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ASSESSMENT OF THE MICROBIAL PARAMETERS ALONG THE PRODUCTION PHASES AT A DAIRY PLANT

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ABSTRACT

The main objective of this paper was to detect the prevalence of pathogenic bacteria (*Listeria monocytogenes, Campylobacter* spp*., Salmonella* spp., *Staphylococcus aureus* and *Escherichia coli*) in raw milk, to assess the hygiene parameters during the milk processing and to evaluate the safety of the final dairy products using standard ISO methods.

Staphylococcus aureus showed highest prevalence of the pathogen microorganisms (85%), followed by *Escherichia coli* (46%) and *Listeria monocytogenes* (9.8%) in bulk tank milk samples. *Campylobacter* spp. and *Salmonella* spp. were not detected in any of the tested samples.

The swab samples taken from employees and working surfaces, water samples and pasteurized milk samples detected inadequate sanitary procedures during the phase of milk processing. Analysis of the swabs from employees hands revealed 1 positive sample for enumeration for *Enterobacteriaceae* and 7 samples with higher total viable count that did not comply with the legislative, while the analysis of the swabs from working surfaces detected 5 samples positive for *Enterobacteriaceae* and 4 samples with higher total viable count that did not comply with the same legislative.

The analysis of the water samples detected 2 samples with higher total viable count on 22°C, 2 samples for total viable count on 37°C and 1 sample for coliform bacteria that did not comply with the national legislative. None of the samples showed presence of *Pseudomonas aeruginosa, Escherichia* coli, sulphite reducing clostridia and intestinal enterococci. The analysis of the pasteurized milk confirmed 3 samples with presence of *Enterobacteriacae*, 1 sample for coagulase-positive staphylococci and 2 samples with high total viable count at 30°C that did not comply with the national legislative.

The analysis of fermented milk products and cheese samples confirmed the need for immediate corective measures and subsequently, improved sanitation procedures. The testing detected 9 samples of fermented milk products for the *Enterobacteriacae* criteria and 1 sample for coagulase-positive staphylococci criteria that did not comply with the national legislative. The analysis of cheese identified 4 samples for *Escherichia coli* and 2 samples for coagulase-positive staphylococci that did not meet the criteria given in the national legislative.

The goal of the food safety system in the food production is to assure a safe and reliable product which will lead to consumer safety, satisfaction and future greater confidence in the company products.

Keywords: Staphylococcus aureus, Listeria monocytogenes, milk, dairy products, food safety

INTRODUCTION

Pathogenic bacteria in milk have been an issue of public health concern since the early days of the dairy industry. Many diseases such as tuberculosis, brucellosis, diphtheria, Scarlet fever, Q-fever and gastroenteritis are transmissible via milk and milk products (1). The potential threat of pathogenic bacteria has been minimized and the numbers of outbreaks involving milk and milk products have steadily declined mainly due to modern milk production practices which emphasize sanitary measures, improved udder health, herd inspections, proper cooling, careful handling and storage of raw milk, and almost universal use of pasteurization. However, a recent increase of well-publicized outbreaks of salmonellosis, listeriosis, campylobacteriosis, and yersiniosis has refocused the attention on milkborne

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pathogens, especially the newly emerging pathogens, such as *Listeria monocytogenes, Yersinia enterocolitica, Campylobacter jejuni, Salmonella* spp., *Staphylococcus aureus* and *Escherichia coli* (2-5).

Confronted with a recent food poisoning outbreaks involving contaminated milk and cheese the dairy industry has taken several initiatives to control pathogenic bacteria in milk and dairy products (6). Emphasis is placed on keeping pathogens out of the plant, eliminating them through proper processing, preventing postprocessing contamination and sanitation. Increased emphasis is focused on proper maintenance of eqipment, use of footbaths and control of the plant environment. The HACCP program, specifically designed to minimize the threat of pathogenic bacteria, is also being reemphasized (7).

Pathogenic bacteria have occurred in milk since the dairy industry began. Because it is impossible to prevent contamination by pathogens at all times, the dairy industry has to implement newer knowledge in an effective manner to minimize the threat of these pathogens. To successfully face the problem of emerging pathogens a mutual effort of the industry, academic and government institutions is needed (8).

