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Hazard Analysis Critical Control Point (HACCP) System Implementation in the Dairy Supply Chain (Karachi-Pakistan)

Sayed Zaheer Abbas[1,2], Muhammad Naseem Khan[1,*], Anjum Zehra Naqvi[2], Kashan Kaif[5], Nargis Tabassum[3], Zulfiqar Ali Mirani[1], Abdul Basit Khan[1], Anila Siddiqui[1], Mazahir Hussain[4]

[1]Microbiology Section, Food and Marine Resources Research Centre,
Pakistan Council of Scientific and Industrial Research (PCSIR) Laboratories Complex Karachi - 75280, Pakistan
[2]Department of Microbiology, University of Karachi – 75270, Sindh, Pakistan
[3]Department of Pharmaceutical Chemistry, Federal Urdu University of Art, Science and Technology, Karachi – 75300, Pakistan
[4]Department of Bioscience, Shaheed Zulfikar Ali Bhutto Institute of Science and Technology, Karachi - 75600, Pakistan
[5]Department of Chemistry, Faculty of Science and Technology, NOVA University of Lisbon, 2829-516, Caparica, Portugal
*Corresponding author: micro_pcsir@yahoo.com

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Abstract The study's objective was to implement the HACCP plan in a local dairy supply chain in Karachi, Pakistan, to eliminate or reduce the risks for safe and high-quality milk available to the general public and to assess the degree of conformance to food safety and to look into the actual complexity involved in the HACCP implementation process. Different food safety hazards may enter the dairy supply chain during milk production, processing, shipping, storage, and serving, which may have an impact on milk safety and quality. The total average bacterial count $8.5x10^8$±0.18 CFU/mL, *Coliforms* 83.1±25 CFU/mL, *E. coli* 96.1±4.1 CFU/mL, and *Staph. aureus* 27±9.7 CFU/mL were recorded in the milk samples before the HACCP implementation. Although after the HACCP implementation, the count reduced to a considerable level as; 53% ($2.9x10^4$±0.15CFU/mL) reduction was noted in the total bacterial count, 80.4% (16±4.8 CFU/mL) in *Coliform* count, 77% (18.4±0.90 CFU/mL) in *E. coli* count and 83% (4.2±1.5 CFU/mL) in *Staph. aureus* count in the final product. Whereas, *Salmonella* and *Listeria* were not detected in any milk sample before and after HACCP system implementation. The results reflected the remarkable improvement in milk microbial hygiene. This means that our HACCP system was effective, however, continual improvement may be achieved by periodic monitoring of risks.

Keywords: *CCPs, dairy, food safety, HACCP, supply chain*

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1. Introduction

Pakistan's traditional milk production methods are similar to those of other underdeveloped nations [1,2]. Unhygienic methods of milk handling increase the possibility of contamination, which harms its composition, quality, and shelf life and makes it unsafe for human consumption [3]. Food safety hazards (biological, chemical, or physical agents) may enter the dairy supply chain during milk production, processing, shipping, storage, and serving, which may have an impact on the safety and quality of the milk [4]. Every nation is currently making an effort to reduce the health risks associated with food through a variety of means, such as improving hygienic conditions, food storage temperature, improper processing, etc. to provide customers with food that is free of contamination [5]. Good manufacturing practices (GMPs), sanitation standard operating procedures (SSOPs), and the hazard analysis critical control point (HACCP) system serve as the basis of food safety. However, HACCP is a preventive strategy that acknowledges the critical control points (CCPs) in the approaches to control product safety, GMPs cover several primary measures and circumstances to assure food safety following specified legislation. The implementation of SSOPs and GMP is a necessary condition for making the HACCP system effective [6].

When the HACCP plan is used, milk safety should be improved throughout the entire supply chain. The Hazard Analysis Critical Control Point (HACCP) is a system for successful food safety program that has gained international recognition. Given that the majority of dairy

products are delicate, have a limited shelf life, and are susceptible to numerous contaminants as a result of poor handling and production procedures, it is crucial to establish a food safety policy and plan for the implementation of the HACCP system [3]. In the dairy food chain, from production through handling and processing to consumption, production, and safety are essentially interrelated. Therefore, a system of preventive measures that progress from the safety of animal feed through useful and effective farming practices to good manufacturing and hygiene practices, consumer safety awareness, and proper application of food safety management systems throughout the dairy chain is required to reduce the food safety risks associated with milk and dairy products [4].

