Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Volume 4, Issue 4 (2026)

Online ISSN 3007-3197
Print ISSN 3007-3189

http://amresearchreview.com/index.php/Journal/about

Microbial Contamination in Milk: Detection, Prevention, and Safety Measures with Emphasis on Salmonella spp. and Escherichia coli

Rimsha Saleem
Department Of Allied Health Sciences, Government College University Faisalabad, Faisalabad, Pakistan

Tariq Saeed
Department of Dairy Technology, University of Veterinary and Animal Sciences, Lahore, Pakistan

Abdul Rehman Tariq
School of Natural Sciences, University of Hull, Uk

Minahil Khan
Institute of Microbiology, University of Veterinary and Animal Sciences, Lahore, Pakistan

Laraib Rasheed
Department of Allied health sciences, Government College University Faisalabad, Faisalabad, Pakistan

ABSTRACT

Milk is a highly nutritious food but is also highly susceptible to microbial contamination, posing significant public health risks. This study aimed to evaluate the prevalence of *Salmonella* spp. and *Escherichia coli* in milk, along with assessing detection methods and identifying key factors contributing to contamination. A total of 50 milk samples were collected from dairy farms, vendors, and retail shops in Lahore, Punjab, Pakistan. Standard microbiological techniques were used for isolation and identification, followed by molecular confirmation using polymerase chain reaction (PCR) targeting *invA* and *uidA* genes. The results revealed that 30% of samples were contaminated with *Salmonella* spp., while 42% were positive for *E. coli*. Higher contamination levels were observed in vendor and retail samples compared to farm samples, indicating poor hygiene and inadequate storage conditions during distribution. Total bacterial counts ranged from 1.2×10^4 to 3.8×10^6 CFU/mL, exceeding acceptable safety limits in several cases. PCR analysis confirmed the accuracy and sensitivity of conventional detection methods. The findings highlight the critical need for improved sanitary practices, proper pasteurization, and implementation of food safety management systems such as HACCP. Public awareness and strict regulatory enforcement are essential to reduce microbial contamination and ensure safe milk consumption. This study provides valuable insights into milk safety and emphasizes the importance of continuous monitoring to protect consumer health.

Keywords: Milk contamination; *Escherichia coli*; *Salmonella* spp.; Food safety; PCR detection

Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Volume 4, Issue 4 (2026)

Online ISSN 3007-3197
Print ISSN 3007-3189

http://amresearchreview.com/index.php/Journal/about

Introduction

Milk is a highly nutritious biological fluid and an essential component of human diet, which contains proteins, fats, vitamins and even minerals, which are required to grow and develop. Rather, it is also in its nutrient content that makes it suitable as a medium through which microorganisms, some of which are pathogenic bacteria, can proliferate and develop, and which can have extreme health effects on the population (1). Contamination of milk by microorganisms can occur at various stages, including; milking process, handling, processing, storage and transportation, particularly in inappropriate and hygienic and sanitary conditions (2).

Of the existing range of milk-borne pathogens, Salmonella spp. and Escherichia coli play the greatest role as they are widespread and are involved in food-borne diseases all over the world. Salmonella spp. are the cause of a disease called salmonellosis that is an illness marked by diarrhea, fever and cramps in the abdomen and is normally transmitted by contaminated dairy products (3). Similarly, the E. coli O157:H7 of the pathogenic E. coli has been reported to cause crippling gastrointestinal diseases such as hemorrhagic colitis and hemolytic uremic syndrome (4). These milk pathogens not only impact on food safety, but also result in huge economic losses in the dairy industry.

Microbial contamination is especially high in developing nations, with a lack of cold chain facilities, proper facilities, and hygienic knowledge of microbial contamination increasing the rate of contamination (5). It has been shown that Salmonella and E. coli in raw and unpasteurized milk vary in magnitude and that good monitoring and control measures are important (6,7). In addition, the development of antimicrobial resistant strains of these bacteria also complicates treatment and control interventions, which is also a threat to the health of the public (8).

Several detection techniques have been established to detect and guarantee quality and safety of milk beginning with the old culture-based detection methods and the advanced molecular-based detection methods such as polymerase chain reaction (PCR) and biosensor-based detection systems (9). Other preventive interventions like sanitation, pasteurization, refrigeration and the use of Hazard Analysis and Critical Control Points (HACCP) in addition to detection are important in minimizing microbial contamination (10). There is also the aspect of education and publicity that can help in making the handling and consumption of milk safe (11).

