

**Original Article****Evaluation of the Adequacy of GMP to Control Microbial Hazards in Dairy Factories in Fars Province**Sajjad Abdi¹, Sahar Jazaeri^{2*}, Zohreh Amiri², Farid Zaeri³, Mehrdad Niakowsari⁴

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Received: February 2016**Accepted:** June 2016**ABSTRACT**

Background and Objectives: Pre-requisite programs (PRPs) are “primary conditions and requirements essential for HACCP operations, which are crucial in food safety programs”. The present study was conducted to evaluate the impact of implementation of PRPs on the microbial parameters of pasteurized milk (according to the National Standard of Iran). Effectiveness of HACCP operation requirements and efficiency of Good Laboratory Practice (GLP) were also evaluated in control of the above-mentioned microbial parameters.

Materials and Methods: According to the approved checklist of the Vice-chancellor in Food and Drug affairs, PRPs of 26 factories were evaluated from March 2014 to March 2015 in two-month intervals, and their total and component scores were obtained along with the microbial parameters of pasteurized milk. Generalized Estimating Equations (GEEs) were used to determine the significance of total score and the impact of its components on controlling microbial hazards.

Results: There was a reverse significant relation between the total scores of the PRPs and microbial hygiene indices (total and coliform count) which approves the effectiveness of operating the programs in controlling the mentioned microorganisms. Efficiency of each pre-requisite program was different in controlling the microbial parameters. Good Laboratory Practice (GLP) had a prominent effect on controlling of the index microorganisms of hygienic operations. Overall, the results showed a little probability of contamination with *E. coli* in the pasteurized milk samples of Fars Province for which the statistical analysis was ignored.

Conclusions: The exact operation of PRPs resulted in reduction of microbial parameters in a way that increasing the total score of PRPs led to decrease in microbial parameters of total count (TC), coliforms, molds and yeasts. The findings further suggest the application of this checklist in evaluation and prediction of microbial parameters.

Keywords: Good manufacturing practices, Microbial parameters, Pasteurized milk

Introduction

Food safety, security and hygiene are important in human's health and well-being, so it is essential that governments pass obligatory laws to protect and promote safety requirements of food products and encourage the producers to implement them (1).

According to the definition of the World Health Organization (WHO), Pre-requisite Programs (PRPs) are “primary conditions and requirements essential for HACCP operations, which are critical in food safety programs”. These programs often include an extensive set of features and measures that are

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preferably considered according to the process or product characteristics. Achieving these conditions reduces the risk of possible hazards (2). Microbial indicators are used to assess food safety and hygiene. Although achieving a zero threshold for all types of organisms is not possible under conditions of Good Manufacturing Process (GMP), the main purpose is the production of goods with the lowest number of microorganisms (3). In Iran, microbial indicator organisms such as bacterial total count, coliform count and *E. coli* are used to assess the safety of pasteurized milk (4). In most countries, microbial indices are applied for evaluation of food safety programs. Fewer pathogenic microbes and low contamination index indicate the effectiveness and proper implementation of food safety management programs (5). For instance, the presence of gram-negative bacteria in pasteurized products like milk, cream and yogurt indicates cross-contamination or leakage of contaminated water into the milk, and other unsanitary conditions (6). In addition to the safety assessment of food consumption, the number of pathogenic microbes and contamination indices represent the effectiveness and implementation of food safety management programs (PRPs, HACCP and ISO22000) as well. Currently, the Ministry of Health's Food and Drug Organization, evaluates GMP to assess its risk identification and monitors Food control laboratories and production units such as dairy products in Fars Province by using a "checklist to evaluate a PRP for food and beverage production units" (accepted checklist). The checklist has 34 sections (total of 1000 points); each section consists of rated audit components including several subunits).

In this study, we used the above mentioned checklist to obtain relations between implementing the PRPs and controlling the microbial parameters in pasteurized milk. Additionally, this is the first time that this official checklist is evaluated in the country.

Materials and Methods

Samples: The production units and samples of pasteurized milk were selected based on the following factors:

1. These products are susceptible to spoilage. They need to be kept at the refrigerator temperature (7, 8), and the inadequacy of the PRPs can cause cross-contamination and product spoilage.
2. The production units were active during the experiments and were not closed.
3. Selected installations include small, medium and large manufacturing units.
4. One of the PRPs is assessment of the suppliers, so the milk delivered to the factory should not have more than 10×10^5 total count.