2. Materials and Methods

2.1. Samples Collection

For the evaluation of microbiological assessment, the dairy supply chains were studied in the five districts of Karachi (District East, West, North, South, and Central) to judge their quality and safety attributes according to the Punjab Pure Food Rules, 2007.

A total of 9 sampling spots were selected throughout the milk supply chain such as; direct milking animals, collecting jug, bucket, storage tank at dairy farms, dispensing cans, receiving cans, storage tank, collecting tubs at shops and selling points.

2.2. Methodology

Ten samples from each sampling point were collected (n-90). For sample collection, sterile, clean polythene plastic bags were used. The sample units were quickly and aseptically transferred to the lab in a clean, chilled container after being properly sealed. Most samples were examined for bacteriological examination as soon as they arrived or within 24 hours after being held at 0-4°C. *Total bacterial count*, *Staph. aureus*, *Salmonella*, *Listeria*, *Coliforms* & *E. coli* isolates were analyzed in milk samples as per the US FDA protocol. *Total bacterial count*, *E. coli* and *Staph. aureus* was enumerated by using (PCA, EMB, and BPA) agar respectively by pour plate method, MPN - presumptive and confirmative tests were performed for *Coliforms*. However, *Salmonella* and *Listeria spp*. were analyzed by enrichment and culturing method. For the detection of *Listeria monocytogenes*; a 25 ml milk sample was transferred to 225 ml *Listeria* broth having 2.5 ml selective supplement and incubated for 24 hours at 35°C. After the incubation 1 ml was transferred to BHI (brain heart infusion) agar plates and incubated for the next 48 hours at 35°C. Similarly, for the *Salmonella* (Isolation): a 25 ml milk sample was mixed in 225 ml lactose broth and incubated for 24 hours at 35°C. 0.1 ml of the incubated sample was transferred to 10 ml RV (Rappaport Vassiliadis) medium and another 1 ml to 10 ml TT (Tetrathionate) broth. RV medium was incubated for 24 hours at 42°C and TT broth tubes for 24 hours at 43°C. A loopful (10 µl) of incubated TT broth was streaked on the BS (Bismuth Sulfite) agar, XLD (Xylose Lysine Deoxycholate) agar, and HE (Hektoen enteric) agar plates and incubated for 24 hours at 35°C. A similar, procedure was repeated for incubated RV medium**.** After incubation, the plates were examined. The same protocol was repeated for microbiological assessment after the implementation of the HACCP plan from farm to retail shops.

2.3. Implementation of the HACCP

To successfully regulate, eliminate or reduce the food safety hazardous elements to an acceptable level, the HACCP plan was created to examine the multiple latent hazard variables that could result in the contamination of raw milk during the supply chain. The research work on the implementation of HACCP was conducted in the local dairy supply chain from December 2021 to June 2022. The research methodology was established on the FAO's provided HACCP checklist and CCPs decision tree. Based on the twelve procedures outlined by the Codex Alimentarius Commission, which are summarized as follows.

2.3.1. Establishment of the HACCP Team

To conclude the HACCP study successfully, the HACCP team was established. The interdisciplinary team consisted of dairy farm owners, employee supervisors, a veterinarian's doctor, the manager of the dairy farm, the transportation coordinator, the owners of the dairy shop, and a research scholar.

2.3.2. Raw Milk Description

Raw milk is typically thought of as a full diet when consumed in its natural state because it contains high food values and nutrients such as water, proteins, lactose, energy-containing fat and solids-not-fat (SNF), carbohydrates, vitamins, organic acids, and enzymes. Along with the main constituent, it also contains about 150 other important nutrients [7,8]. Due to various factors, including seasonal variations, animal species, stage of lactation, feed, milking, breed diversity, age, frequency of milking, interval, diseases, stress, abnormal conditions, drug and hormone injection, all lactating animals have nearly the same chemical composition but differing concentrations [9]. As illustrated in Table 1.

