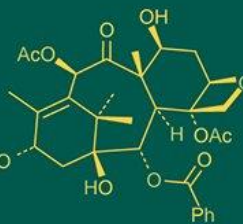
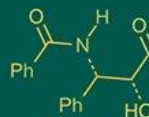
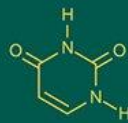


International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
ISSN Online: 2617-4707
NAAS Rating (2026): 5.29
IJABR 2026; 10(7): 567-570
www.biochemjournal.com
Received: 28-05-2026
Accepted: 30-06-2026

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Clinicopathological evaluation of *Escherichia coli* infection in broiler chickens of the north eastern plain zone of Uttar Pradesh

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DOI: <https://www.doi.org/10.33545/26174693.2026.v10.i7h.9083>

Abstract

Colibacillosis in birds caused by *E. coli* is a serious bacterial disease that causes considerable economic losses to the poultry industry. This study determined the presence and bacteriological characteristics of *E. coli* in broilers from the North Eastern Plain Zone of Uttar Pradesh. A total of 677 naturally dead broilers from the districts of Basti, Bahraich, and Siddharth Nagar from October 2023 to September 2024 were collected. Samples from liver, lung, heart, and intestines of dead broilers were used for isolation and characterization of bacteria through cultural and biochemical tests. The maximum percentage of prevalence was found in Basti (53.49%), while Bahraich (42.85%) and Siddharth Nagar (39.77%) had the lower percentages of prevalence. Isolated bacteria had typical cultural and biochemical characteristics of *E. coli*.

Keywords: *Escherichia coli*, avian colibacillosis, broiler chickens, bacteriology, prevalence

Introduction

The poultry industry is the fastest growing sector in animal agriculture and is crucial for providing food security, nutritional supplement, job creation and economic development. India is currently among the top producing countries of poultry meat where commercial broiler production is a major contributor to the livestock economy. Productivity and profitability of poultry industry is greatly hindered by the occurrence of infectious diseases, whereby bacterial diseases are among the major sources of economic losses through higher mortality rate, poor growth performance, inefficient feed conversion ratio and condemnation of carcasses (Hofstad, 1975; Gross, 1991) [7, 6].

Escherichia coli bacteria are one of the important pathogens associated with disease in commercial poultry. Though *E. coli* is a normal constituent of intestinal flora of healthy avian and mammalian animals, some of the strains of the bacterium have the capacity to cause diseases to poultry. These strains are collectively referred to as avian pathogenic *Escherichia coli* (APEC) because of their ability to develop localized and systemic infections. The disease complex called avian colibacillosis is characterized by airsacculitis, pericarditis, perihepatitis, omphalitis, cellulitis, salpingitis and septicemia, causing immense economic losses to the poultry industry (Jawetz *et al.*, 1984; Gross, 1991; Dho-Moulin and Fairbrother, 1999) [8, 6, 4].

Escherichia coli is a Gram-negative, facultative anaerobe, motile, sporeless, lactose-fermenting bacillus that belongs to the bacterial family Enterobacteriaceae. Traditional bacteriological methods based on colony appearance, Gram staining, motility, and biochemical tests are the standard tools used in laboratories to diagnose *E. coli* infections. They are accurate, cost-effective, and commonly used to identify pathogenic *E. coli* strains in birds (Croxen *et al.*, 2013) [2].

Avian colibacillosis is usually associated with stress conditions, lack of good biosecurity, improper ventilation, overcrowding, and simultaneous respiratory infections that lead to the susceptibility of birds to opportunistic *E. coli* infection (Gross, 1991; Dho-Moulin and Fairbrother, 1999) [6, 4]. Despite the number of reports about the occurrence of *E. coli* infection in broiler chickens from various parts of India, there is no sufficient information

available on the prevalence and isolation of this infection in the North Eastern Plain Zone of Uttar Pradesh. Especially in the districts of Basti, Bahraich, and Siddharth Nagar, very few studies are reported. This study has been conducted for the isolation, identification, and characterization of *Escherichia coli* from broiler chickens of the North Eastern Plain Zone of Uttar Pradesh by traditional bacteriological techniques.

Materials and Methods

Study Area and Duration

The current research study was carried out from October 2023 to September 2024 in the North Eastern Plain Zone of Uttar Pradesh, including the districts of Basti, Bahraich, and Siddharth Nagar. A total of 12 commercial broiler units were chosen, having one unit per block in each district.

Sample Collection

A total number of 677 naturally dead broiler chickens, suspected of having *E. coli* infection, were submitted to postmortem examination. Out of which, 372 samples were collected from Basti, 217 samples from Bahraich, and 88 samples from Siddharth Nagar. The tissue samples were collected from liver, lungs, heart, and intestines with gross lesions indicating colibacillosis and aseptically transferred into sterile jars and brought to the Department of Veterinary Microbiology, Faculty of Veterinary and Animal Sciences, Acharya Narendra Deva University of Agriculture and Technology, Kumarganj, Ayodhya.