The purpose of this study is to research the incidence of microbial contamination of milk, in particular Salmonella spp. and Escherichia coli, and to assess effective methods of detection, prevention and safety to avoid health risks to the population (12).

Study Area and Duration

The research was carried out during six months (January- June 2025) in a selected dairy farms and the local milk collection points in South Punjab, Pakistan. The study area was selected because it produces a lot of milk and consumes raw milk frequently, which contributes to the risk of microbial contamination.

Sample Collection

It has been able to collect 50 milk samples that were of various sources (n = 20 dairy farms, n = 15 milk vendors, and n = 15 retail shops). Aseptically collected around 100 mL of raw milk in sterile screw-capped bottles. All samples were labeled with date, location, and source of collection. The insulated ice box was kept at **4°C** and samples were taken to the microbiology laboratory within 4-6 hours of collection to avoid alteration of the microbial load.

Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Volume 4, Issue 4 (2026)

Online ISSN 3007-3197
Print ISSN 3007-3189

http://amresearchreview.com/index.php/Journal/about

Sample Preparation

All the milk samples were homogenized with a slight shaking. Sterile normal saline (0.85% NaCl solution) was used to prepare serial dilutions up to 10 -6. These were dilutions on which microbial enumeration and isolation of particular pathogens were carried out.

Isolation and Identification of Salmonella spp.

Pre-enrichment

To detect Salmonella spp., pre-enrichment was done to enable the recovery of stressed or injured bacterial cells which might be found in the milk samples. In short, 10 mL of each milk sample was aseptically transferred into 90 mL of sterile buffered peptone water (BPW) and this gave a 1:10 dilution. The mixture was thoroughly mixed to make sure that microorganisms were evenly distributed and incubated at **37°C** during 18-24 hours under aerobic conditions. This is a non-selective step of enrichment that helps to resuscitate and first multiply Salmonella cells without the suppression of rival microflora.

Selective Enrichment

After the pre-enrichment, selective enrichment was performed to stimulate the growth of Salmonella spp. and to inhibit non-target organisms. The pre-enriched culture was aliquoted (1 mL) into 10 mL of Rappaport-Vassiliadis (RV) broth, which is a selective media that is specifically used to isolate Salmonella. Inoculated broth was incubated at 42°C within a 24 hours period of time since Salmonella spp. are more resistant to high temperatures than other bacteria. The malachite green and magnesium chloride, which are selective agents in RV broth, also prevent the growth of other microorganisms.

Selective Plating

After selective enrichment, a loopful of the enriched culture was streaked onto Xylose Lysine Deoxycholate (XLD) agar plates, a differential and selective medium commonly used for the isolation of enteric pathogens. The plates inoculated were incubated at **37°C**. Based on their typical appearance, which is the red colonies with black centres caused by the production of hydrogen sulfide (H 2 S) and lysine decarboxylase, presumptive Salmonella colonies were identified. Selective ingredients of the XLD agar prevent Gram-positive bacteria and permit the differentiation between lactose-fermenting bacteria and non-fermenting ones.

Biochemical Identification

The colonies with a typical Salmonella-like morphology were further tested in a battery of routine biochemical tests to confirm. These were Triple Sugar Iron (TSI) agar test to find out the patterns of fermentation of carbohydrates and production of hydrogen sulfide, urease test to determine the presence of urease enzyme, citrate utilization test to determine whether the organism can use citrate as a sole source of carbon or not and indole test to detect the presence of tryptophan degradation. A typical biochemical profile of Salmonella spp. includes alkaline slant/acid butt with H_2S production in the TSI test, negative urease and indole, and positive citrate reduction. These confirmatory tests assure proper identification of Salmonella isolates.

Isolation and Identification of Escherichia coli.

Selective Culturing

In isolating the Escherichia coli, the milk samples were inoculated on selective and differential media with appropriate dilutions. Each serially diluted sample was aseptically inoculated on MacConkey agar plates specifically to isolate Gram-negative

Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Volume 4, Issue 4 (2026)

Online ISSN 3007-3197
Print ISSN 3007-3189
http://amresearchreview.com/index.php/Journal/about

enteric bacteria, and differentiate lactose fermenters and lactose non-fermenters. The plates inoculated were incubated at **37°C** in aerobic conditions in a 24-hour period. After incubating, colonies that appeared with typical pink to reddish coloration were counted as presumptive E. coli because it is an indication of lactose fermentation resulting in the formation of acid and subsequent color change of the neutral red indicator included in the medium.