Table 1. Raw Milk Description

Product	Characteristics
Raw Milk	Fat: 3.5%
	Ash: 5.56%
	Water: 87%
	pH: 6.4 to 6.8
	Lactose: 4.3%
	Proteins: 3.9%
	Minerals: 0.8%
	Vitamins: 0.1%
	SNF: 12 to 16%
	Acidity: .10 to 0.
	Viscosity: 0.2 Centipoise
	Shelf life 2 hours after milking
	Specific Gravity: 1.028 to 1.032
	Storage temperature between 0 to 4°C
	Freezing point between -0.522 to -0.540°C
	Natural cow’s milk is off-white and shining white (buffalo)

2.3.3. Identification of Intended Use

Due to milk's nutrient-rich nature, it is consumed by more than 6 million people worldwide and is especially popular with those who may have lowered immunity [7,8]. Milk is a very healthy and nourishing food for all individuals at any age [10,11]. In Pakistan, it is reported that 97% of milk is sold in raw form and the rest is pasteurized, the biggest yearly per capita milk consumption in Pakistan is in Sindh 246 kg, in Punjab, it is roughly 132 kg, in KPK 86 kg, in Baluchistan 108 kg [1].

2.3.4. Flow Diagram of the Complete Process

The purpose of the flow diagram was to provide a clear, concise path of the procedures necessary in the entire supply chain from farms to retail stores. The flow diagram covered almost all stages of the raw milk supply chain. Through an on-farm checklist review with the stick holders, several data on farm features related to animal health and cleanliness, feeding, milking method, storage, transportation, and milk quality were gathered. Aspects of management practices were carefully investigated to complete the diagram. As shown in Figure 1.

2.3.5. On-site Confirmation of Flow Diagram

Once the flow diagram was constructed, the HACCP team visually checked it for accuracy and completeness on the work site. The status of the animal's body cleansing, the milking technique, pre and post teat treatment, storage temperature, transportation, distribution, and various management practices were carefully noted as some of the various modifications, procedures, and activities that were required for on-site confirmation.

2.3.6. Hazard Analysis

The HACCP team used a checklist to conduct a hazard analysis to prevent any changes in milk quality across the supply chain. Each phase of the flow chart's possible risks was initially identified, with an emphasis on how they might affect the pre and post-milk quality. Three groups of risks were identified: biological, chemical, and Physical. As shown in Table 2.

2.3.7. Determination of the CCPs (Critical Control Points)

The most crucial component of a HACCP plan was identifying critical control points (CCPs) or situations when risks should be avoided, eliminated, or reduced to a manageable level. Finding CCPs based on the hazard analysis was done using the decision tree given by Codex. The members of the HACCP team established the following CCPs: animal washing/cleaning (CCP-1), (CCPs-2 & 3) pre and post-milking udder preparation, milk storage temperature (CCP-4), milk transportation (CCP-5) and (CCP-6) was fixed during milk handling at retail stores.

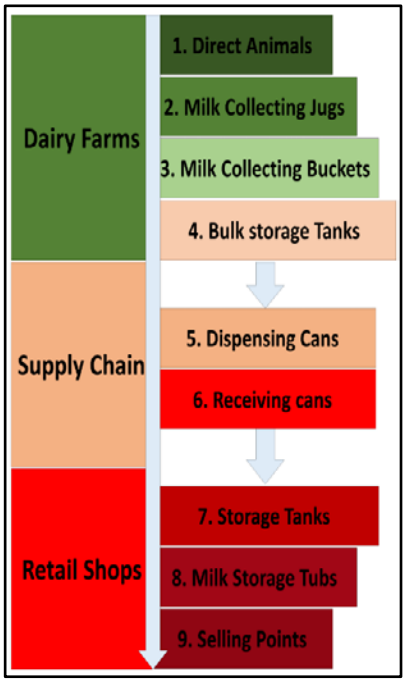

Figure 1. Flow diagram

Table 2. Hazards analysis of dairy supply chain

Steps	Hazard			Control Measures
	Microbial	Chemical	Physical	
Animals body washing	Introduction & transmission of zoonotic pathogens	Unauthorized detergents or disinfectants	Extraneous matters (Soil, dirt, manure, hairs, dust, flies)	Proper Implementation of SSOP
Pre-milking udder preparation	Presence of mastitis pathogens	Unauthorized cleaning solvent	Personnel hygiene, Contaminated or uncleaned towels for drying	Proper cleaning and GMP Implementation
Milking	Unhygienic handling, Infected person, use of contaminated water, cross contamination	Strong smell, painted non-food grade utensils, pesticides, smoking, spit or cough	Soiling, bedding, manure, feeding	Implementation of proper GMP
Post-milking udder disinfection	Open teat orifice, Recontamination	Unauthorized cleaning solvent, insufficient rinsing	Personnel hygiene, Contaminated or uncleaned towel use for drying	Implementation of SSOP
Milk storage	Increase bacterial count and zoonotic pathogens	Cross-contamination, copper made nonfood grade materials, unauthorized detergent or disinfection insufficient rinsing	Exposure to sunlight, dust flies, strong smell, or any other physical residue	Implementation of GMP
Milk Transportation & Distribution	Improper washing and disinfection, improper temperature control, cross-contamination	Non-food grade materials	Extraneous matters	Usage of food-grade containers and Implementation of GMP
Milk handling at shops	Increase bacterial count, improper utensils washing & disinfection, uncontrolled temperature, cross-contamination	Non-food, damaged, cracked, and rusted utensils Milk handler communicating disease symptoms, Fuels, chemicals, food, etc.	Extraneous matters	Implementation of GMP

