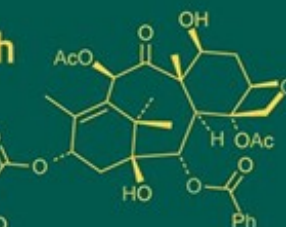


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Prevalence, isolation and characterisation of *Escherichia coli* in milk and milk products from Meerut region

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ABSTRACT

Foodborne infections can result from consuming contaminated milk and dairy products, especially if appropriate hygiene and temperature control are neglected. Because they are frequently handled during preparation and may be stored in environments that promote the growth of germs, milk-based sweets and other ready-to-eat dairy products may pose a higher risk. Foodborne disease are a major public health concern worldwide. *Escherichia coli* O157:H 7 is a recently emerged and widely reported food-borne pathogen. The South East Asia has the highest burden of diarrhoeal deaths, especially among children. Many studies in developed countries have examined the role of *E. coli* O157:H 7 as a key cause of foodborne outbreak (Singha S, *et al*, 2022). In this research it was conducted to isolate and identify *E. coli* from Raw, pasteurized milk, and cheese. This cross sectional study was conducted from October 2025 to March 2026 at Sardar Vallabhbhai Patel University of Agriculture & Technology and dairy farms in the Meerut city. Eight dairy farms provided a total of 70 samples of bulk milk (40), and cheese (20), and (10) milk samples taken from pasteurized milk (Ame, Mohammed Kemal *et al.*, 2023). *E. coli* was isolated and identified based on cultural, staining, and biochemical characterisation, including Indole, Citrate, Catalase, Triple sugar iron (TSI), Methyl red test for confirmation. The result revealed a higher prevalence of *E. coli* and cheese samples compared to pasteurized milk, indicating inadequate hygiene during milking, handling, or storage of raw milk. (Ugwu, Celestina (Chibuzo *et al.*, 2025). The detection of *E. coli* in pasteurized milk samples indicates likely contamination occurring after pasteurization.

The study highlight the importance of strict hygienic practices, effective pasteurization, and proper handling of milk to ensure microbiological safety and protect public health.

Keywords: Foodborne illness, *Escherichia coli* O157:H 7, Dairy products, Biochemical Characterisation

Introduction

Milk and Milk products are most widely consumed foods worldwide due to its nutritional value; however, it is also highly susceptible to microbial contamination. Contamination may milking, processing, storage, or distribution, making milk an important vehicle for the transmission of food-borne pathogens. Among these pathogens, *Escherichia coli* (*E. coli*) holds particular significance as an indicator organism of faecal contamination and hygienic quality of milk and milk products. Enterobacteriaceae family member *E. coli* is a Gram-negative, Rod shaped bacteria. It is commonly found in the intestinal tract of humans and warm-blooded animals. Their pathogenic strains are capable of causing severe gastrointestinal illness and extra-intestinal infections. The presence of *E. coli* in milk, especially pasteurized milk, indicates inadequate sanitation or post-processing contamination and poses a potential risk to public health. *E. coli* is grouped into distinct pathotypes including Enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAEC), diffuse adherent *E. coli* (DAEC), Shiga toxin-producing *E. coli* (STEC) and enteroaggregative-haemorrhagic *E. coli* (EAHEC). Although most *E. coli* strains are non-pathogenic, some have acquired specific virulence determinants that enable them to cause disease. For example Shiga toxin-producing *E. coli* (STEC) are important foodborne zoonotic pathogen, and the main risk foods for STEC infection in human. Even though STEC infections in ruminants are typically asymptomatic, these animals represent the primary reservoir of STEC and the consumption of unpasteurized milk has been associated with many foodborne outbreaks (Drugea *et al.*, 2025) [1]

In view of this, the current study concentrated on cheese, pasteurised milk, and raw bulk milk that were gathered from dairy farms and retail establishments in the Meerut area. Using selective and differential culture conditions, the study sought to detect probable *E. coli*. The isolates were then characterised using standard biochemical tests, colony morphology, and Gram-staining features. Additionally, the prevalence of *E. coli* in the three categories of dairy products was compared. The results were further examined in terms of

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hygienic procedures, pasteurisation efficacy, and the potential for contamination following processing. This study aims to improve routine microbiological monitoring and enhance preventive actions across the local dairy supply chain by offering evidence unique to the Meerut region.