Confirmation of Eosin Methylene Blue (EMB) Agar

Colonies presumed to be E. coli on the MacConkey agar were sub-cultured on Eosin Methylene Blue (EMB) agar to confirm identification. EMB agar is a selective and differentiating culture, preventing Gram-positive bacteria but allowing fermentation of lactose to differentiate. The plates were incubated at **37°C**, 24 hours. E. coli colonies normally cause a characteristic metallic green halo on EMB agar because of the high production of acids and dye precipitation, which is regarded as a characteristic feature to identify it. This measure increases specificity and minimizes chances of identifying false-positives.

Biochemical Identification (IMViC Tests)

Additional confirmation of E. coli isolates was done through a set of standard biochemical assays commonly referred to as the IMViC tests, including Indole production, Methyl Red (MR), Voges Proskauer (VP) and Citrate utilization tests. The tests are commonly employed in the separation of E. coli and the other members of the family Enterobacteriaceae.

Indole Test: Tests the capacity of the organism to catabolize tryptophan to indole; E. coli usually gives a positive result.

Methyl Red Test: Determines mixed acid fermentation; a positive test of E. coli means that acid production is stable.

Voges Proskauer Test: The test identifies the production of acetoin; E. coli is usually negative.

Citrate Utilization Test: Test of the capability to use citrate as a single source of carbon; E. coli is typically negative.

The typical IMViC image of E. coli is (++−−), therefore, confirming its identity. Such biochemical tests are dependable to differentiate and guarantee the correct detection of E. coli isolates collected in milk samples.

Molecular Confirmation (PCR Analysis)

A commercial DNA extraction kit was used to extract genomic DNA of confirmed isolates according to the protocol of the manufacturer. Species-specific primers of amplification were used to amplify PCR with species-specific primers targeting:

InvA gene of Salmonella spp.

uidA gene for E. coli

The conditions of PCR were as follows: the initial denaturation was performed at **42°C** 5 minutes, and 35 cycles were performed: denaturation (**95°C** 30 seconds), annealing (55-60**°C** 30 seconds), and extension (**72°C** 1 minute). The final extension was **72°C** 7 minutes. The amplified products were subjected to 1.5% agarose gel electrophoresis and visualized by using UV light.

Enumeration of Total Bacterial Count (TBC)

A standard plate count was used to determine the total viable bacterial counts. Plate Count Agar (PCA) was prepared with diluted samples and was incubated at **37°C** and 24-48 hours. Colonies were counted and expressed as colony-forming units per

Online ISSN 3007-3197
Print ISSN 3007-3189

milliliter (CFU/mL).

Quality Control Measures

All microbiological tests were done in aseptic conditions. Positive controls were standard reference strains of E. coli and Salmonella spp. and negative controls were sterile media.

Statistical Analysis

Statistical software (SPSS version 25) was used to analyze data collected in terms of microbial counts and prevalence of pathogens. Mean and standard deviation were calculated using descriptive statistics. The chi-square test was used to establish significant variations in the levels of contamination in various sources of milk. The p-value of less than 0.05 was the statistical significance level.

Results

Prevalence of *Salmonella* spp. and *E. coli*

Among the 50 milk samples analyzed, a significant percentage of the sample was identified to be contaminated by Salmonella spp. and Escherichia coli, and this showed the prevalence of microbial contamination in raw milk. These pathogens were found in differing quantities based on the origin of the samples, which suggests that hygiene practices, handling procedures, and storage conditions differ across the dairy supply chain are important factors that impact microbial load.

In general, E. coli was detected in 42.0% (21/50) of the samples, and Salmonella spp were detected in 30.0% (15/50) of the samples. The prevalence rate of E. coli is high relative to Salmonella spp. indicates that there is a high level of fecal contamination and indicates unsanitary conditions in the process of collecting and distributing the milk. Since E. coli is often used as an indicator organism of fecal contamination, its high concentration highlights the existence of the possibility of environmental contamination, animal feces, or improper milking.