Production capacity of units under study varied according to Table 1. They were divided into three categories of small factories with daily milk production up to 15 tons, medium factories with the average milk production of more than 15 and up to 50 tons daily, and large factories with more than 50 tons of daily milk production.

Table 1. Factories under study

Daily milk production (Ton/day)	Scale	Number	Percentage
Up to 15 tons	Small	15	57.7
15 to 50 tons	Medium	7	26.9
50 tons and more	Large	4	15.4
Total		26	100

Material: The PRPs' assessment checklist approved by the Food and Drug Administration (FDA) was used in this study: the checklist includes 34 audits as follows: 1) The area around the plant (20), 2) Construction equipment (55) 3) Plant layout (40), 4) Doors (20), 5) Windows (25), 6) Floor (20), 7) Walls (25), 8) Ceiling (15), 9) Sewerage (16), 10) Lighting (6), 11) Ventilation (25), 12) Water treatment plants (25), 13) Water Treatment (31), 14) Toilets (48), 15) Welfare services (17), 16) Workers hygiene (45), 17) Capabilities of personal hygiene (20), 18) Warehouse/refrigerator/oven (78), 19) Parts manufacturing (84), 20) Control of insects and rodents (14), 21) Washing/disinfection/cleaning (33), 22) Maintenance (11), 23) Conditions of technical attendant (30), 24) Labeling system (10), 25) Identification and tracking (30), 26) Customer complaints (20), 27) Call (20), 28) Risk identification and monitoring (47), 29) Training (15), 30) Suppliers (35), 31) Communication within and outside the organization (16), 32) Research and development (10), 33) Quality management certificate (20), and 34) Inspection and testing (74). The audit consists of several components that make up the total score of the audit. In the end, the final total score of the audit formed "total points of prerequisite programs". It is to be noted that the checklist combined with GMP (Good Manufacturing Practice), GLP (Good Laboratory Practice), HACCP (Hazard Analysis of Critical Control Point) and Institutional Relations are collectively called "Prerequisite Programs of Food and Beverage Units" to evaluate PRPs for monitoring and inspecting the production units, which have been implemented in the country since 2010.

Pasteurized milk microbial tests were conducted by the Food and Drug Reference Laboratory: These tests were carried out according to the Iranian National Standard No. 2406 (Microbiology of milk and its products-features) by measuring the biological parameters listed in Table 2 (4).

Operational description of the work: First, the inspectors of Food and Drug Deputy of Shiraz Medical University audited the PRPs during March 2014-March 2015 in every two-month period (6 times a year), and scored all 34 sections of the PRPs. Each part consisted of several parameters, and total scores showed the score of each parameter during each step of the audit. Sampling of the production units was conducted, and the samples were sent to the reference laboratory of Food and Drug Deputy of Shiraz Medical School in less than one hour under controlled temperature; then the results of microbial tests for pasteurized milk were collected. According to the standard, microbiological properties of milk per milliliter or gram product must be in accordance with those given in Table 2.

Table 2. Microbial specifications of pasteurized milk (4)

Parameters	Pasteurized milk
Total count	Max $7/5 \times 10^4$
Coliforms	10
E.Coli	0

Statistical analysis: In addition to studying the significance of the relationship between microbial parameters and total score of PRPs, marginal modeling method or GEE (Generalized Estimating Equations) was used to evaluate the impact of each auditing section on controlling the microbial hazards.

Table 3. Results of the side model of relationship between the total scores prerequisite programs and the total count in pasteurized milk

Audit item	P-value	Estimate (CFU/ml)	SE
Total score	<0.001	-9.34×10^3	1998.85
Identifying and traceability	0.002	-8.81×10^3	2792.05
Wash out, cleaning and disinfection	0.008	-8.68×10^3	3297.64
W.C.	0.005	-8.34×10^3	416.20
Customer complain	0.001	-7.85×10^3	2295.70
Storage/Refrigerator/Incubator	<0.001	-7.21×10^3	1990.90
Hazard analysis and monitoring	0.001	-7.03×10^3	2033.36
Research & Development	0.002	-6.78×10^3	2229.68
Production and processing areas	0.009	-6.70×10^3	2564.67
Personal hygiene abilities	0.006	-6.68×10^3	2451.17
Inspection and laboratory	0.002	-6.62×10^3	2161.03
Pest control	0.002	-6.61×10^3	2084.46
Recall	0.008	-6.50×10^3	2442.87
Training	0.20	-6.28×10^3	2691.16
Structural facility	0.026	-6.14×10^3	2761.70
Supplier	0.028	-6.04×10^3	2755.54
Labeling	0.026	-4.34×10^3	1955.10

Estimate (β) of this method indicates the change of dependent variable per unit change in the independent variable. Correlations between different repeats were checked, and since there was no particular structure, so the unstructured correlation was used. there was no missing responses in variable response amounts during the time.

