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# EVALUATION OF QUALITY INDICATORS AND BIOFILM FORMATION TO DETERMINE MICROBIOLOGICAL SAFETY IN A MILK PROCESSING PLANT

*Avaliação dos indicadores de qualidade e da formação de biofilme para determinar a segurança microbiológica em um laticínio*

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**ABSTRACT:** The aim of this work was to verify the presence of microbiological indicators and the biofilm production capacity of the microorganisms found on the surfaces, in a milk processing plant, in order to estimate the hygienic sanitary conditions. For this purpose, a Good Manufacturing Practices (GMP) checklist and microbiological analysis were applied. Through to use of GMP checklist was possible to determine sites for sample collection and to establish the respective microbiological analyses. The presence of *Enterococcus* and high total bacterial counts (TBC) suggested the presence of biofilms on equipment, being also evidenced by the microplate technique. Although acceptable results were obtained for pasteurized milk, the indicators show that the risk was present in this dairy processing plant.

**Key words:** Good manufacturing practice, indicators of contamination, pasteurized milk, *Enterococcus*

**RESUMO:** O objetivo deste trabalho foi verificar a presença de indicadores microbiológicos e a capacidade de produção de biofilme dos microrganismos encontrados nas superfícies, em uma usina de processamento de leite, para estimar as condições higiênico sanitárias. Para esse fim, uma lista de verificação de Boas Práticas de Fabricação (BPF) e análise microbiológica foram aplicadas. Através do uso da lista de verificação das BPF foi possível determinar os locais para coleta de amostras e estabelecer as respectivas análises microbiológicas. A presença de *Enterococcus* e as contagens bacterianas totais (CBT) elevadas, sugeriram a presença de biofilmes nos equipamentos, sendo também evidenciada pela técnica de microplacas. Embora tenham sido obtidos resultados aceitáveis para o leite pasteurizado, os indicadores mostram que o risco estava presente nessa planta de processamento de laticínios.

**Palavras-chave:** Boas práticas de fabricação, indicadores de contaminação, leite pasteurizado, *Enterococcus*

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## INTRODUTION

The microbiological quality of the pasteurized milk depends on several factors, from the milking and storage of the raw milk, to the transportation to the dairy, the hygiene of the whole process and training of the manipulators, to the conditions of transport and storage of the finished product. This is necessary because of milk is considered an ideal culture media for different microorganisms due to its composition and, thus it is susceptible to contamination (SALVADOR et al, 2012).

In order to sell safe products, it is necessary to comply with certain microbiological standards established in each country. In Brazil, for pasteurized milk, Instruction normative N° 61 (BRASIL, 2019b) establishes the maximum detection limit for *Enterobactericeae*/mL (n = 5, m = 10). According to BRASIL (2018), which established the Technical Regulation on Milk Production, Identity and Quality (Instruction Normative 76), the microbiological standards is: *Enterobactericeae* (UFC/mL). A RDC n° 331 (BRASIL, 2019a) recommends that the microbiological standards established by this Resolution should be achieved by the application of Good Manufacturing Practices (GMP) and other quality control programs. The use of GMP to process food is mandatory worldwide and it is considered one of the most important tools for quality controlling of a process or product (TAVORALO; OLIVEIRA, 2006). They are based on procedures that take into account principles and rules, to define the correct handling of food. These recommendations range from caring for the raw material to the final product in order to achieve the identity and quality standard established for a particular product (SILVA JÚNIOR, 2007).

In Brazil, RDC N° 275 (BRASIL, 2002) established Standard Operating Procedures (POPs) to be applied to Food Producing Establishments and a Good Manufacturing Practices Checklist (GMP) for these establishments. After that, RDC N°. 216 (BRASIL, 2004b) established the Good Practices procedures for food services and made it mandatory to train the manipulators and work on improving health control actions, in order to protect the health of the consumer.

Microbiological indicators are very useful to evaluate the efficiency of GMP (DIAS et al.; 2012). The most widely used method is total bacterial count (TBC). However, it is not considered a safe indicator since it does not differentiate the microorganisms present in a sample nor does it determines the presence of pathogens or toxins (NASCENTES; ARAÚJO, 2012; OLIVEIRA, 2008; SALVADOR et al., 2012). The use of the coliform group as an indicator of hygienic and sanitary quality is well established, and high counts indicate that hygiene practices may have been neglected (OLIVEIRA, 2008; SALVADOR et al., 2012). The coliform group was the most common indicator used in dairy products, but the *Enterobactericeae* group has already been used in some countries as an indicator. In the case of dairy products, it is recommended to use these two standards for GMP and Good Hygiene Practices (GHP) (BAYLIS et al., 2011).