There was a distinct source-wise difference in contamination with the dairy farms showing less contamination than vendors and highest contamination in retail shops. Those samples of milk taken out of dairy farms had comparatively lower contamination, and had little exposure to external contaminants. Meanwhile, vendor samples were moderately contaminated, probably because of repeated handling, poor hygiene, and poor refrigeration conditions during transportation. The greatest contamination levels were observed in retail shop samples with a long storage period, ambient conditions of the environment, and cross-contamination of the samples due to handling potential contributions to microbial growth.

This upward trend in the milk supply chain is a strong indication that post-milking factors, such as transportation, storage time and retail handling practices are significant sources of microbial contamination. The results highlight the fact that although milk can be collected at the farm level in relatively hygienic conditions, poor downstream practices may severely undermine the quality and safety of milk.

On the whole, the presence of both Salmonella spp. and E. coli in high prevalence in milk samples is a severe health issue among the population, especially in the areas where raw milk is widely used. The findings underscore the importance of better hygienic measures, effective cold chain management, and stringent monitoring systems all along the dairy supply chain to guarantee milk safety and safeguard consumer health.

Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Volume 4, Issue 4 (2026)

Online ISSN 3007-3197
Print ISSN 3007-3189

http://amresearchreview.com/index.php/Journal/about

Table 1. Prevalence of bacterial pathogens in milk samples

Source	Total Samples (n)	*Salmonella* Positive n (%)	*E. coli* Positive n (%)
Dairy Farms	20	4 (20.0%)	6 (30.0%)
Vendors	15	5 (33.3%)	7 (46.7%)
Retail Shops	15	6 (40.0%)	8 (53.3%)
Total	**50**	**15 (30.0%)**	**21 (42.0%)**

Source-wise Distribution of Contamination

The bar graph shows that Salmonella spp. and Escherichia coli are most common in various source of milk samples such as dairy farms, vendors and retail shops. It is evident that there is an increasing trend in microbial contamination at farm level up to retail level. The samples of retail shops had the highest rate of contamination, then the samples of the vendors and finally had the lowest level of contamination, the samples of dairy farms. This trend shows that the microbial load increases in a progressive manner up the milk supply chain. The high contamination at the retail level can be blamed on the poor hygienic conditions, handling several times, exposures of the product to environmental contaminants and poor refrigeration of the product during storage and transportation.

Additionally, E. coli was always more prevalent than the Salmonella spp. at all sources of sampling. This implies extensive fecal pollution and poor sanitation in the collection and distribution of milk. The indicator organism, E. coli, also highlights the possible threat of contamination by the external sources. Generally, the contamination pattern indicates that after harvest conditions, such as handling, transportation, and storage conditions are major causes of milk quality deterioration and increase the levels of microbial contamination at the retail stage.

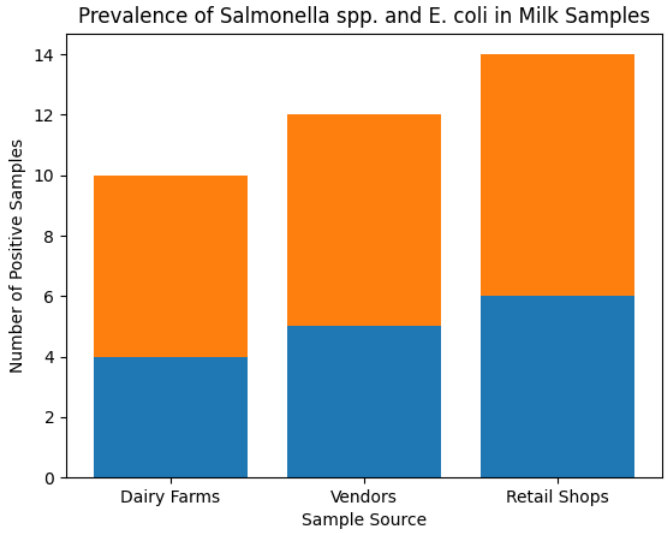


Figure 1. Bar graph showing the prevalence of *Salmonella* spp. and *Escherichia coli* in milk samples from different sources. The **blue bars represent** *Salmonella* **spp.**, while the **orange bars represent** *E. coli*. The graph demonstrates a progressive increase in contamination from dairy farms to retail shops, with *E. coli* showing

Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Volume 4, Issue 4 (2026)

Online ISSN 3007-3197
Print ISSN 3007-3189

http://amresearchreview.com/index.php/Journal/about

consistently higher prevalence compared to *Salmonella* spp.