The main objective of this paper was to detect the prevalence of pathogenic bacteria in raw milk, to determine the hygiene parameters during the milk processing and to evaluate the safety of the final dairy products.

MATERIAL AND METHODS

In this study the analysis were made at three phases of the dairy production: milk production phase, milk processing phase and the phase of finished dairy products (fermented dairy products and cheese)

The first phase of milk production in which 133 samples of bulk tank milk were collected and analysed for the presence of pathogen microorganisms (*Listeria monocytogenes, Campylobacter jejuni, Salmonella* spp., *Staphylococcus aureus* and *Escherichia coli*).

The second phase of milk processing at the dairy plant was assessed through collecting and analysing samples from the working surfaces and employees (60 swabs), analysis of the water used for processing (40 samples) and pasteurized milk (30 samples).

The third phase estimation consisted of analysis of finished dairy products. In this phase the analysis of the finished products consisted of analysis of fermented milk product (30 samples) and cheese (30 samples).

All of the analysed parameters according to the tested matrix and reference methods (9-20) used are given in Table 1.

Table 1. Overview of the parameters, matrices and methods used in the study

No.	Parameter	Matrix	Reference method
1	*Salmonella* spp.	raw milk	ISO 6579:2002
2	*Campylobacter* spp.	raw milk	ISO 10272-1:2006
3	*Listeria monocytogenes*	raw milk	ISO 11290-1:1996
4	*Escherichia coli*	raw milk, cheese	ISO 16649-3:2005
5	*Staphylococcus aureus*	raw milk, pasteurized milk, fermented dairy products, cheese	ISO 6888-1:1999
6	*Enterobacteriaceae*	swabs, pasteurized milk, fermented dairy products	ISO 21528-2:2004
7	Total viable count	swabs, pasteurized milk	ISO 4833:2003
8	Total viable count (22°C and 37°C)	water	ISO 6222:1999
9	*Pseudomonas aeruginosa*	water	ISO 16266:2006
10	Intestinal enterococci	water	ISO 7899-2:2000
11	Sulphite reducing clostridia	water	ISO 6461-2:1986
12	*Escherichia coli* and coliform bacteria	water	ISO 9308-1:2000

RESULTS AND DISCUSSION

In the first phase i.e. analysis of the bulk tank milk samples the testing showed no presence of *Campylobacter* spp. and *Salmonella* spp. In a similar study D'Amico *et al*. (21) published the following informations: *Listeria monocytogenes* showed prevalence of 2.3% and *Salmonella* spp. was not detected in any of the samples. In this study the highest prevalence of 73% was confirmed for *Staphylococcus aureus*.

The results from the microbiological analysis of the raw milk in this study are given in Table 2.

Table 2. Prevalance of the pathogen microorganisms in bulk tank milk

No.	Pathogen microorganism	Prevalence
1	*Salmonella* spp.	0%
2	*Campylobacter* spp.	0%
3	*Listeria monocytogenes*	9.8%
4	*Escherichia coli*	46%
5	*Staphylococcus aureus*	85%

The high prevalence of *Staphylococcus aureus* and *Escherichia coli* in bulk tank milk samples show overal lack of hygiene measures in the production of raw milk. This problem can be easily transmited to the next phase of milk processing in case of unsatisfactory sanitation procedures or inadequate pasteurisation.

The results from the next phase – the milk processing phase - present an information about the hygiene practices of the employees and the sanitation procedures of the dairy plant.

The results from the second phase of milk processing that included swabs from employees and working surfaces, water and pasteurized milk are presented in Table 3, Table 4 and Table 5.

Table 3. Swabs from employees and working surfaces that do not comply with the legislative

No.	Sample type	>1 cfu/cm^2 Enterobacteriacae	> 10 cfu/cm^2 Total viable count
1	Employee hand swab	1 (3.33%)	7 (23.3%)
2	Working surface swab	5 (16.6%)	4 (13.3%)

In this study the analysis of the swabs from employees hands revealed only 1 sample positive for *Enterobacteriaceae* and 7 samples for total viable count that did not comply with the legislative (EU Directive 471/2001), while the analysis of the swabs from working surfaces detected 5 samples for *Enterobacteriaceae* and 4 samples for total viable count that also not comply with the same legislative.