*Enterobactericeae* comprises a group of gram negative, glucose fermenting and thermolabile bacteria with deteriorating microorganisms and coliforms, as well as other enteric pathogens (HERVERT et al, 2016), such as *Salmonella*, *Shiguella* and *Escherichia coli*. The maximum counting standard established for this group in Europe for pasteurized milk is m = 10UFC / mL, according to ISO 21528-2 (BAYLIS et al., 2011).

*Staphylococcus* is naturally found in the skin, upper respiratory mucosa and intestines of humans, and are responsible for a considerable number of foodborne disease outbreaks (NASCENTES; & Araújo, 2012; OLIVEIRA, 2008). Because of this, staphylococcal contamination of food may occur through poor sanitation of handlers, or lack of care in handling practices, where the hands function as vectors of contamination. With this, there may be growth of the microorganism, under favorable conditions, with formation of enterotoxins that can result in gastroenteritis (HO, 2015).

*Enterococcus* has the ability to live in diverse environments such as vegetables, various foods, especially those of animal origin, as well as on surfaces of food processing plants. They are used as indicators of hygienic-sanitary contamination (GIRAFFA, 2002). Previous studies have shown the presence of *Enterococcus* in dairy cow feces (KAGKLI et al, 2007), which shows that this microorganism can reach milk through cross-contamination, although they are also considered natural milk organisms. However, due to its ability to grow in other environments, there is concern about its growth in processing plants (HARTMAN, 2001), because for the presence of *Enterococcus* may indicate that hygienic practices were inadequately performed, and usually forming biofilms on equipment and utensils (ROSADO, 2017).

Biofilm can be defined as communities of microorganisms attached to a surface, which produce exopolysaccharide substances that protect them against antimicrobial agents, increasing their ability to survive (CHAI CHU, 2008; GEORGE, 2005), with the possibility of fixing pathogenic microorganisms in this biofilm, with difficulty to remove them if the surfaces are not flat enough or adequately sanitized (SREY et al., 2013).

Therefore, the purpose of this work was to verify the presence of microbiological indicators and the biofilm production capacity of the microorganisms found on the surfaces, in a milk processing plant, in order to determine the hygienic sanitary conditions.

## MATERIAL AND METHODS

The present work investigated a milk processing plant, which processes 50 thousand liters per month of pasteurized milk, in the city of Virmond, Paraná state, Brazil, in September 2016. It was a small cooperative that collected milk from small farms. Firstly, a GMP checklist was applied (BRASIL, 2002) in order to have an overview of the facilities and to establish the sampling points for the microbiological analyses.

### Application and evaluation of the GMP checklist

The Good Manufacturing Practices checklist for food processing industry is part of the RDC N°. 275 (BRASIL, 2002). It is divided into five blocks, namely: (1) buildings and facilities; (2) equipment, furniture, and utensils; (3) food handlers; (4) food production and transportation; (5) documentation. Each block comprises several items. The possible answers for each item are "Yes", "No" or Not Applicable "NA".

The questionnaire was evaluated and as a result, the items were scored as: (4) Essential, (2) Necessary, (1) Advisable and (0) Not Accomplished. Each block was assigned a score (Eq. 1), where: BS = Block Score; Y = number of 'Yes' answers, K = maximum score of the block and NA = number 'Not Applicable' items.

$$\mathrm{BS} = \frac{\mathrm{Y}}{(K - \mathrm{NA})} \quad (1)$$

The percentage of 'Indispensable' items in each block (%I) (Eq. 2) and the 'Block Weight' (BW) (Eq. 3) were calculated, where: ΣI = Total of Indispensable items of the block; ΣTot = Total of items in the block; Σ% I = sum of %I of all blocks (TOMICH et al., 2005).

$$\%I = \left(\frac{\sum I}{\sum \mathrm{Tot}}\right) \times 100 \quad (2)$$

$$BW = \left(\frac{\%I}{\sum \%I}\right) \times 100 \quad (3)$$

The BS and BW values resulted in the Weighted Block Score (WBS). The sum of all WBS provided an Establishment Weighted Score (EWS). The relevance of each block in the final score was calculated by the percentage contribution of each block in relation to the Establishment Weighted Score (EWS). The score was considered of 96 to 100 as Excellent, of 89-95 as Very Good, of 76 to 88 as Good, of 41 to 75 as Regular and <41 as Unsatisfactory.