Isolation of *Escherichia coli*

Each of the tissue samples used in the study was mixed with 10 ml of sterile normal saline. Approximately 5ml of the tissue sample was then inoculated in nutrient broth and was incubated at 37 °C for 24 hours. The enriched culture obtained from nutrient broth was streaked onto MacConkey agar and was incubated at 37 °C for 24 hours. Lactose fermenters were sub cultured into new medium and maintained on nutrient agar slants at 4 °C.

Identification and Characterization of *E. coli*

E. coli was identified on the basis of colony morphology, Gram staining, motility, and biochemical properties as described by Cruickshank *et al.*, (1975) [3] and Edwards and Ewing, (1972) [5]. Colony characteristics were studied on MacConkey and Eosin Methylene Blue (EMB) agar. Gram

staining was done to know the morphology and staining pattern. Motility test was done by the hanging drop method. The biochemical tests included Indole, Methyl Red, Voges-Proskauer, Citrate utilization, Urease, Triple Sugar Iron (TSI), Nitrate reduction, Hydrogen sulphide production, and carbohydrate fermentation for glucose, lactose, sucrose, mannitol, dulcitol, adonitol, and salicin.

Statistical Analysis

Prevalence of *Escherichia coli* infections was calculated in percentages in each district. The outcomes of culturing and biochemical identification were presented descriptively.

Results

Isolation and Identification of *Escherichia coli*

A total of 677 samples from naturally dead broilers were used to conduct bacteriological investigations. Out of these, 327 (48.30%) isolates were confirmed to be *Escherichia coli*. Cultural prevalence of *Escherichia coli* isolates varied between districts. The maximum isolation rate was found in Basti (53.49%, 199/372), then in Bahraich (42.85%, 93/217) and Siddharth Nagar.

Table 1: District-wise cultural prevalence of *Escherichia coli* in broiler chickens

District	Total samples	Positive samples	Prevalence (%)
Basti	372	199	53.49
Bahraich	217	93	42.85
Siddharth Nagar	88	35	39.77

Culture and Morphology

The colonies grown on MacConkey agar had the appearance of being smooth, round, elevated and bright pink colonies that fermented lactose. The colonies grown on EMB agar had a metallic green sheen, which helped to confirm the identification of *E. coli*. Cultures grown in nutrient broth resulted in uniform turbidity. Gram staining indicated Gram-negative, short rods in single or pair formation.

Biochemical Characterization of *Escherichia coli*

The isolates showed positive results for indole test, methyl red, TSI test, nitrate reduction, glucose, lactose and mannitol fermentation tests. Most of the isolates were negative for Voges-Proskauer test, citrate test, urease test and hydrogen sulphide. Variable results were shown by sucrose, dulcitol, adonitol and salicin fermentation tests (Table 2).

Table 2: Biochemical characterization of *Escherichia coli* isolates based on IMViC reactions, carbohydrate fermentation, and other diagnostic biochemical tests.

Medium/Test	Characteristics observed
MacConkey agar	Bright pink, smooth, circular, raised lactose-fermenting colonies
EMB agar	Characteristic metallic green sheen
Nutrient broth	Uniform turbidity
Gram staining	Gram-negative, short rod-shaped bacilli occurring singly or in pairs

Biochemical Characterization of *Escherichia coli* Isolates

Biochemical tests conducted on the isolates indicated that they had biochemical traits of *Escherichia coli* bacteria. The indole test and methyl red test showed positive results for all the isolates while the Voges-Proskauer test and hydrogen

sulfide test showed negative results. Most of the isolates failed to ferment citrate and also exhibited negativity for urease enzyme. The carbohydrate fermentation test revealed the formation of acid and gas in glucose, lactose, and mannitol.

Table 3: Biochemical characteristics of *Escherichia coli* isolates based on IMViC reactions, urease activity, hydrogen sulfide production, and carbohydrate fermentation tests.