Table 3. Critical Control Points Analysis

Step	Hazards	CCPs	Critical Limit	Monitoring	Corrective Action	Verification
Animals washing & cleaning	Chemical	CCP - 1	Soak 250ml of turpentine/taramira oil in a mixture of cool water and 100g salt	Non-compliance with the cleaning procedure	Buy chemicals from authorized dealers and apply salt & a mixture of taramira oil with sufficient rinsing of cold water	In-charge should verify the system to ensure that the activities are performed accordingly
Pre & post-milking udder preparation	Microbial	CCP - 2	-SSC – 25000 cells/ml, Thermoduric count should not be detected or less than 200cfu/ml	Visual udder Inflammation	Dry with a clean individual towel and disinfect hands after washing with clean water	Recent infection rate, Total bacterial & thermoduric count
	Chemical	CCP - 3	70% iodine or hypochlorite solution	Prevision of disinfectant analytical data	Immerse at least ¾ of the teat into pre-dip disinfectant (70% iodine or hypochlorite solution) and allow for 30 sec contact time	Tractability of the non-compliance with the instructions
Milk Storage	Microbial	CCP - 4	Temperature >4-7°C for short time	Hygiene and cold storage temperature	Install calibrated thermometer	Cold storage temperature
Milk Transportation	Microbial	CCP - 5	Temperature not to rise above 10°C	Hygiene and cold storage temperature	Cool insulated facility to maintain the milk temperature not rising above 10°C and reach within a short time shops	Tractability of the non-compliance with the instructions
Milk handling at shops	Microbial	CCP - 6	*TPC* <50000cfu/ml, *Coliforms*-5cfu/ml, *E. coli*-3cfu/ml, *S. aureus*-0cfu/ml, *Listeria* and *Salmonella*-0cfu/25ml	Cold storage temperature and hygiene, Non-compliance with the hygienic milk handling	Ensure a clean hot (80°C) water system for utensils washing, and maintain milk temperature at 7°C or below	Tractability of the non-compliance with the instructions

2.3.8. Establishment of the Critical Limits for Each CCP (principle 3)

A type of regulating index called a "critical limit" is employed to ensure the food's safety and quality. For each CCP that was discovered, a critical limit was established and specified. Critical limits were the parameters that were used to assess whether a procedure was producing safe milk. These constraints may be physical, chemical, or microbial. Table 3 provides some illustrations of activities that are regarded to represent important boundaries (CCPs) and their restrictions.

2.3.9. Establish Monitoring Procedures for CCPs (principle 4)

To ascertain whether the HACCP plan is being implemented under control, that is, within the critical limits, a monitoring system that includes testing, assessing, monitoring, and reviewing all of the actions carried out across the supply chain has been built. Monitoring techniques used during the operation were recorded and saved for future use.

2.3.10. Establish Corrective Measures/Actions (Principle 5)

Corrective actions were taken when the observation showed that a specific CCP is not under control, i.e. if the deviation is from the critical limit.

2.3.11. Establish Verification Procedures (Principle 6)

To ensure that the HACCP approach is being used properly, the internal audit was conducted as part of the verification process. When critical limits are crossed, authentication made sure that the proper alternative procedure plans are in place. 1) The HACCP team's internal audit was provided as a backup for the verification procedures. They made sure the methods were used properly, after which they issued a report explaining the flaws and possible areas for development.

2.3.12. Establish a System of Documentation and Record Keeping (Principle 7)

The HACCP system's first written records of the correct development of all activities, from farms to retail stores, were documented with references to ensure the traceability of violations of the procedures in each step. These records allowed for reviewing, evaluations, and confirmation of the system's proper implementation. Records were kept throughout the entire HACCP plan.