**Analysis of microbiological quality indicators analysis**

Three samplings were carried out, on different days (SANTANA et al. 2009). In our study, the methodologies proposed by the American Public Health Association (APHA) were adopted. The sampling sites and the microbiological analysis carried out were determined after the application of the GMP checklist.

Surfaces were sampled with sterile cotton swabs using a template of 100cm$^2$ or 25cm$^2$ and homogenized in peptone water supplemented with a 0.1% sodium thiosulphate 0.25% solution. Finally, 200mL of milk and 200mL of water were collected in sterile flasks also containing the sodium thiosulphate solution. All samples were transported to the laboratory in cool boxes containing ice packs, where they were analyzed, in no longer than 2 h.

The analyses performed for surfaces were: total bacterial count (TBC) by pour plate technique, on Plate Count Agar (PCA), and incubated at 37°C for 48h; *Enterococcus* by spread plate technique on Kanamycin Esculin Azide (KEA), and incubated at 37°C for 48h; *Enterobacteriaceae* by pour plate technique, in Violet Red Bile Glucose Agar (VRBG), and incubated at 35°C for 24h and Coliforms at 35°C and at 45°C, using Petrifilm EC$^{TM}$.

For the milk samples, TBC, *Enterobacteriaceae*, Coliforms at 35°C and at 45°C by Most Probable Number (MPN) method, and *Salmonella* analyses were performed by the AOAC method 2011.03 (AOAC, 2012). For the water, analyses of *Enterobacteriaceae* and Coliforms were carried out, following the same methodology adopted for milk.

The disinfectant activity of the cleaning products was tested by the suspension test, evaluating the number of decimal reductions of *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 25923) in contact with the sanitizer for 30s at 20°C. The efficiency was determined by a 5 or greater log reduction in bacterial counts (AOAC, 2000 with modifications).

Indoor air quality was analyzed for aerobic mesophilic bacteria by passive deposition on Nutrient Agar. Samples were collected by leaving a petri dish opened for 15min, approximately 1m above the floor and 1m away from the wall, and then they were incubated at 36ºC for 24h (PASQUARELLA, 2000).

In order to verify the biofilm formation potential of the microorganisms found on the surfaces, the microplate method was used (STEPANOVIC et al., 2007 with modifications), The inoculum from the surface swabs was grown overnight in Brain and Heart Infusion Broth (BHI), at 37°C, under aerobic conditions. Then, an aliquot was transferred, again, to fresh BHI broth, at 37°C for 24h, and after 200µL of growth was transferred to a sterile 96-well polystyrene microplate and incubated under the same conditions. After, the contents of the wells was discard and washed with 300 µL of sterile saline (pH 7,2), three times and heat-fixed at 60°C for 60min. The well was stained, at room temperature, with 150 µL of 2% Hucker crystal violet for 15min and aspirated with a pipette and washed by running water. The microplate was dry at room temperature and eluted from attached cells with 150 mL of 33% glacial acetic acid. The optical density $OD_{540}$ was measured using a microtiter-plater reader. The bacteria from places were classified as follows: OD sample ≤ OD control = Non biofilm producer; OD control < OD sample ≤ 2 x OD control = Weak biofilm producer, 2 x OD control < OD sample ≤ 4 x OD control = Moderate biofilm producer and 4 x OD control < OD sample = Strong biofilm producer. The tests were carried out in triplicate.

## RESULTS AND DISCUSSION

**Application of the GMP checklist**

The results obtained from the checklist based on the items analyzed (Table 1) and the classification of the GMP level that was being applied in the milk processing plant are shown on the Table 2.

According to the Weighted Score (EWS), the processing plant was classified as "Regular" (Tomich et al., 2005). To the Percentage Contribution Weighted Score (%EWS), the 'Food handlers' (27,13), 'Equipment, furniture, and utensils' (33,16) and 'Food production and transportation' (26,48) blocks influenced the most, since these were the ones that scored as 'Indispensable' more frequently, being 5, 11 and 12, respectively.

These results were similar to those found by SANTOS; HOFFMANN (2010) and DIAS et al. (2012), who evaluated a "Minas frescal" and ricotta cheese factory in São Paulo-Brasil, and a mozzarella cheese factory in Paraná-Brasil, respectively, and also classified the establishments as 'Regular'. Those studies also revealed the 'Food handlers' block as responsible for many non-conformities. The items classified as 'Indispensable' were submitted to microbiological analysis.