3. Overall Contamination Status

The total microbial contamination of samples of milk was determined regardless of the source in order to indicate the overall microbiological quality of raw milk. As shown in Figure 2, the results suggest that a significant percentage of the samples under analysis were polluted with pathogenic microorganisms.

According to the total number of samples 42.0% of the samples were found to be contaminated and 58.0% were non-contaminated. Even though the percentage of samples without any noticeable contamination was slightly higher, the percentage of pathogens in almost half of the samples is also a serious indication. Such contamination indicates the prevalence of microbial hazards in raw milk. The fact that contamination was detected at such a significant percentage implies that there are poor hygienic standards during the collection of milk, its handling, transportation, and storage. The Salmonella spp. and Escherichia coli presence are other indications of the possible fecal contamination of the dairy supply chain, as well as other signs of poor sanitation conditions.

These results highlight the possible health hazards in the use of raw or unpasteurized milk by the population. Even when contamination is moderate, foodborne diseases can occur and especially in the susceptible groups. Thus, the findings highlight the importance of stringent measures in hygienic control, correct pasteurization, and routine control to guarantee the safety of milk and prevent disease in consumers.

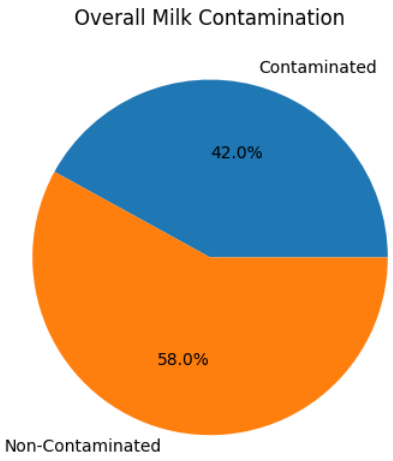


Figure 2. Pie chart representing the overall microbial contamination of milk samples. The **blue portion indicates contaminated samples (42.0%)**, while the **orange portion represents non-contaminated samples (58.0%)**, highlighting the proportion of samples affected by microbial contamination.

4. Total Bacterial Count (TBC)

The total viable bacterial count (TVC) of milk samples was determined to assess the overall microbial load and hygienic quality across different sources. The results revealed that the bacterial count ranged from **1.2×10^4 to 3.8×10^6 CFU/mL**, indicating substantial variation in microbial load among the sampled groups.

Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Volume 4, Issue 4 (2026)

Online ISSN 3007-3197
Print ISSN 3007-3189
http://amresearchreview.com/index.php/Journal/about

Table 2. Mean Total Bacterial Count (CFU/mL) in Milk Samples by Source

Source	Mean TBC (CFU/mL)
Dairy Farms	1.2×10^4
Vendors	6.5×10^5
Retail Shops	3.8×10^6

The findings show a definite rising trend in bacterial load at dairy farms towards vendors and retail shops. The samples of milk taken straight off the dairy farms showed the lowest average number of bacteria (1.2 x **10^4** CFU/mL), which indicates somewhat improved hygienic conditions on the sampling point. Conversely, the bacterial load in vendor samples was average (6.5 × **10^5**CFU/mL) and retail shop samples had the highest bacterial load (3.8 × **10^6** CFU/mL). Statistical analysis indicated a significant difference in the bacterial load of the samples in retail outlets and dairy farm samples ($p < 0.05$), showing that the bacteria contamination of the various sources was significantly different. This gradual rise in bacterial numbers indicates that post-milking factors, such as handling, transportation conditions, storage time and non-refrigerated condition, play a significant role in bacterial growth.

The high bacteria count in the retail samples reveal poor hygiene and poor cold chain management that may contribute to bacteria growth. The high TVC values reflect low quality of milk and high risks of spoilage, and health risks of consuming contaminated milk. Such results highlight the significance of stringent hygiene and proper storage environments along the dairy supply chain to safeguard and maintain the quality of milk.

5. Molecular Confirmation (PCR Results)

PCR analysis was performed to confirm the presence of target genes in the biochemically identified isolates. The results demonstrated high confirmation rates for both pathogens.

Table 3. PCR Confirmation of Isolates

Pathogen	Target Gene	Biochemical Isolates (n)	PCR Positive (n)	Confirmation Rate (%)
Salmonella spp.	invA	15	14	93.3%
E. coli	uidA	21	20	95.2%

Out of 15 biochemically identified *Salmonella* isolates, 14 (93.3%) were confirmed positive for the *invA* gene. Similarly, 20 out of 21 (95.2%) *E. coli* isolates were confirmed by amplification of the *uidA* gene. These results indicate a high level of agreement between biochemical and molecular methods, confirming the reliability and accuracy of the detection procedures used in this study.

