International Journal of Veterinary Sciences and Animal Husbandry

ISSN: 2456-2912
NAAS Rating (2025): 4.61
VET 2025; SP-10(10): 97-101

www.veterinarypaper.com
Received: 05-08-2025
Accepted: 06-09-2025

Manikkavasagan Ilangopathy
Assistant Professor, Department of Food Safety and Quality Assurance, College of Food and Dairy Technology, Tamil Nadu Veterinary and Animal Sciences University, Tamil Nadu, India

Vijayarani Kumanan
Professor and Head (Retd.), Bioinformatics Centre, Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University, Tamil Nadu, India

Murugan Balaraman
Professor and Head, Department of Food Safety and Quality Assurance, College of Food and Dairy Technology, Tamil Nadu Veterinary and Animal Sciences University, Tamil Nadu, India

Meignanalakshmi Sundaram
Professor, Department of Animal Biotechnology, Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University, Tamil Nadu, India

Eswari Soundian
Professor and Head, Centre for Stem Cell Research and Regenerative Medicine, Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University, Chennai, Tamil Nadu, India

Ragavendiran Venkatesan
M.Tech Scholar, Department of Food Safety and Quality Assurance, College of Food and Dairy Technology, Tamil Nadu Veterinary and Animal Sciences University, Tamil Nadu, India

Corresponding Author:
Vijayarani Kumanan
Professor and Head (Retd.), Bioinformatics Centre, Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University, Tamil Nadu, India

Biofilm formation and genetic profiles of *Escherichia coli* isolated from milk and flavoured milk

Manikkavasagan Ilangopathy, Vijayarani Kumanan, Murugan Balaraman, Meignanalakshmi Sundaram, Eswari Soundian and Ragavendiran Venkatesan

DOI: https://www.doi.org/10.22271/veterinary.2025.v10.i10Sb.2653

Abstract
Biofilm-forming *Escherichia coli* is an important concern in the food industry because it can cause persistent contamination of food products and lead to diseases in consumers.
E. coli has the ability to form biofilms through the action of various genes involved in their development in food and food-processing environments. In the present study, biofilm-forming *E. coli* was evaluated in milk and flavoured milk samples. Positive *E. coli* strains were tested for the presence of different genes associated with biofilm formation. Among the isolates from raw milk samples, 42.8% were strong biofilm producers, while 28.5% were weak or moderate producers. In the flavoured milk samples, two isolates were weak producers and one isolate was a strong producer. Molecular characterisation results revealed that the biofilm-associated gene *luxS* was prevalent in all strains isolated from both milk and flavoured milk samples, followed by *csgA* and *fimH*. No amplification of *papC* was detected in any of the strains. This study provides insights into the prevalence of genes associated with the biofilm-forming capability of *E. coli* and highlights the importance of addressing these threats in the food industry.

Keywords: *Escherichia coli*, food industry, flavoured milk, Biofilm formation

1. Introduction
The World Health Organization has reported that hundreds of millions of people worldwide suffer from foodborne diseases each year in both developed and developing countries. More than 100 million cases of food-related illnesses are anticipated to occur each year in India by 2030 (Bisht *et al.*, 2021) [2]. The increasing frequency of foodborne disease outbreaks across various states in India further underscores this burden National Health Portal. (2019).
Bacterial biofilms as complex microbial communities encased in extracellular polymeric substances, emphasizing the matrix components such as polysaccharides, proteins, and extracellular DNA and their role in biofilm formation and resilience (Zhao, Sun and Liu, 2023) [16]. Microbes embedded in biofilms exhibit far greater resistance to cleaning agents than their free-floating (planktonic) counterparts. Several human pathogens are involved in biofilm formation, including *Bacillus cereus*, *Escherichia coli*, *Listeria monocytogenes*, *Salmonella enterica*, and *Staphylococcus aureus* (Galié *et al*., 2018) [6].
Biofilms consist of a collection of microbial cells that are permanently attached to a surface and are surrounded by a matrix mainly composed of polysaccharide substances, offering certain benefits to the bacteria and making them resistant to cleaning and disinfection (Flemming *et al*., 2016) [4].
Biofilms are a persistent issue on food contact surfaces such as stainless steel, plastic, and rubber in processing environments. These biofilms not only threaten food safety but can also cause equipment degradation and reduce processing efficiency (Liu *et al*., 2023) [16].
Escherichia coli is a gram-negative commensal gastrointestinal microflora in humans and animals. They may be difficult to detect and treat owing to their short doubling time, ability to adhere to surfaces, and biofilm formation (Bhardwaj *et al.*, 2021) [1].