The results obtained from the water analysis were evaluated by the current legislative in Macedonia (Official Gazette of RM, No. 46/2008). This study detected a small number of samples that did not comply with the current legislative. None of the samples showed presence of *Pseudomonas aeruginosa, Escherichia* coli, sulphite reducing clostridia and intestinal enterococci.

Water is needed for many operations in the dairy: washing, indirect heating, cooling and for adjusting product composition (dillution). The goal is to make sure that all the water is clean, with good bacteriological quality. This will prevent contamination and deterioration in the quality of milk and milk products.

Table 4. Percentage share of samples of water used for processing that do not comply with the legislative

Analysis	Result
Total viable count 22°C	2 (5%)
Total viable count 37°C	2 (5%)
Coliform bacteria	1 (2.5%)
Pseudomonas aeruginosa	0%
Sulphite reducing clostridia	0%
Escherichia coli	0%
Intestinal enterococci	0%

Pasteurization is a process that kills harmful bacteria by heating milk to a specific temperature for a set period of time. Inadequate pasteurisation (low temperature, shorter time) can cause many problems in the next phases of production. The current legislative in Macedonia (Official Gazette of RM, No. 78/2008) was used for interpreting the results obtained from the analysis of pasteurized milk.

In a previous study in Republic of Macedonia, Prodanov *et al*. (22) detected 9.52% positive samples for *Enterobacteriacae*, 3.33% samples with presence of coagulase-positive staphylococci and 3.33% with high total viable count at 30°C.

Table 5. Pasturized milk that do not comply with the legislative

Analysis	Result
Enterobacteriaceae	3 (10%)
Coagulase-positive staphylococci	1 (3.3%)
Total viable count 30°C	2 (6.6%)

The results from the third phase of milk processing are most important because they show the hygiene and safety of the final products placed on the market.

During this study the analysis were made on fermented milk products (Table 6) and cheese samples (Table 7) and the results were evaluated with the national legislative (Official Gazette of RM, No. 78/2008). The testing detected smaller number of samples positive for *Enterobacteriacae* than the study taken by Prodanov *et al*. (23) where that percent was 35.3%. In the same study the authors confirmed that 0.82% of the samples were positive for coagulase-positive staphylococci.

Table 6. Results of the analysis of fermented milk products

Analysis	Comply	Do not comply
Enterobacteriaceae	21 (70%)	9 (30%)
Coagulase-positive staphylococci	29 (96.6%)	1 (3.33%)

The cheese samples were tested on two criteria: *Escherichia coli* and coagulase-positive staphylococci. The results for coagulase-positive staphylococci were in accordance with the previous study by Ratkova *et al*. (24) in which the analysis identified 5.9% of positive samples, while for the *Escherichia coli* criteria that study detected larger number (24.4%) of positive samples.

Table 7. Results of the analysis of cheese

Analysis	Comply	Do not comply
Escherichia coli	26 (86.6%)	4 (13.3%)
Coagulase-positive staphylococci	28 (93.3%)	2 (6.66%)

In this last phase of milk processing the analysis on finished dairy products (fermented milk products and cheese) detected a number of products that are not safe for consumption. This imply on a critical problem in this phase that need immediate action.

CONCLUSION

The goal of the food safety system in the dairy production is to assure a safe and reliable products which will lead to consumer safety, satisfaction and

future greater confidence in the company products. Therefore, every mistake in the earlier steps of the production will have great impact on the finished dairy products.

The results of this study indicate that pathogen microorganisms can be found in the bulk tank raw milk. Inappropriate handling of the raw milk can result in future bacterial growth and increase of the potential risk for contamination of the final products.

The inside surfaces of the dairy plant and the workers hygiene present another obstacle in the food safety system because of the inadequate sanitary measures taken. In this area the conclusion is that a corrective measures must be taken to assure the food safety of the dairy products.

Finally, the results from the analysis of the fermented dairy products and cheese emphasizes the need for stricter hygienic conditions during milking and processing.

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