Isolation and characterisation of *E. coli* from milk are essential for assessing microbiological quality and ensuring consumer safety. Isolation typically involves selective and differential culturing techniques, followed by identification based on colony morphology, Gram staining, and some confirmatory biochemical tests. Characterisation through confirmatory biochemical tests like indole, citrate, Triple Sugar Iron (TSI) tests, motility, oxidase, and catalase test enables accurate confirmation of the organism. These conventional methods remain widely used due to their reliability, simplicity, and cost-effectiveness, particularly in routine microbiological laboratories. Raw milk is more prone to contamination due to direct exposure to environmental sources, animal feces, and unhygienic handling practices. Pasteurization is designed to eliminate pathogenic microorganisms; however, the detection of *E. coli* in pasteurized milk may occur due to insufficient heat treatment or contamination during post-pasteurization handling, packaging, or storage. Therefore, monitoring both raw and pasteurized milk is critical for evaluating processing efficiency and hygiene standards. The present study focuses on the isolation and characterisation of *Escherichia coli* from raw and pasteurized milk samples using standard microbiological and biochemical techniques. The findings of this work contribute to understanding the prevalence of *E. coli* in milk and highlight the importance of strict hygienic practices and effective quality control measures in the dairy industry.

2. Materials and Methods

2.1. Milk sampling

70 dairy samples were collected during the study period. These included:

- Raw bulk cow milk samples ($n = 40$) collected from eight different dairy farms

- Cheese samples ($n = 20$) prepared from raw milk obtained at the same farms
- Pasteurized milk samples ($n = 10$) collected from local retail outlets in Meerut city

Selection of eight dairy farms was based on logistical accessibility and the farm owners' consent to take part in this study. Samples were collected randomly to ensure representative coverage of different farms and production batches.

2.2 Sample collection and transportation

Raw milk samples were aseptically collected directly from bulk milk containers into sterile, screw-capped bottles. Cheese samples were collected using sterile forceps and placed into sterile sample containers. Pasteurized milk samples were collected in their original sealed packages. All samples were labelled properly with date, sample type, and source, and immediately transported to the Microbiology Laboratory of Sardar Vallabhbhai Patel University in an insulated ice box maintained at 4 °C. Microbiological analysis was initiated within 4 hours of sample collection to prevent changes in microbial load.

2.3 Isolation of *Escherichia coli*

For the detection of *Escherichia coli*, milk samples were enriched for 24 hrs in peptone water at 35–37 °C and then streaked onto MacConkey, EMB and Rapid Hi-coliform agar plates for isolation and identification.

2.4 Characterization and identification of isolates

Cultural examination of raw and pasteurised milk and cheese samples was conducted following ICMSF guidelines (1985), and for the identification colony morphology, Gram staining and standard biochemical test including TSI, indole, MR (Methyl red test), VP (Voges-Proskauer) test, catalase, Citrate Utilisation test was performed according to the standard method (Cheesebrough, 1985) [2].

3. Results

3.1. Prevalence of *E. coli* in dairy products

Table 1. Prevalence of *E. coli* in dairy products

Name of samples	Number of samples	Number of cultural positive samples	Positive percentage	Overall prevalence of isolated <i>E.coli</i> (%)
Raw milk	40	15	37.5	40
Pasteurised milk	10	01	10	
Cheese	20	08	40	

Out of the 70 dairy samples examined, 60 samples were found to be positive by the culture method, resulting in an overall culture-positive rate of 85.7%. The high proportion of positive samples indicates a substantial level of bacterial contamination among the dairy products analysed.

3.2. Cultural characteristics of *E. coli*

E. coli isolates were assessed on following different media, and pure cultures were obtained by streaking mixed cultures on simple and selective agar plates.