Biofilm formation in *E. coli* is a coordinated process that requires multiple genes for surface sensing and attachment (*fimH*, *csgA*), (Foroogh *et al*., 2021; Bu, Dee and Liu, 2024) [5, 3], motility, quorum sensing (*luxS*), (Laganenka and Sourjik, 2018) [7] and maturation. These gene products interact to allow the bacteria to colonise surfaces, resist environmental stress, and disperse when conditions are favourable.

This study investigated the biofilm-forming capacity of *Escherichia coli* isolated from raw and flavoured milk collected from local markets. Subsequently, the genes associated with biofilm formation were assessed in the field isolates

2. Materials and Methods

2.1 Food samples

Raw milk (20), flavoured milk (10) samples were collected in separate containers from the retail outlet in and around Chennai, Tamil Nadu.

2.2 Isolation, identification and characterization of foodborne microorganisms

A total of 25 g of the sample was mixed with 225 mL of nutrient broth for enrichment and incubated at 37 °C for 18-24 hours. After enrichment, 100 µL of the inoculum was plated onto MacConkey agar (MCA) plates, which were then incubated at 37 °C for 18-24 hours. Pink bacterial colonies on the MCA were identified as *E. coli*. The *E. coli* isolates were inoculated into BHI broth and incubated at 37 °C for 24 hours. The cultures were stored at-20 °C in glycerol for future use.

2.3 Identification of biofilm forming organism and quantification of biofilm: Positive isolates were grown in 10 mL of BHI broth, and the bacterial culture turbidity was adjusted to an optical density (OD) of 0.1 (range: 0.08-0.1) at 600 nm. In microtiter plate wells, 180 µL of BHI broth and 20 µL of the inoculum were added in triplicate, with broth-only wells serving as negative controls. The plates were incubated at 37 °C for 48 h to allow biofilm formation. After incubation, the contents were removed, and the wells were washed three times with sterile distilled water to remove planktonic cells, then air-dried for 15 min. The attached cells were stained with 0.1% crystal violet solution for 30 min, washed, and air-dried. A mixture of 75% ethanol and 25% acetone was added to solubilize the bound dye. Biofilm formation was quantified by measuring absorbance at 597 nm, and isolates were classified as weak (< 0.3), moderate (0.3-0.6), or strong (> 0.6) biofilm producers (Obe *et al.*, 2021) [11].

2.4 Molecular identification and amplification of adhesion genes

Genomic DNA was extracted from *E. coli* isolates using the QIAamp DNA Mini Kit (QIAGEN) according to the manufacturer's instructions. All positive colonies were confirmed by PCR using *16S rRNA* primers specific for *E. coli*. Adhesion-associated genes involved in biofilm formation by *E. coli* were identified using primers targeting various genes (Table 1). The PCR protocol consisted of 30 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 1.5 min, following an initial denaturation at 95 °C for 3 min and ending with a final extension at 72 °C for 7 min. The amplified products were analysed by electrophoresis on a 1.8% agarose gel using a 100 bp molecular weight marker and were documented with a UV light transilluminator.

Table 1: Biofilm associated gene specific primers for *E. coli*

Gene	Nucleotide sequences (5′→3′)	Amplicon size (bp)	Annealing temperature	Reference
16S rRNA	CGGACGGGTGAGTAATGTCT CTCAGACCAGCTAGGGATCG	200	57ºC	(Bhardwaj *et al.*, 2021) [1]
Type 1 fimbriae (*fimH*)	TGCAGAACGGATAAGCCGTGG GCAGTCACCTGCCCTCCGGTA	508	55ºC	(Wang *et al.*, 2016) [15]
Curli major subunit (*csgA*)	GGTAATGGTGCAGATGTTG GTCACGTTGACGGAGGAGTT	188	55ºC	(Bhardwaj *et al.*, 2021) [1]
Regulation of AI-2, cell signaling *(luxS)*	TTCTTCGTTGCTGTTGATGC TGGAAGACGTGCTGAAAGTG	152	57ºC	(Bhardwaj *et al.*, 2021) [1]
Type 1 fimbriae major subunit *(fimA)*	ATCGTTGTTCTGTCGGCTCT GCGGTACGAACCTGTCCTAA	167	57ºC	(Bhardwaj *et al.*, 2021) [1]
P fimbriae *(papC)*	TGATATCACGCAGTCAGTAGC CCGGCCATATTCACATAAC	482	55ºC	(Wang *et al.*, 2016) [15]