Results

The relationship between scores of prerequisite programs and the total count (TC) of microorganisms in pasteurized milk: There was a reverse significant relationship between the total score of PRPs and the total count of microorganisms in milk ($P < 0.001$) so that for every unit increase in the total scores of PRPs, the total count of microorganisms in the pasteurized milk reduced to 9340 CFU/ml.

The relationship between the scores of PRPs and coliform count in pasteurized milk: There was a significant relationship between the total scores of prerequisite PRPs and coliform number in the pasteurized milk ($0.01/0 > P$) so that for every unit increase in the total scores of PRPs, there was a 1.61 CFU/ml decline on the coliform counts in pasteurized milk.

The relationship between the scores of PRPs and coliform count in pasteurized milk: There was a significant relationship between the total scores of prerequisite PRPs and coliform number in the pasteurized milk ($0.01/0 > P$) so that for every unit increase in the total scores of PRPs, there was a 1.61 CFU/ml decline on the coliform counts in pasteurized milk.

Table 4. Results of the side model of relationship between the total score of PRPs and coliform count in the pasteurized milk

Audit item	P-value	Estimate (CFU/ml)	SE
Total score	0.013	-1.61×10^0	0.14
Hazard analysis and monitoring	<0.001	-1.55×10^0	0.29
W.C.	<0.001	-1.54×10^0	0.32
Pest control	<0.001	-1.34×10^0	0.18
Production and processing areas	<0.001	-1.30×10^0	0.24
Inspection and laboratory	<0.001	-1.29×10^0	0.23
Supplier	<0.001	-1.26×10^0	0.21
Research & Development	<0.001	-1.21×10^0	0.27
Training	0.002	-1.21×10^0	0.38
Wash out, cleaning and disinfection	<0.001	-1.07×10^0	0.23
Ventilation	0.021	-1.07×10^0	0.46
Storage/Refrigerator/Incubator	<0.001	-1.04×10^0	0.25
Identifying and traceability	0.008	-1.02×10^0	0.38
Waste treatment	<0.001	-0.95×10^0	0.25
Personal hygiene	0.012	-0.76×10^0	0.30
Windows	0.025	-0.74×10^0	0.33
Soil	0.034	-0.62×10^0	0.29
Labeling	0.030	-0.62×10^0	0.28
welfare services	0.047	-0.61×10^0	0.30
Structural facility	0.042	-0.55×10^0	0.27

Discussion

The relationship between the total scores of PRPs and the total count (TC) of microorganisms in pasteurized milk: Generally, pasteurized milk spoilage is the result of contamination after pasteurization caused by Psychrotrophic Gram-negative and rod-shaped bacteria, such as *Pseudomonas*, *Alcaligenes*, *Acinetobacter* and *Psychrobacter* (3). In order to prepare a safe dairy product, all hygienic points including GHP, GLP and GMP programs should be considered from milk production to consumption (9-11). There is a highly significant correlation between total score of PRPs of approved checklist that includes good manufacturing, laboratory and health and total count of microorganisms in both pasteurized milk and cream products ($P < 0.001$); this indicates that microbial quality of the product depends on implementation of the above-mentioned programs.

As can be seen in Table 3, out of the 34 auditing sections, 19 cases (57%) had negative correlation ($P < 0.05$) with the total count of microorganisms in pasteurized milk. This result is consistent with the that of other studies, Smigic and colleagues (2012) showed that before the establishment of PRPs and HACCP in milk producing farms, more than 55% of the milk received from the farms to dairy factories were of grade 3 milks and only 35% of them were in the group of high-quality grade; however, after settling PRPs and HACCP, this amount reached to 53%, and amount of grade 3 milk declined to 20.3%. The results indicated the role of improving PRPs in farms, milk collection centers and milk transport

vehicles, on the one hand, and raising the awareness of farmers through training, in improving the quality of raw milk on the other. In the case of pasteurized milk, similar results were obtained and after implementing the PRPs, the total count of microorganisms decreased to 2-3 CFU/ml (20) because implementation of prerequisite programs such as washing, cleaning, disinfection and staff hygiene could prevent cross-contamination of the pasteurized milk, too (10). The present study demonstrated that the total count of microorganisms in pasteurized milk was affected by "identification and tracing", "washing, cleaning and disinfection", "toilets", "structural facilities" and "training". For example, for every unit increase in PRPs for washing, cleaning and disinfection, the total count of microorganisms decreased to 8689 CFU/ml ($P < 0.05$).

