli* within the respiratory tract. However, their observation of perihepatitis with a thick fibrinous layer producing the classic “bread and butter” appearance was not evident in the present investigation. Swollen and congested liver was predominantly observed in nearly one-quarter of examined carcasses, reflects the liver's role as a primary site of bacterial localization following septicemia. The frequency of intestinal lesions suggests that enteric infection may represent an important route of APEC entry in Kadaknath birds, potentially facilitated by intestinal barrier disruption due to concurrent infections, dietary factors or management-related stressors. The observation of splenomegaly in a substantial proportion of birds indicates systemic dissemination with involvement of lymphoid organs, consistent with the pathogenesis of colisepticemia. Respiratory manifestations, including pulmonary congestion and air sac involvement, support the respiratory tract as both a portal of entry and target organ for APEC. Elnagar *et al.*, 2021 [6] isolated *E. coli* from various internal organs with highest isolation rate from lungs and liver. The pericardial and myocardial lesions reflect the organism's tropism for serosal surfaces and its capacity to cause fibrinous polyserositis, a hallmark of severe colibacillosis. Wilczynski

et al. (2022) ^[20] reported reproductive tract involvement with hyperaemia of the yolk sac, presence of cloudy fluid in the oviduct, manifested as salpingitis and egg peritonitis, represents a significant economic concern in layer-type birds, as these conditions can severely impact egg production and lead to mortality.

Similar to our findings recent study of Tersue *et al.* (2024) ^[18] reported gross lesions in the *E. coli* infected layers as congested lungs, enlarged liver, hyperaemic intestine and congested ovarian follicles. Present study enlisted the different lesions observed in Kadaknath birds during necropsy. These comparisons with the earlier prevalence studies emphasize on both the shared pathological features of colibacillosis among different breeds and the possible breed specific and strain related variations in lesion expression.

Prevalence Assessment and Epidemiological Significance

The determined prevalence of 24.66% positions the investigation within the range of 15-25% prevalence of APEC reported in various poultry breeds by Ramadan *et al.* (2016) ^[15] and Sarba *et al.* (2019) ^[17]. When compared specifically to previous studies in Kadaknath, the present findings indicate a moderately higher prevalence than some earlier reports of Chakravarti *et al.*, 2018 ^[5] and Gumasta *et al.* (2021) ^[8], which documented rates between 15.51% and 17.1%. This variance may be attributable to several factors including temporal changes in pathogen circulation, seasonal influences at the time of sampling, variations in farm management practices or may be due to differences in the age distribution of sampled populations. The substantially higher prevalence rates of 53.2% in broilers in Egypt reported by Elnagar *et al.* (2021) ^[6], while Tan *et al.* (2025) observed an even higher prevalence of 57.5% in China reaching 50-60%, likely reflect the influence of intensive production systems, higher stocking densities, specific environmental stressors, co-infections with immunosuppressive agents.

The relatively moderate prevalence observed in the present study may indicate that traditional or semi-intensive management systems commonly employed for Kadaknath production, combined with the breed's inherent hardiness, provide some protective benefit against extremely high infection rates.

Serological Diversity and Epidemiological Implications

The results obtained revealed O8 as the most prevalent serogroup in different Kadaknath chicken farms in and around Mhow. Fancher *et al.* (2021) ^[7] reported O8 in about 31% of cases, notably higher than most global data and suggesting emergence of new trend within "no antibiotic ever" (NAE) broiler farms. Recent study of Tan *et al.* (2025) also reported O8 serogroup as one of the predominant serotypes in China (7.9%). On the contrary to these research findings some researchers have reported lower prevalence of O8 (< 2%) (Koutsianos *et al.*, 2022 and Wilczyński *et al.*, 2022) ^[12, 20]. Serogroup O8 has observed increasing importance in global APEC epidemiology in recent years and is generally recognized as a significant contributor to colibacillosis.