3. Results and Discussion

3.1 Isolation and identification

In this study, 20 raw milk and 10 flavoured milk samples were collected for the isolation of foodborne microorganisms using MacConkey agar for the isolation of *E. coli*. In the present study, Out of 20 raw milk samples, 12 formed pink colonies on MacConkey agar (60 %) and in flavoured milk samples out of 10, three formed pink colonies on MacConkey agar (30 %). The results of the present study are consistent with Ravi *et al.* (2025), who reported that the prevalence of *E. coli* was 22.50%, 30.55%, and 12.5% in retail raw milk and traditional milk products, respectively. The prevalence of *E. coli* isolates was higher in traditional dairy products than in pasteurized dairy products (Madani *et al.*, 2022) [9].

3.2 Identification of biofilm forming organism

The biofilm forming ability of *E. coli* isolates from raw milk and flavoured milk are given in Table 2. In raw milk out of seven isolates screened, three were strong biofilm producers (42.8%), two were moderate biofilm producers (28.5%), and two were weak biofilm producers (28.5%). Out of three isolates from flavoured milk, two were weak producers and one was a strong biofilm producer. Madani *et al.* 2022 [9] reported that 28.9% isolates classified as strong biofilm producers, 10.5% isolates showed moderate biofilm formation, 28.9% isolates showed weak biofilm formation, and 12 (31.6%) isolates did not form any biofilms. The present study revealed a higher rate of strong biofilm formation in *E. coli* isolates from raw milk samples.

Table 2: Biofilm production assay of *E. coli* isolates from raw milk and flavoured samples

Sample	Biofilm formation
RM2	Strong
RM3	Weak
RM4	Weak
RM5	Strong
RM6	Moderate
RM12	Strong
RM13	Moderate
FM2	Weak
FM4	Strong
FM6	Weak

3.3 Molecular identification and amplification of adhesion genes

The PCR amplification and the agarose gel electrophoresis results are presented in Figure 1, 2, 3, and 4. Molecular characterisation of seven isolates from raw milk and three isolates from flavoured milk were screened for 16S *rRNA* specific primer to *E. coli*. All the samples were positive for *E. coli*. The amplification of adhesion genes in *E. coli* associated with biofilm production were *luxS* (100%), *csgA* (71%), and *fimH* (57%). All the isolates from raw milk were negative for *fimA* and *papC* in the milk samples. The PCR results are presented in Table 3. Molecular characterisation of three isolates from flavoured milk samples were screened for 16S *rRNA* specific primer to *E. coli*. All the samples were positive for *E. coli*. The amplification of adhesion genes in *E. coli* associated with biofilm production were *luxS* (100%), *csgA* (66%), and *fimH* (33%). All the isolates from flavoured milk samples tested negative for *fimA* and *papC*. High prevalemce of biolfilm genes such as *luxS* and *csgA* in all biofilm producing isolates studied in this study. Our study are correlated with the Soares *et al.*, (2023) reported that 100% of *fimH*, *fimA* (100%) *papC* gene prevalence was 0% gene prevalence in *E. coli* isolated from raw milk sample.