Pasteurized milk with high bacterial load (200000 CFU/ml) was observed among the samples. By examining the sources of raw milk supply of this unit, it was identified that there were drawbacks in the milking parlor so that when the cows entered the milking parlor, their teats were dirty; the udders were washed down but they were not dried out before being connected to the milking machine. This provides a moist environment full of germs and microbes that enter the milk tank. These agents can increase the total count of microorganisms, as confirms by the results of the present study.

Relationship between the scores of PRPs and coliform count of pasteurized milk: Some factors affecting the amount of coliforms in pasteurized milk

are presented in Table 4 according to the degree of their effect. "Processing section", "washing, cleaning and disinfection", "warehouse" and "sanitation" are important in controlling the amount of coliforms.

Coliforms are organisms that are able to ferment lactose in the presence of bile salts at 37°C. This group includes many sera of *E. coli*, as well as microorganisms such as *Citrobacter* and *Enterobacter* that do not ferment fecal sources (3). Lopez and Stamford (1997) investigated the contamination centers of raw and pasteurized milk and found out that 60% of the samples were contaminated due to improper cleaning of milk storage tanks and the inadequate temperature (12). The results also showed a significant inverse relationship between the number of coliforms in the product and the degree of implementation of the PRPs of washing and cleaning that after the "Inspection and testing" audit had the greatest impact on the number of coliforms in the product. It is to be mentioned that in a study carried out by Lopes and Stamford (1997), inspection and testing (GLP) were not included in the audits.

Auditing regarding hazard and coliform count in the products is significant. By increasing every auditing unit, coliform could reduce to 1.55 CFU/ml. Milk spoilage is caused by thermophile microbes and cross-contamination. Cross-contamination during milking and transportation, and other environmental factors cause *Listeria* contamination. Reduction of *Listeriosis* in the developed countries indicates microbial control procedure during production. The number of bacteria has been reduced by implementing HACCP, critical control points, and training public regarding food safety (13). Coliforms are destroyed by pasteurization so they are used as microbial indicators of pasteurization products. The auditing factor of production units has a reverse relation with coliform counts (14).

Relation of total scores of PRPs and the number of *E.coli* in pasteurized milk: *E.coli* existence indicates the possibility of pathogenic bacteria such as *Salmonella* and *Shigella* as well as other pathogenic organisms including viruses and parasites. According to the standards, there should be no *E.coli* in food and water.

Three samples of pasteurized milk were contaminated with *E. coli*; though the analysis was not statistically significant but their low levels in 468 samples were considerable. It seems that *E.coli* contamination of pasteurized milk above all is related to inadequate pasteurization process. Among the three samples contaminated with *E.coli*, two cases were

from the production units; according to the industry authorities, their pasteurization machines were not updated based on the standards. Although food infections caused by *E. coli* from pasteurized milk are rare, but in an outbreak in Scotland in 1994, one hundred people were infected by contamination of the transmitter tube from pasteurizer machine to the filling machine (17). Concerning the contamination of four samples of cream and three samples of the pasteurized milk with *E.coli*; however, it is statistically negligible, but the presence of these microorganisms in small production and non-automatic units (the same result as *Staph. aureus*) is considerable and needs further investigation.

Conclusion

According to the results of this study, approved PRPs assessment checklist has the ability to predict microbial parameters of pasteurized milk. So if the PRPs are better implemented and the score of production unit is higher, microbial indices will be significantly reduced. Regarding the relationship of checklist components and microbial indicators, it can be said the "training" audit has a reverse significant effect on all microbial indicators in pasteurized milk. Due to the high impact of this audit on microbial indicators, it is suggested that the audit points should be raised in the checklist. The audits "inspection and testing", "hazard identification and monitoring", and "washing, cleaning and sterilization" have a reverse significant effect ($P < 0.05$) on the microbial indicators of pasteurized milk. It is noteworthy that this checklist includes comprehensively the requirements of PRPs, HACCP and ISO 22000 program. More than 80% of the checklist score is in compliance with pre requisites, hence the title "checklist to evaluate a PRP for food and beverage production units" is appropriate but shouldn't be confused by universal acceptance of pre requisite definition. Since most of the microbial parameters were evaluated in this study, the audit "inspection and testing" received a relatively high score, indicating its importance in dairy product factories. While, despite its great effect in controlling and predicting the microbial indicators in some countries, this audit is not part of PRPs; it can be concluded that that the PRP checklist used in Iran is superior to that in other countries in this respect, but the only question is "how to name it and its discrepancy with international PRPs that, for instance, hazard identification and monitoring is not considered in them.

