Short Communication

ISOLATION AND IDENTIFICATION OF *ESCHERICHIA COLI* FROM BROILERS AND LAYERS IN NAMAKKAL

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ABSTRACT

A study was undertaken to isolate, identify and determine the prevalence of Escherichia coli in broilers and layers in Namakkal, a major poultry production hub in South India. A total of 200 samples were collected aseptically, consisting of 100 broiler meat samples obtained from retail outlets and 100 cloacal swabs collected from commercial layer farms. Isolation was carried out using standard cultural procedures, followed bybiochemical characterization to confirm presumptive E. coli. Molecular identification was performed by targeting the 16S rRNA gene. All 129 isolates that were presumptively identified as E. coli yielded the expected 410 bp amplicon, validating the accuracy of phenotypic identification. The prevalence of E. coli was 66.00 per cent in broiler meat samples, indicating considerable contamination at the retail level and emphasizing the need for improved hygienic handling and processing practices. In layer birds, 63.00 per centpositivity in cloacal swabs reflected persistent intestinal carriage and the potential for environmental dissemination. Overall, the findings demonstrate a high occurrence of E. coli across different stages of the poultry chain and underline the importance of continuous microbiological surveillance to support food safety, guided intervention strategies and reduced public health risks.

Key words: Broiler, layers, *Escherichia coli,* molecular identification.

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INTRODUCTION

Poultry meat and eggs are the major sources of high-quality animal protein and contribute significantly to global food and nutritional security. Poultry meat provides highly digestible proteins along with essential B-complex vitamins and minerals

such as iron, zinc and copper (Marangoni *et al.*, 2015). Eggs are naturally encapsulated, nutrient-dense foods containing balanced macro- and micronutrients, making them affordable and valuable components of the human diet (Rehault-Godbert *et al*., 2019).

Despite their nutritional importance, poultry products are highly perishable and susceptible to microbial contamination during production, processing and handling. In broiler meat, contamination commonly arises due to accidental rupture of intestine during evisceration and unhygienic slaughterhouse environment (Tegegne *et al.*, 2024). Raw eggs may also tend to be contaminated, as faecal material on the shell supports the growth and penetration of Gram-negative bacteria, increasing the risk of internal infection (Board, 1968). *Escherichia coli*, a commensal bacterium serves as an important indicator of faecal contamination of food and inadequate hygiene (Barnes and Gross, 1997).

This study was to assess the prevalence of *E. coli* in broiler meat and layer cloacal swabs to understand the extent of contamination in poultry products. The findings will help improved hygiene measures, guide preventive strategies and contribute to the production of safe and wholesome poultry foods.

MATERIALS AND METHODS

Study design

The present investigation was carried out to isolate *Escherichia coli f*rom broilers and layers in Namakkal. A total of 200 samples were aseptically collected for the study, comprising 100 broiler meat samples collected from local retail meat shops and 100 cloacal swabs from commercial layer farms. All samples were processed on the day of collection itself (Cruickshank *et al.,* 1972).

Cultural isolation

For primary isolation, samples were first enriched in Nutrient broth and subsequently streaked onto MacConkey agar (MAC). After incubation, representative lactose-fermenting colonies were transferred onto Eosin Methylene Blue (EMB) agar and incubated at 37°C for 24 h. Colonies showing the characteristic morphology of *E. coli* such as pink colonies on MAC and metallic-sheen colonies on EMB were selected for further study.

Biochemical identification

Presumptive isolates were examined using a set of standard biochemical reactions. The IMViC panel (Indole, Methyl Red, Voges–Proskauerand Citrate utilization) together with Triple Sugar Iron (TSI) reactions were used to confirm the identity of *E. coli.*

Molecular confirmation

Genomic DNA was extracted using the boiling and snap-chilling method. Polymerase Chain Reaction (PCR) amplification targeting the 16S rRNA gene was carried out using specific primers under standardized cycling conditions. The PCR

protocol consisted of an initial denaturation at 94°C for 5 min, followed by 32 cycles of denaturation at 94°C for 30s, annealing at 54°C for 30s, extension at 72°C for one minute and a final extension at 72°C for 10 min.Amplicons were separated by agarose gel electrophoresis and visualized using a gel documentation unit.