Table 3: Amplification of adhesion genes of *E. coli* isolates from raw milk and flavoured milk

Sample	*16S rRNA*	Genes associated with biofilm formation				
		luxS	*csgA*	*fimH*	*fimA*	*papC*
RM2	+	+	+	+	-	-
RM3	+	+	-	-	-	-
RM4	+	+	-	-	-	-
RM5	+	+	+	+	-	-
RM6	+	+	+	-	-	-
RM12	+	+	+	+	-	-
RM13	+	+	+	+	-	-
FM2	+	+	+	-	-	-
FM4	+	+	-	+	-	-
FM6	+	+	+	-	-	-

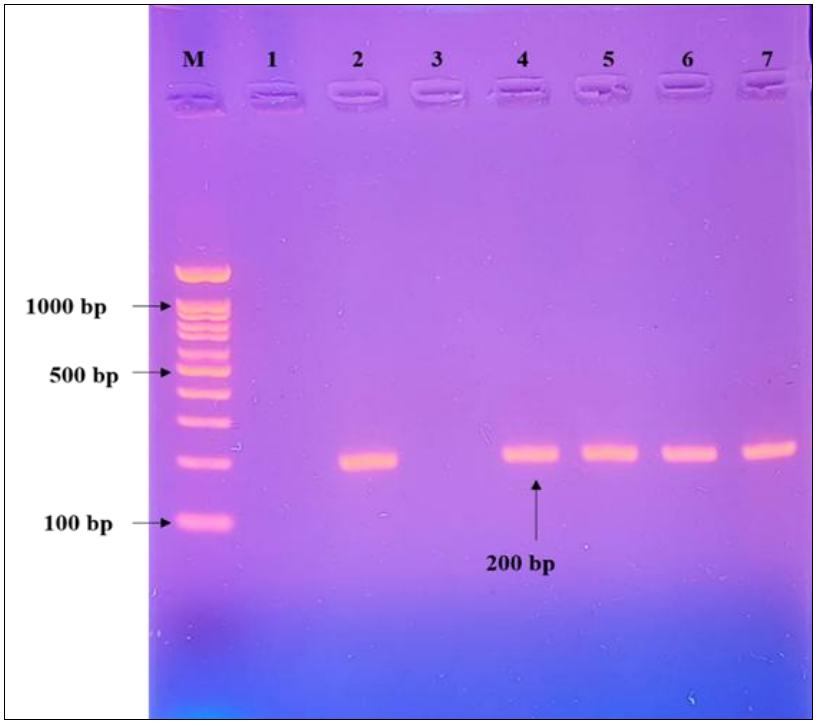

Fig 1: 1.8% agarose gel electrophoresis for identification and confirmation of *E. coli* field isolates by PCR (16S rRNA). M-Marker 100bp, Lane 1-7 *E. coli* field isolates

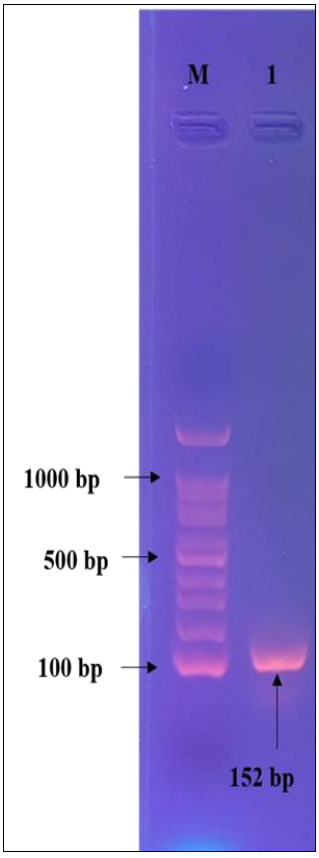

Fig 2: 1.8 % Agarose gel electrophoresis for PCR products for detection of biofilm forming *E. coli* field isolates with *luxS* gene. M-Marker 100bp, Lane 1 *E. coli* field isolates