2.4. Pre-requisites

To ensure the excellent quality of raw milk, the first step was to bring all pre-requisite programs that were placed under the framework of the HACCP program and provided a widespread path to achieve zero defects in the end product. A strong system of checks against potential failures of crucial control points was ensured by several precondition programs, which formed the basis for the HACCP model. Sanitation program, which maintained effective sanitary conditions necessary for high-quality milk production, storage, and transportation under good GMP and hygienic practices to control hazards, are prerequisite programs used in the complete processing. The Risks of Milk Contamination Preventive (RMCP) program was created to produce, safeguard, and market high-quality milk without any risks of contamination. This included workers' hand wash drying, milk utensils coverage and its food grade nature, drying of the bodies of animals, sterilizing and disinfecting milking equipment, keeping an eye on worker hygiene standards, milk filtration through muslin cloth, providing clean transportation facilities and ensuring that employees behave safely and responsibly when handling milk to

reduce or prevent recontamination. For each CCP that was identified, a critical limit was established and specified. Critical limits were the criteria that were used to assess if a procedure was generating safe milk.

2.5. Statistical Analysis

The collected (pre and pro-HACCP) samples were analyzed using IBM SPSS statistics 22. Results were recorded as mean ± SE, and analysis of variance was performed by ANOVA procedure to compare the results by at least significant difference (LSD) at $P<0.05$.

3. Results and Discussion

Before HACCP implementation, the *total bacterial count* (mean log 9.3±0.18), 83.1±25 CFU/mL *Coliform* count, 96.1±4.1 CFU/mL *E. coli* and 27±9.7 CFU/mL *Staph. aureus* count was recorded in the selling points milk samples, and the count for each microbiology parameter was beyond the acceptable level (Table 4) according to the national microbiological food standards.

Higher bacterial counts attributed to the unsatisfactory level of hygiene milk handling, ineffective cleaning procedures, lack of cooling facilities, and workers training. After HACCP implementation, the *total bacterial count* (mean log 4.4±0.15), 16±4.8 CFU/mL *coliform* count, 18.4±0.90 CFU/mL *E. coli* and 4.2±1.5 *Staph. aureus* count was noted in the final product (raw milk) collected from the selling points, the count was reduced to a considerable level but not to an acceptable level according to the Punjab Pure Food standards. The results obtained revealed that the mean log values of total colony count (CFU/mL) of examined samples were significantly reduced at ($P<0.05$) after HACCP implementation. The *total colony count*, *coliform*, *E. coli*, and *Staphylococcus aureus* counts (CFU/mL) in raw milk samples were reduced by 53.76%, 80.4%, 77%, and 83.7% to the mean value respectively, as presented in Table 5.

Table 4. Microbiological assessment (CFU/mL) of raw milk from farm to shops

Organisms	Sampling Points								
	Dairy Farms				Supply Chain		Retail Shops		
	1	2	3	4	5	6	7	8	9
APC	2	135	$3.9x10^3$	$2.1x10^5$	$4x10^5$	$5.8x10^6$	$7.8x10^7$	$9.9x10^7$	$8.5x10^8$
Coliforms	0	2	8	34	56	85	115	162	240
E. coli	0	0	0	15	20	25	49	70	90
S. aureus	0	0	5	12	15	19	30	37	77

1; Direct Animals **2**; Milk Collecting Jug **3**; Milk Collecting Bucket **4**; Bulk Storage Tank, **5**; Dispensing Cans, **6**; Receiving Cans **7**; Storage Tank **8**; Milk storage tub **9**; Selling Point

Table 5. Pre and post-HACCP implementation significance values

Total samples of selling points (n-90)	Pre-HACCP implementation system	Post-HACCP implementation system	Percentage of reduction calculated to mean	Significance value
1. *Total bacterial count*	9.3±0.18*	4.4±0.015*	53.76	0.0036
2. *Coliforms*	83.4±25	16±4.8	80.4	0.016
3. *E. coli*	96.1±4.1	18.4±0.90	77	0.053
4. *Staph. aureus*	27±9.7	4.2±1.5	83.70	0.042

Bacterial Counts, *Coliform* Counts, *E. coli*, and *Staphylococci* counts in selling points milk samples before and after implementation of the HACCP system.
NOTE: n: number of the Samples; * Mean log (10) ±SE; The Mean Difference is Significant at the 0.05 Level.