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References

1. Jaxsens L, Kussaga J, Luning PA, Van der Spiegel M, Devlieghere F, Uyttendaele M. A Microbial Assessment Scheme to measure microbial performance of Food Safety Management Systems. *International Journal of Food Microbiology*. 2009 8/31/;134(1–2):113-25.
2. Organization WH. Strategies for Implementing HACCP in Small and/or Less Developed Businesses: Report of a WHO Consultation in Collaboration with the Ministry of Health, Welfare and Sports, the Netherlands, the Hague, 16-19 June, 1999: Food Safety Programme, World Health Organization; 1999.
3. Jay JM, Loessner MJ, Golden DA. *Modern Food Microbiology* Modern Food Microbiology. 2005.
4. Iran IoSaIRo. Microbiology of milk and milk products: Specifications, 2nd. revision. Tehran, Iran: Institute of Standards and Industrial Research of Iran; 2008. p. 14.
5. Doménech E, Amorós J, Pérez-Gonzalvo M, Escriche I. Implementation and effectiveness of the HACCP and pre-requisites in food establishments. *Food Control*. 2011;22(8):1419-23.
6. Bylund G, Pak T. *Dairy processing handbook: Tetra Pak Processing Systems* AB Lund; 2003.
7. Iran IoSaIRo. Pasteurized and UHT cream: Specifications and test methods. Institute of Standards and Industrial Research of Iran; 2011.
8. Iran IoSaIRo. Doogh: Specifications and test method. Institute of Standards and Industrial Research of Iran; 2008.
9. Valeeva NI, Meuwissen MPM, Oude Lansink AGJM, Huirne RBM. Improving Food Safety within the Dairy Chain: An Application of Conjoint Analysis. *Journal of Dairy Science*. 2005 4;88(4):1601-12.
10. Whitley L. *Improving the Safety and Quality of Milk* (2010). Wiley Online Library; 2011. 43656000912104005999- 5895
11. Valeeva N, Meuwissen M, Lansink AO, Huirne R. Improving food safety within the dairy chain: an application of conjoint analysis. *Journal of Dairy Science*. 2005;88(4):1601-12.
12. Lopes A, Stamford T. Critical control points in the pasteurized milk processing fluxogram. *Archivoslatinoamericanos de nutricion*. 1997;47(4):367-71.
13. Mossel D, Pflug I. Occurrence, prevention, and monitoring of microbial quality loss of foods and dairy products. *Critical Reviews in Environmental Science and Technology*. 1975;5(1):1-139.
14. Alizadeh Behbahani B, TabatabaeiYazdi F, Shahidi F, Mohebbi M, Zanganeh H. Investigation of the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the Aqueous and Ethanolic Avicennia Marina Extracts on Gram Positive and Gram Negative Bacteria "in Vitro". *Sadra Medical Sciences Journal*. 2014;2(2 Apr.)
15. Opiyo BA, Wangoh J, Njage PMK. Microbiological performance of dairy processing plants is influenced by scale of production and the implemented food safety management system: a case study. *Journal of Food Protection®*. 2013;76(6):975-83.
16. Simsek B, Sagdic O, Ozcelik S. Survival of *Escherichia coli* O157: H7 during the storage of Ayran produced with different spices. *Journal of Food Engineering*. 2007;78(2):676-80.
17. Fernandes R. *Dairy products: microbiology handbook: Leatherhead Food*; 2009.
18. Bagheripoor Fallah N, Mortazavian Farsani, S. A. M., Hosseini, H. Shahraz, F., BahadoriMonfared A. Identification of microorganisms in industrial Iranian Doogh. *Iranian Journal of Nutrition Sciences & Food Technology*. 2014;13(57):18.
19. Jakobsen M, Narvhus J. Yeasts and their possible beneficial and negative effects on the quality of dairy products. *International Dairy Journal*. 1996 8;6(8–9):755-68.
20. Nada S, Ilija D, Igor T, Jelena M, Ruzica G. Implication of food safety measures on microbiological quality of raw and pasteurized milk. *Food Control*. 2012 6;25(2):728-31.