The prevalence of serogroup O78 (14.28%) in this study aligns with its recognized global importance, although at a comparatively lower frequency than earlier reports. Previous studies have consistently identified O78 as the predominant

serogroup among APEC isolates, with an occurrence of 27.8% in broiler chickens (Elnagar *et al.*, 2021) ^[6], and its association with increased mortality in both pullets and layers (Koutsianos *et al.*, 2022) ^[12]. Similarly, Wilczyński *et al.* (2022) ^[20] reported a global prevalence of approximately 14% in both broiler and layer populations, which is comparable to the findings of the present study. This suggests that while O78 remains epidemiologically significant, its prevalence may vary regionally due to differences in management practices, host factors, or environmental conditions. The present research identified underreported serogroup O84 at a notable frequency of 10.7%, indicates a possible emerging serotype in poultry. However, O84 has previously been documented in psittacine birds, where it exhibited virulence characteristics similar to APEC strains (Knöbl *et al.*, 2011) ^[11]. Research highlights the potential for cross-species dissemination or adaptation of virulent strains, warranting further epidemiological surveillance. Serogroup O7 was detected at a prevalence of 10.71% in the present study. Although earlier reports describe O7 as less frequent in avian hosts, its significance lies in its overlap with human extraintestinal pathogenic *E. coli* (ExPEC) strains. These strains are known to cause urinary tract infections, neonatal meningitis, and septicemia in humans (Maluta *et al.*, 2016) ^[14]. The presence of O7 in poultry isolates therefore raises concerns regarding its zoonotic potential and underscores the need for integrated "One Health" approaches in monitoring APEC strains. Furthermore, the identification of serogroup O115 (7.1%) in the present study is noteworthy. This serogroup has been reported as the most predominant in broiler chickens in Egypt (Awad *et al.*, 2020) ^[1] and is characterized by high virulence and pathogenicity scores. Its detection in the current study suggests possible geographical spread or independent emergence of highly pathogenic strains within local poultry.

Conclusions

This comprehensive investigation has established that avian pathogenic *E. coli* infection represents a significant disease challenge in Kadaknath chicken populations in the Mhow region, affecting approximately one-quarter of examined birds. The disease manifests as a multisystemic condition with diverse pathological presentations, most commonly involving the hepatic, gastrointestinal, lymphoid, and respiratory systems. The remarkable serological diversity demonstrated through the identification of 15 distinct serogroups, with O8, O78, O84, O7, and O115 being most prevalent, reveals a complex epidemiological landscape that necessitates comprehensive surveillance and multifaceted control approaches.

These findings provide essential baseline epidemiological data for developing evidence-based disease prevention and control strategies tailored to indigenous Kadaknath production systems. The detection of both commonly reported and novel serogroups underscores the importance of ongoing regional surveillance programs that can track emerging strains and temporal changes in pathogen populations. The substantial serological heterogeneity observed argues for control strategies that emphasize management-based interventions and biosecurity measures rather than exclusive reliance on vaccines targeting limited serogroups.

As Kadaknath farming enterprises continue to expand in response to growing market demand for this indigenous breed, implementation of effective disease control measures based on sound epidemiological understanding will be essential for ensuring the sustainability and profitability of this sector. The present study contributes foundational knowledge toward this goal while highlighting important areas for future investigation that can further refine our understanding of APEC epidemiology in indigenous poultry breeds.

The present investigation provides baseline epidemiological insights into APEC infection in Kadaknath chickens and identifies the circulating serogroups in the study region. However, further research is warranted to deepen understanding and improve control strategies. Molecular characterization of virulence genes across serogroups would help elucidate pathogenic mechanisms and identify markers of high virulence. Antimicrobial susceptibility profiling is essential to guide effective treatment and monitor the emergence of resistance. Longitudinal surveillance studies are needed to determine whether the serogroup distribution represents a stable trend or temporal variation. Additionally, analytical studies exploring risk factors within Kadaknath production systems could identify management and environmental determinants of infection. Comparative pathogenicity assessments of different serogroups would clarify their relative virulence. Evaluation of zoonotic potential, particularly for serogroups associated with human ExPEC strains, is important for public health considerations. Finally, controlled studies assessing interventions such as vaccines, probiotics, and improved management practices are necessary to develop effective and sustainable disease control strategies.

Acknowledgement

The authors are thankful to the Director of Instructions, Nanaji Deshmukh Veterinary Science University Jabalpur for providing the necessary facilities to carry out this investigation and authors acknowledge the cooperation of the National Salmonella and Escherichia Centre, Central Research Institute, Kasauli, for serotyping services.

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