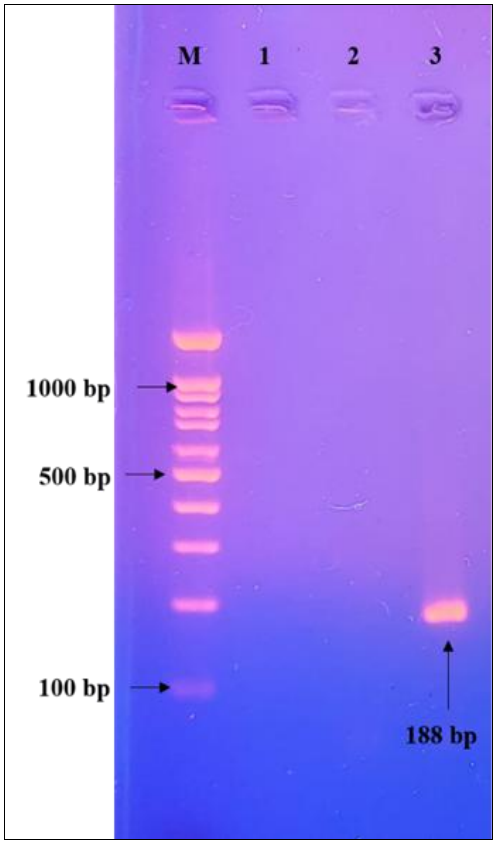

Fig 3: 1.8 % Agarose gel electrophoresis for PCR products for detection of biofilm forming *E. coli* field isolates with *csgA* gene. M-Marker 100bp, Lane 1-3 *E. coli* field isolates

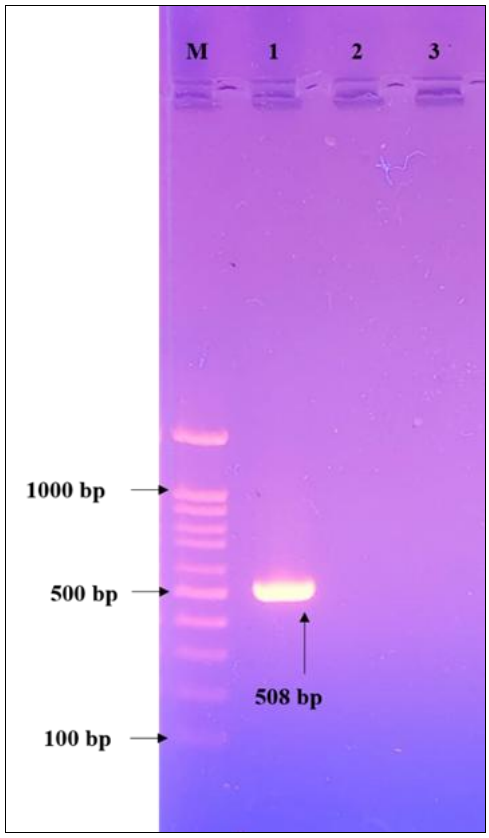

Fig 4: 1.8 % Agarose gel electrophoresis for PCR products for detection of biofilm forming *E. coli* field isolates with *fimH* gene. M-Marker 100bp, Lane 1-3 *E. coli* field isolates

4. Conclusion

The prevalence of *E. coli* in raw and flavoured milk samples was high, and the majority of E. coli isolates were strong biofilm producers. The prevalence of biofilm-associated genes in this study confirmed that *luxS*, cs*g*A, and *fimH* are responsible for biofilm formation by *E. coli* isolates. This highlights the importance of hygienic practices during the production and processing of milk and milk products to minimise contamination associated with biofilm-forming E. coli in the dairy industry.

Acknowledgement

The authors thank the Department of Animal Biotechnology, Madras Veterinary College, Chennai-600 007, and College of Food and Dairy Technology, Chennai-600 052 for providing laboratory facilities to carry out the research work.

Funding

This research was funded by the Tamil Nadu Veterinary and Animal Sciences University for conducting the PhD dissertation work.

Conflict of Interests

The authors declare no conflict of interest.