Table 2. Cultural characteristics of *E. coli*

Name of the culture media	Cultural properties	Remarks
Nutrient agar media	Whitish, mucoid colony	<i>E.coli</i>
MacConkey agar media	Rose pink large mucoid colony	
Eosin Methylene Blue media	Green metallic sheen	
Rapid Hi coliform agar	Blue-green colour colonies	

(continued on next page)

3.3 Morphological and staining characteristics of *E. coli*

Table 3. Morphological and staining characteristics of *E. coli*

Staining	Morphological characteristics	Result	Remarks
Gram staining and viewed under Microscope	Single, paired or in short chain	Negative	<i>E.coli</i>

3.4 Biochemical characteristics of *E. coli*

Of the 60 samples that showed positive growth by the culture method, 40 were confirmed as *Escherichia coli* on the basis of their characteristic biochemical reactions. Thus, 66.7% of the culture-positive samples and 57.1% of the total 70 samples were confirmed as *E. coli*. The confirmed isolates were catalase positive and oxidase negative and exhibited the typical IMViC reaction pattern of indole positive,

methyl red positive, Voges–Proskauer negative, and citrate negative (++)-. On Triple Sugar Iron agar, the isolates produced a yellow slant and yellow butt (A/A) with gas formation but no hydrogen sulphide production. These combined biochemical characteristics supported their identification as *E. coli*, while the remaining 20 culture-positive samples did not show the complete biochemical profile required for confirmation.

Table 4. Biochemical characteristics of *E. coli*

Catalase	oxidase	Cit	Indole	Methyle red	VP	TSI	Result
Positive	Negative	Negative	Positive	Positive	Negative	Slant=yellow colour butt=yellow colour Gas=(+) ve H ₂ S=(-)ve	<i>Eshcherichia coli</i>

Note: IMViC test = Indole, Methylene Red, Voges-Proskauer, citrate test MR= Methyl red, VP= Voges-Proskauer, TSI=Triple sugar iron ,cit= Simmon citrate,(+)=Positive, (-)=Negative.

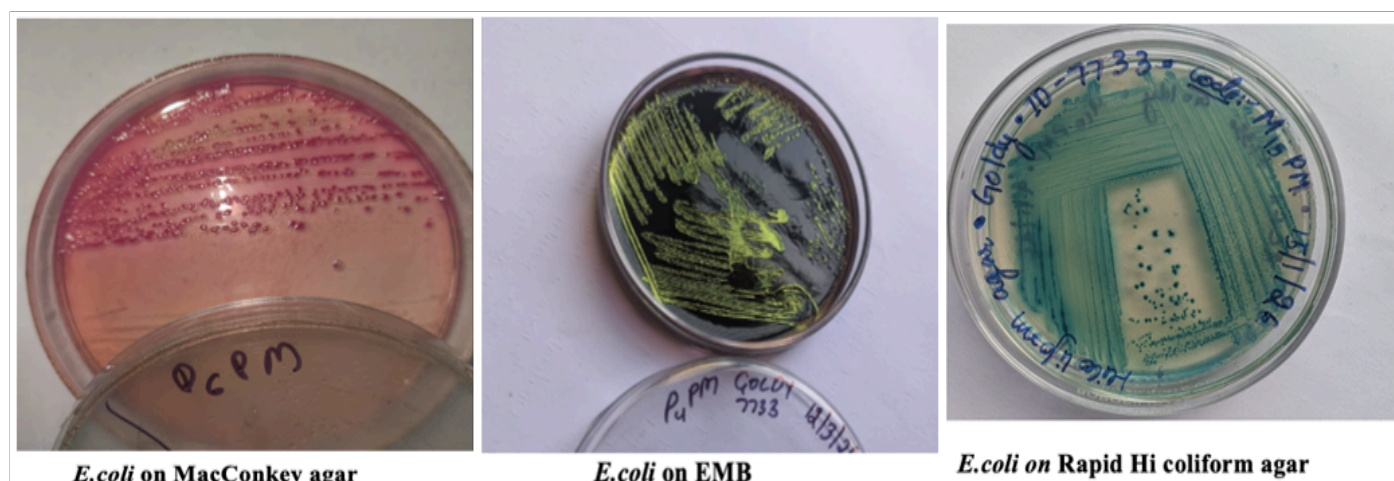


Figure 1. Cultural characterization of *E. coli*

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