Statistical analysis

The chi-square test was applied to assess the association between the type of sample and the detection of *E. coli.*

RESULTS AND DISCUSSION

The enrichment step yielded turbidity in all the 200 samples. More than half of the broiler meat and layer cloacal samples produced lactose fermenting colonies on MacConkey agar and all presumptive isolates developed a metallic sheen on EMB agar (Fig.1 and 2). Sannat *et al.* (2017) and Mandal *et al*. (2022) reported the same sequential cultural pattern which is consistent with standard diagnostic criteria for *E. coli*. All isolates demonstrated the classical IMViC pattern + + – – and produced an acid/acid reaction with gas in TSI agar, reaffirming their typical biochemical behaviour as *E. coli* (Fig. 3). This supports earlier observations who highlighted the reliability of IMViC reactions for confirming *E. coli.*

Molecular confirmation through amplification of the 16S rRNA gene yielded the expected 410 bp product in about 129 isolates (Fig.4).The present study recorded a 66 per cent prevalence of *E. coli* in broiler meat, suggesting that retail poultry remains a major contamination source and it is comparable with the reports of Vasanthi *et al.* (2023). But, comparatively lower valueswere reported by Arul Kumar *et al.* (2014), highlighting substantial variability. These differences likely stem from variations in sampling methods, slaughterhouse hygiene, storage conditions and regional production systems. The relatively higher recovery in this study may be linked to intensified broiler production and inconsistent hygienic practices during processing.

Cloacal swabs from layers showed 63 per cent positivity, indicating continued intestinal carriage of *E. coli.* This aligns with reports of Panchal *et al.* (2020). However, several studies have documented considerably higher prevalence, such as Balasubramaniam *et al.* (2014). Such variability reflects differences in farm practices, environmental hygiene and methodological approaches. The statistical association of *E. coli* prevalence between broilers and layers were not statistically significant ($p>0.05$).

CONCLUSION

The present study demonstrated a high prevalence of *Escherichia coli* in both broiler meat and cloacal swab samples of layerscollected from the Namakkal region. With 66 per cent of broiler meat samples testing positive, the findings indicate considerable microbial contamination at the retail level, likely influenced by handling

practices, processing and storage conditions. Similarly, the 63 per cent prevalence observed in cloacal swabs of layers reflects the continued intestinal carriage of *E. coli* in live birds, which is consistent with the organism's natural commensal status and the potential for intermittent shedding. The successful molecular confirmation of all isolates through 16S rRNA gene amplification further validates the accuracy of identification and underscores the reliability of the detection methods employed. Overall, the results emphasize the widespread distribution of *E. coli* within poultry production and marketing environments. These findings highlight the need for strengthened farm biosecurity measures, improved slaughter hygieneand routine microbiological surveillance to minimize contamination risks and ensure safer poultry production.

Table.1. Primer used in the current study

Target Gene	Primer sequence		Amplicon size
16S rRNA	F	5' - CCCCCTGGACGAAGACTGAC - 3'	410bp
	R	5' - ACCGCTGGCAACAAAGGATA - 3'	

Table.2. Prevalence of *E. coli* among chicken

Sample type	No.ofsamples collected	No. of positive samples	Percentage	P value
Broilers	100	66	66.00	0.6575^{NS}
Layers	100	63	63.00	
Total	200	129	64.50	
^{NS} - Not Significant				