Financial Support

Not available

Reference

1. Bhardwaj DK, Taneja NK, DP S, Chakotiya A, Patel P, Taneja P, *et al*. Phenotypic and genotypic characterization of biofilm forming, antimicrobial resistant, pathogenic *Escherichia coli* isolated from Indian dairy and meat products. Int J Food Microbiol. 2021;336:108899. DOI: 10.1016/j.ijfoodmicro.2020.108899.
2. Bisht A, Kamble MP, Choudhary P, Chaturvedi K, Kohli G, Juneja VK, Sehgal S, Taneja NK. A surveillance of food borne disease outbreaks in India: 2009-2018. Food Control. 2021;121:107630. DOI: 10.1016/j.foodcont.2020.107630.
3. Bu F, Dee DR, Liu B. Structural insight into *Escherichia coli* CsgA amyloid fibril assembly. mBio. 2024;15(4):e0041924. DOI: 10.1128/mbio.00419-24.
4. Flemming HC, Wingender J, Szewzyk U, Steinberg P, Rice SA, Kjelleberg S. Biofilms: An emergent form of bacterial life. Nat Rev Microbiol. 2016;14(9):563-575. DOI: 10.1038/nrmicro.2016.94.
5. Foroogh N, Rezvan M, Ahmad K, Mahmood S. Structural and functional characterization of the FimH adhesin of uropathogenic *Escherichia coli* and its novel applications. Microb Pathog. 2021;161:105288. DOI: 10.1016/j.micpath.2021.105288.
6. Galié S, Gutiérrez GC, Miguélez EM, Villar CJ, Lombó F. Biofilms in the food industry: Health aspects and control methods. Front Microbiol. 2018;9:898. DOI: 10.3389/fmicb.2018.00898.
7. Laganenka L, Sourjik V. Autoinducer 2-dependent *Escherichia coli* biofilm formation is enhanced in a dual-species coculture. Appl Environ Microbiol. 2018;84(5):e02638-17. DOI: 10.1128/AEM.02638-17.
8. Liu X, Yao H, Zhao X, Ge C. Biofilm formation and control of foodborne pathogenic bacteria. Molecules. 2023;28(6):2432. DOI: 10.3390/molecules28062432.

9. Madani A, Esfandiari Z, Shoaei P, Ataei B. Evaluation of virulence factors, antibiotic resistance, and biofilm formation of *Escherichia coli* isolated from milk and dairy products in Isfahan, Iran. Foods. 2022;11(7):960. DOI: 10.3390/foods11070960.
10. National Health Portal. State/UT-wise details of outbreak of foodborne diseases during 2017 [Internet]. 2019 [cited 2025 Aug 8]. Available from: https://www.nhp.gov.in/state-wise-details-of-outbreak-of-foodborne-diseases-during2017
11. Obe T, Nannapaneni R, Schilling W, Zhang L, Kiess A. Antimicrobial tolerance, biofilm formation, and molecular characterization of *Salmonella* isolates from poultry processing equipment. J Appl Poult Res. 2021;30(4):100195. DOI: 10.1016/j.japr.2021.100195.
12. Ravi P, Jain UP, Barkha S, Raghavendra MP, Satyendra SP, Gurvinder, *et al*. Evaluation of biofilm production capability of Shiga toxin producing *Escherichia coli* isolated from milk, milk products and hand swabs. Asian J Dairy Food Res. 2025; DOI: 10.18805/ajdfr.DR-2330.
13. Soares SJA, Guimarães FF, Rossi GAM, Guerra ST, Dalanezi FM, Lopes BC, *et al*. Virulence potential, biofilm formation, and disinfectants control of *Escherichia coli* from raw milk bulk tanks in the southeast of Brazil. Dairy. 2023;4(4):541-53. DOI: 10.3390/dairy4040037.
14. Vysakh A, Midhun SJ, Jayesh K, Jyothis M, Latha MS. Studies on biofilm formation and virulence factors associated with uropathogenic *Escherichia coli* isolated from patients with acute pyelonephritis. Pathophysiology. 2018;25(4):381-7. DOI: 10.1016/j.pathophys.2018.07.004.
15. Wang Y, Yi L, Wang Y, Wang Y, Cai Y, Zhao W, *et al*. Isolation, phylogenetic group, drug resistance, biofilm formation, and adherence genes of *Escherichia coli* from poultry in central China. Poult Sci. 2016;95(12):2895-901. DOI: 10.3382/ps/pew252.
16. Zhao A, Sun J, Liu Y. Understanding bacterial biofilms: From definition to treatment strategies. Front Cell Infect Microbiol. 2023;13:1137947. DOI: 10.3389/fcimb.2023.1137947.

How to Cite This Article
Ilangopathy M, Kumanan V, Balaraman M, Sundaram M, Soundian E, Venkatesan R. Biofilm formation and genetic profiles of *Escherichia coli* isolated from milk and flavoured milk. International Journal of Veterinary Sciences and Animal Husbandry. 2025;SP-10(10):97-101.