Statistical Analysis

Chi-square (χ^2) analysis was performed to evaluate the association between milk source and contamination levels.

Table 4. Association Between Milk Source and Contamination

Source	Contaminated (n)	Non-Contaminated (n)	Total (n)
Dairy Farms	8	12	20
Vendors	9	6	15
Retail Shops	12	3	15

The analysis revealed a statistically significant association ($p < 0.05$) between milk

Online ISSN 3007-3197
Print ISSN 3007-3189
http://amresearchreview.com/index.php/Journal/about

source and contamination level. Retail samples showed significantly higher contamination compared to dairy farm samples.

Discussion

The current research showed that microbial contamination was very high in milk with Escherichia coli (42.0) being more common compared to Salmonella spp. (30.0%). These results are in line with other reports that have reported E. coli as a major predictor of fecal contamination and inadequate hygienic standards in milk handling and processing (13). The prevalence of E. coli indicates extensive sanitation problems and the possibilities of the milk being exposed to the environmental and fecal contaminations.

It was found that there was a significant rise in contamination of milk farm to retail stores thus showing that handling, transportation, and storage of milk are key factors in facilitating microbial growth. Other previous studies have also given similar trends, in which poor hygiene and temperature control have been cited as significant factors contributing to heightened contamination in milk distribution systems (14). The overall total viable counts of the bacteria especially in retail samples ($3.8 \times \mathbf{10^6}$ CFU/mL) were well beyond the acceptable safety limits of bacteria and indicated poor cold chain control and long periods of storage. Similar observations have been made in developing countries whereby lack of refrigeration and poor sanitation conditions contribute to rapid growth of bacteria (15,16). Such gradual build-up of microbial load along the supply chain supports the essence of high hygienic standards in the production to consumption.

The Salmonella spp. identification in 30% of the samples is a significant health issue to the populations because it has low infectious dose and is characterized by serious gastrointestinal infections (17). At the same time, the high rate of E. coli, especially of the pathogenic type, presents other dangers like hemolytic uremic syndrome, especially in the vulnerable populations (18).

PCR targeting invA and uidA genes provided high sensitivity and specificity to molecular confirmation to confirm the reliability of the standard methods of identification. The results are consistent with the literature that has been promoting the use of molecular diagnostics to properly identify the presence of milk-borne pathogens (19,20). In general, the continued high levels of contamination indicate lapses in the implementation of regulations, low awareness among stakeholders, and poor infrastructure. To enhance milk safety, Hazard Analysis and Critical Control Points (HACCP), regular monitoring of microbes, and awareness programs should be put in place. Moreover, treating antimicrobial resistance of foodborne pathogens should be a priority to reduce the future treatment difficulties (21,22).

Prevention and Safety Measures

Effective prevention and control of microbial contamination in milk require a multi-level approach spanning production, processing, and distribution stages. At the farm level, strict adherence to hygienic milking practices, including proper udder cleaning, sterilization of equipment, and use of clean water, is essential to minimize initial contamination. Implementation of Good Hygienic Practices (GHP) and Good Manufacturing Practices (GMP) can significantly reduce microbial load during handling and processing. Pasteurization remains one of the most reliable methods for eliminating pathogenic microorganisms, including *Salmonella* spp. and *Escherichia coli*, and should be consistently applied before consumption. In addition, maintaining

Online ISSN 3007-3197
Print ISSN 3007-3189
http://amresearchreview.com/index.php/Journal/about

an uninterrupted cold chain during transportation and storage is critical to inhibit bacterial growth. The adoption of Hazard Analysis and Critical Control Points (HACCP) systems can further help identify and control potential contamination points across the milk supply chain. Regular microbial monitoring and quality assessment should be enforced by regulatory authorities to ensure compliance with food safety standards. Furthermore, training and awareness programs for dairy farmers, vendors, and consumers are crucial to promote safe milk handling practices. Collectively, these measures can significantly reduce contamination risks and enhance the overall safety and quality of milk.