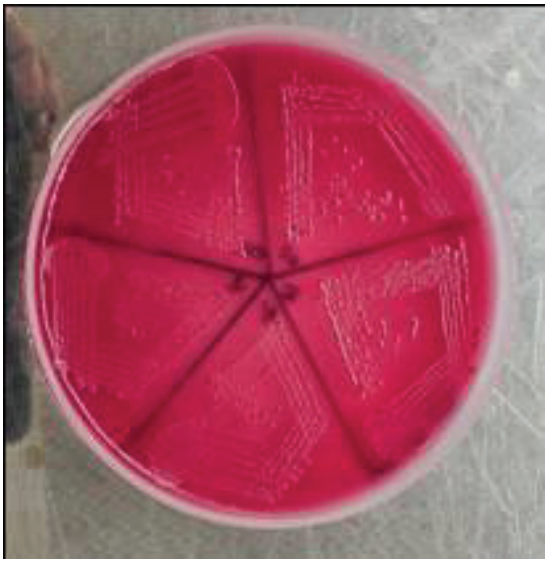

Fig.1. Lactose fermenting colony on MAC

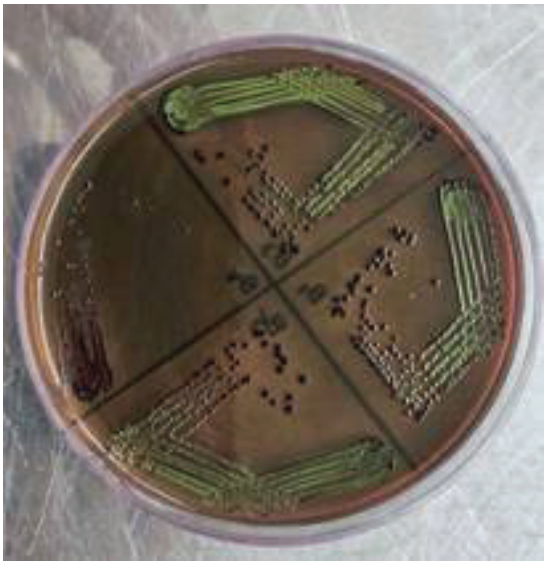

Fig.2. Greenish metallic sheen colony on EMB

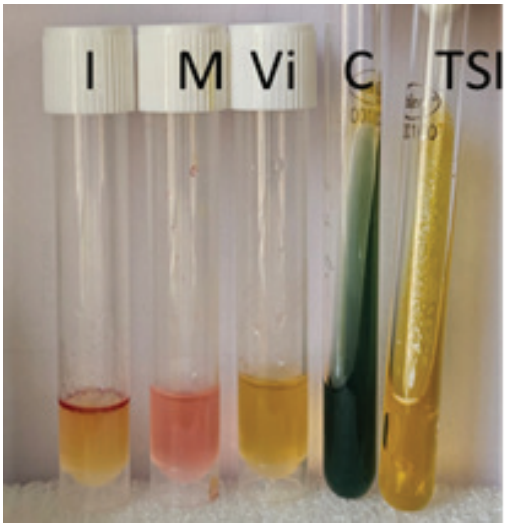

Fig.3. Indole, Methyl Red, Vogues Proskauer, Citrate: ++--
TSI: Yellow colour change in slant and butt along with gas production

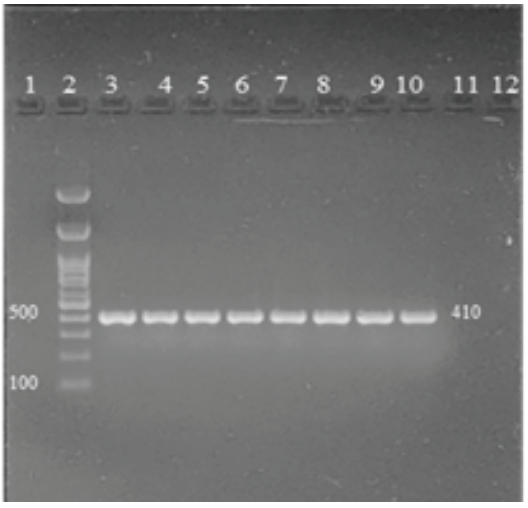

Fig.4 PCR amplification of 16Sr RNA gene (410 bp) 1- nil sample, 2- ladder, 3 to 10- positive sample, 11 and 12- negative sample

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