Bio-chemical reaction		Basti (%)	Bahraich (%)	Siddharth Nagar (%)
Indole test at 37°C	+ ve	199(100)	93 (100)	35 (100)
	-ve	-	-	-
Methyl red	+ ve	199(100)	93 (100)	35 (100)
	-ve	-	-	-
Vogas Proskauer	+ ve	-	-	-
	-ve	199(100)	93 (100)	35 (100)
Citrate utilisation	+ ve	4 (1.88)	2 (2.03)	1 (1.46)
	-ve	195(98.12)	91 (97.37)	34 (98.54)
Urease activity	+ ve	5 (2.38)	2 (1.88)	3 (3.08)
	-ve	194 (97.62)	91 (98.12)	34 (96.92)
T.S.I. Test	+ ve	199 (100)	93 (100)	35 (100)
	-ve	-	-	-
H2S Production	+ ve	-	-	-
	-ve	199 (100)	93 (100)	35 (100)
Nitrate Reduction	+ ve	199 (100)	93 (100)	35 (100)
	-ve	-	-	-
Motility	+ ve	196 (98.36)	91 (97.82)	34 (97.92)
	-ve	3 (1.64)	2 (2.18)	1 (2.08)
D-glucose	+ ve	199 (100)	93 (100)	35 (100)
	-ve	-	-	-
Lactose	+ ve	199 (100)	93 (100)	35 (100)
	-ve	-	-	-
Sucrose	+ ve	163 (81.68)	76 (83.36)	30 (84.72)
	-ve	36 (18.32)	15 (16.64)	5 (15.28)
Manitol	+ ve	199 (100)	93 (100)	35 (100)
	-ve	-	-	-
Dulcitol	+ ve	173 (86.69)	78 (84.37)	30 (85.77)
	-ve	26 (13.31)	21 (15.63)	5 (14.23)
Adonitol	+ ve	4 (1.78)	2 (2.37)	1 (1.73)
	-ve	195 (98.22)	91 (97.63)	34 (98.27)
Salicin	+ ve	152 (76.63)	73 (78.62)	27 (77.13)
	-ve	47 (23.37)	20 (21.38)	8 (22.87)

Legends: + = Positive; - = Negative; TSI = Triple sugar iron.

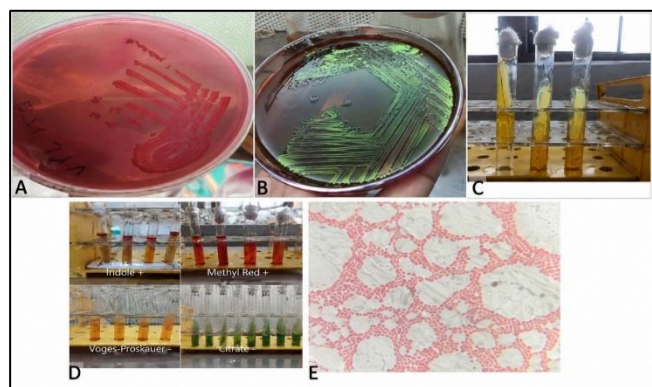


Fig 1: Identification of *Escherichia coli* isolates. **A.** Pink coloured colonies on MacConkey agar; **B.** Metallic sheen on Eosin Methylene Blue agar; **C.** TSI agar slant showing A/A with gas (positive); **D.** Biochemical tests (IMViC): Indole (+), Methyl Red (+), Voges-Proskauer (-), Citrate (-); **E.** Gram-stained smear showing Gram-negative, short rod-shaped bacteria (1500X).

Discussion

This study revealed the high incidence of *E. coli* infections in broiler chicken birds belonging to the North Eastern Plain Zone of Uttar Pradesh, which is an indication that colibacillosis remains to be one of the prominent bacterial diseases in commercial poultry production. The high incidence of *E. coli* infection observed in the Basti district might be due to differences in management, stocking density, hygiene, and biosecurity among others. Similar

findings were made by Gross (1991) [6], Dho-Moulin and Fairbrother (1999) [4], where *E. coli* was found to be one of the prevalent bacteria causing economic losses in poultry production.

Pink colored lactose fermenter colonies in the MacConkey agar medium and metallic green colonies in the Eosin Methylene Blue agar medium obtained in the current study agree with the cultural characteristics of *E. coli* described by Edwards and Ewing (1972) [5] and Cruickshank *et al.* (1975) [3]. Similarly, Gram staining showed Gram negative rods in the samples.

The biochemical characterization of the isolates, which included positive reactions for indole, methyl red, Triple sugar Iron medium, nitrate reduction, glucose, lactose, and mannitol fermentation, along with negative reactions for Voges-Proskauer, hydrogen sulfide production, citrate utilization, and urease test, was consistent with the standard biochemical properties of *Escherichia coli* reported by Croxen *et al.* (2013) [2]. Any deviations from the standard reactions with respect to citrate utilization, urease reaction, and carbohydrate fermentation could be due to strains differences.

The results obtained from this study provide further evidence regarding the prevalence of *Escherichia coli* in broiler flocks of the current study area and highlight the significance of regular bacteriological survey, biosecurity, and better farm management practices to prevent and control avian colibacillosis.

Conclusion

From the above study, it has been found that the bacterium *Escherichia coli* is commonly found in broiler chickens within the North Eastern Plain Zone of Uttar Pradesh. Cultural, morphological, and biochemical techniques were successfully used to isolate and identify the bacteria. It is important to emphasize the need for regular monitoring of diseases to reduce the economic loss from avian colibacillosis in the poultry industry.

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