Conclusion

This paper has shown that there is a high degree of microbial contamination in milk, and Escherichia coli and Salmonella spp. present a big concern to the general population. The greater levels of contamination in the vendor and retail sample in relation to the dairy farm's points to the fact that the mismanagement, transportation, and storage are essential factors that lead to the growth of microbes. High total number of bacteria are other indicators of poor hygiene and mismanagement of cold chains. A combination of the traditional and molecular methods proved the validity of the ways used to detect pathogens used in this research. Milk safety must be ensured by adhering to the hygienic standards, the pathogenic process of milk pasteurization, and regular monitoring system like HACCP. Moreover, mass education and tightened control systems are needed to reduce the risk of contamination and ensure the health of consumers.

References

Jay JM, Loessner MJ, Golden DA. *Modern Food Microbiology*. 7th ed. New York: Springer; 2005.

Oliver SP, Jayarao BM, Almeida RA. Foodborne pathogens in milk and the dairy farm environment. *Foodborne Pathog Dis*. 2005;2(2):115–129.

Foley SL, Nayak R, Hanning IB, Johnson TJ, Han J, Ricke SC. Population dynamics of *Salmonella enterica* serotypes in commercial egg and poultry production. *J Food Prot*. 2011;74(4):690–703.

Karmali MA. Infection by Shiga toxin-producing *Escherichia coli*: An overview. *Clin Microbiol Rev*. 2004;17(4):936–968.

Food and Agriculture Organization (FAO). *Milk and dairy products in human nutrition*. Rome: FAO; 2013.

Van Kessel JS, Karns JS, Lombard JE, Kopral CA. Prevalence of *Salmonella enterica*, *Listeria monocytogenes*, and *Escherichia coli* O157:H7 in bulk tank milk. *J Dairy Sci*. 2011;94(12):5707–5713.

Hussein HS, Sakuma T. Prevalence of Shiga toxin-producing *Escherichia coli* in dairy cattle and their products. *J Dairy Sci*. 2005;88(2):450–465.

Marshall BM, Levy SB. Food animals and antimicrobials: impacts on human health. *Clin Microbiol Rev*. 2011;24(4):718–733.

Law JW, Ab Mutalib NS, Chan KG, Lee LH. Rapid methods for the detection of foodborne bacterial pathogens: principles, applications, advantages and limitations. *Front Microbiol*. 2014;5:770.

Codex Alimentarius Commission. *Code of hygienic practice for milk and milk products (CAC/RCP 57-2004)*. Rome: FAO/WHO; 2004.

Online ISSN 3007-3197
Print ISSN 3007-3189

World Health Organization (WHO). *Estimates of the global burden of foodborne diseases*. Geneva: WHO; 2015.

Oliver SP, Boor KJ, Murphy SC, Murinda SE. Food safety hazards associated with consumption of raw milk. *Foodborne Pathog Dis*. 2009;6(7):793–806.

Doyle MP, Erickson MC. Closing the door on the fecal coliform assay. *Microbe*. 2006;1(4):162–163.

Griffith CJ, Livesey KM, Clayton D. Food safety culture: the evolution of an emerging risk factor? *Br Food J*. 2010;112(4):426–438.

Olatunji EA, Jubril AE, Oladejo AO. Microbial quality of raw milk in Nigeria. *Afr J Microbiol Res*. 2012;6(7):1234–1238.

Parekh TS, Subhash R. Molecular and bacteriological examination of milk from different milch animals. *J Food Sci Technol*. 2008;45(2):143–148.

Eng SK, Pusparajah P, Ab Mutalib NS, Ser HL, Chan KG, Lee LH. *Salmonella*: A review on pathogenesis, epidemiology and antibiotic resistance. *Front Life Sci*. 2015;8(3):284–293.

Pennington H. *Escherichia coli* O157. *Lancet*. 2010;376(9750):1428–1435.

Malorny B, Hoorfar J. Toward standardization of diagnostic PCR for *Salmonella*. *Appl Environ Microbiol*. 2005;71(12):7139–7147.

Law JW, Ab Mutalib NS, Chan KG, Lee LH. Rapid detection methods for foodborne pathogens. *Front Microbiol*. 2015;5:770.

Wallace CA, Sperber WH, Mortimore S. *Food Safety for the 21st Century: Managing HACCP and Food Safety Throughout the Global Supply Chain*. Oxford: Wiley-Blackwell; 2011.

Ventola CL. The antibiotic resistance crisis: causes and threats. *Pharm Ther*. 2015;40(4):277–283.