The results used in this study were to evaluate the effectiveness of the sanitary, hand washing, and overall personal hygiene practices of food handlers for the entire assessment period. Milk handling workers were trained to carry out GVP, GMP, and GHP to assure the best quality of raw milk. The critical control in animal washing, pre, and post-udder preparation, utensils sterilization, hand washing, and temperature control was monitored to reduce high levels of pathogenic microorganisms, to a safe level. Purchase chemicals from authorized dealers and apply with sufficient rinsing cold water to avoid any chance of contamination, cool insulated facility maintained the milk temperature to protect it from spoilage. For proper implementation of the HACCP system, effective implementation of prerequisite programs was employed. As data revealed that before HACCP implementation any of the collected samples (100%) did not meet the microbiological criteria but after HACCP system implementation the *total bacterial count* in 58 (64%), *coliforms* in 67 (64%), *E. coli* in 77 (85%) and *Staph. aureus* in 67 (64%) samples out of a total 90 (n-90) were accounted in acceptable microbiological criteria according to the Punjab Pure Food Standards. Application of effective cleaning procedures combined with carefully planned parameters such as efficient animal washing and drying, sanitization and disinfection of pre and pro-milking udder preparation, controlled cooling temperature system in the storage tank, transportation cans, and hygienically milk handling throughout the supply chain significantly improved the bacteriological condition of the final product as shown in Table 6.

Table 6. Acceptability of raw milk samples (final product) according to the Punjab Pure Food Rules, 2007

The examined organisms	The Punjab Pure Food Rules, 2007	Before Implementation			After implementation		
			Acceptable	Un-acceptable		Acceptable	Un-acceptable
			%	No.		%	No.
**Total bacterial counts*	10^5 CFU/mL	n=90	0	90	n=90	64	32
Coliform count	5 CFU/mL		0	90		74	23
E.coli	3CFU/mL		0	90		85	13
Staph. aureus	0/mL		0	90		74	23

n: Number of Samples.

At a local site in Karachi, the HACCP system for the entire supply chain was established step-by-step using the twelve steps described in the methodology section above. The prerequisite program was made available to reduce risks and simplify the HACCP plan. The identification of hazards, monitoring of critical limits, and validation of corrective actions were carried out based on literature, manuals, and standards. The HACCP team found the CCPs by logically responding to queries presented in the decision trees. The critical control points (CCPs) were selected using the decision tree technique because it is a visual, simple-to-understand alternative to numerical charts and statistical probabilities used in other decisions. For the identified hazards, control measures were advised and for the identified CCPs, appropriate monitoring procedures and corrective actions were proposed.

3.1. Constraints in Adopting the HACCP System

The need for awareness and responsiveness of HACCP, a lack of dairy-related personnel training, uneducated employees and owners, resistance to traditional changes, ignorance of food safety and security, a lack of management commitment, uneven animal care, a lack of government support in the implementation of food safety management program and a lack of technical and professional expertise were just a few of the obstacles in implementing the HACCP system. Best management practices were followed in the HACCP program as preventative measures to ensure food safety. The HACCP method was found to have a better preventative approach than traditional quality milk control systems since it places more emphasis on risk management than end product analysis. Additionally, the working personnel played a major role in the HACCP methodology's performance. The benefits of employing HACCP procedures were confirmed by the high levels of satisfaction indicated by owners who took part in the implementation of this technique.

4. Conclusion

The microbiological assessment of raw milk showed a high bacterial count before the HCCAP implementation, the count was beyond the acceptance criteria in all the collected samples from selling points. This reflects the poor sanitation and hygienic practices within the supply chain.

After the HACCP implementation, the bacterial count was reduced to a considerable level in the collected milk sample from the same site. Due to limited resources, still positive progress was witnessed in the study. The results of this study reflect that there is a remarkable improvement in milk microbial hygiene, which means that our HACCP system is effective. However, continual improvement may be achieved by periodic monitoring of risk.

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Conflict of Interest

The authors declared no conflict of interest.

Abbreviation:	**Full name**
HACCP:	Hazard Analysis Critical Control Point
CCPs:	Critical Control Points
GMPs:	Good manufacturing practices
SSOPs:	Sanitation Standard Operating Procedures
FDA:	Food and Drug Administration
BAM:	Bacteriological Analytical Manual
PCA:	Plate Count Agar
EMB:	Eosin Methylene Blue
BPA:	Baird-Parker Agar
MPN:	Most Probable Number
BHI:	Brain Heart Infusion
RV:	Rappaport Vassiliadis
TT:	Tetrathionate
XLD:	Xylose Lysine Deoxycholate
HE:	Hektoen enteric
FOA:	Food and Agriculture Organization

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