**Table 1** – Results from the Good Manufacturing Practices (GMP) checklist applied to the dairy industry (RDC Nº 275).

| Block | Items | Non Applicable Items | Non Conforming Items | Conforming Items | Indispensable Items |
|---|---|---|---|---|---|
| **Buildings and facilities** | 79 | 10 | 39 | 30 | 17 |
| **Equipment, furniture, utensils** | 21 | 0 | 14 | 7 | 11 |
| **Food handler** | 14 | 4 | 8 | 2 | 5 |
| **Food production and transportation** | 33 | 3 | 23 | 7 | 12 |
| **Documentation** | 17 | 7 | 3 | 7 | 1 |
| **Total** | **164** | **24** | **87** | **53** | **46** |

Source: The author. Analyses were performed with 5 replicates.

**Table 2** – Quantity assessment based on the checklist.

| Block | Block Score (BS) | Indispensable Items (%I) | Block Weight (WB) | Weighted Block Score (WBS) | Establishment Weighted Score (EWS) | Block Percentage Contribution (%EWS) |
|---|---|---|---|---|---|---|
| **Buildings and facilities** | 0.57 | 21.52 | 14.17 | 8.01 | 69.34 | 11.55 |
| **Equipment, furniture, utensils** | 0.67 | 52.38 | 34.49 | 23.00 | | 33.16 |
| **Food handler** | 0.80 | 35.71 | 23.52 | 18.81 | | 27.13 |
| **Food production and transportation** | 0.77 | 36.36 | 23.95 | 18.36 | | 26.48 |
| **Documentation** | 0.30 | 5.88 | 3.87 | 1.16 | | 1.68 |

*Source: The author. Analyses were performed with 5 replicates.*

**Analysis of microbiological quality indicators**

The Figure 1 presents the flow diagram with the sites for sample collection to microbiological analyses chosen to be performed at each site. Except for the disinfectant activity test, the results for the analyses of the first block, 'Equipment, furniture, and utensils' are presented Table 3. Total bacterial counts (TBC) and *Enterococcus* counts were present in the truck piping, in the bulk tank and in the pre-milk pasteurizer piping and truck tank may be due to contamination of the raw material, the mistaken concentration of detergents and sanitizers, inadequate hygiene procedures in equipment, and workers. The pasteurizer post-piping and storage tank showed lower counts, probably because they were cleaned before and after use, and because they were better protected being located inside the industry.

For coliforms at 35ºC and *Enterobacteriaceae* the highest counts were in the truck piping, in the pasteurizer pre-piping, in the truck tank and the bulk tank. In the pasteurizer post-piping and in the storage tank there was no counting. All tested samples were negative for coliforms at 45ºC, except for the truck tank. As the coliform counts at 35ºC were high, the processes for obtaining the raw material, cleaning the facilities and equipment must be improved.

Table 4 presents the results of the microbiological analysis of the milk. Raw milk samples were negative for coliform at 45°C and *Salmonella*. However, the presence of coliforms at 45°C in the truck tank suggests that they are adhered to the tank surface, probably as a result of previous cargoes and inefficient cleaning process.

Results for TBC in raw milk in the truck and in the bulk tank ($3.5x10^6$ CFU/mL) are considered high, however, after pasteurization, it decreased to $1.3x10^4$ CFU/mL and, since it resulted negative for coliforms at 35°C, at 45°C and *Salmonella*, it was therefore considered acceptable for consumption. Although, once more, it reveals the bad quality of raw milk. According to Commission Regulation 2073/2005 on microbiological criteria for foodstuffs and subsequent amendments (No. 1441/2007 and 365/2010) (BAYLIS et al., 2011), the allowed count for pasteurized milk is 10 UFC/mL for the group *Enterobactericeae*, so the milk would also be accepted according to international standards. The average count of *Enterobacteriaceae* in milk sample was $1.51x10^5$ CFU/mL. PICOLI et al., (2014), had similar results when evaluating raw milk samples from dairy farms in Rio Grande do Sul - Brazil ($3.96x10^5$ CFU/mL). *Enterobacteriaceae* count for raw milk was 3log higher than the coliform at 35ºC, this is an efficient indicator of poor sanitation or post-pasteurization contamination in dairy products.

**Figura 1.** Selected sites for sample collection and respective microbiological analyses. [1] TBC (Total Bacterial Count), [2] *Enterococcus*, [3] Coliforms at 35°C and at 45°C, [4] *Enterobactericeae*, [5] Biofilm, [6] *Staphylococcusaureus*, [7] *Salmonella*, [8] Mesophilic aerobes, * Suspension test (Standards: *Escherichia coli* and *Staphylococcus aureus*). Source: The autors.

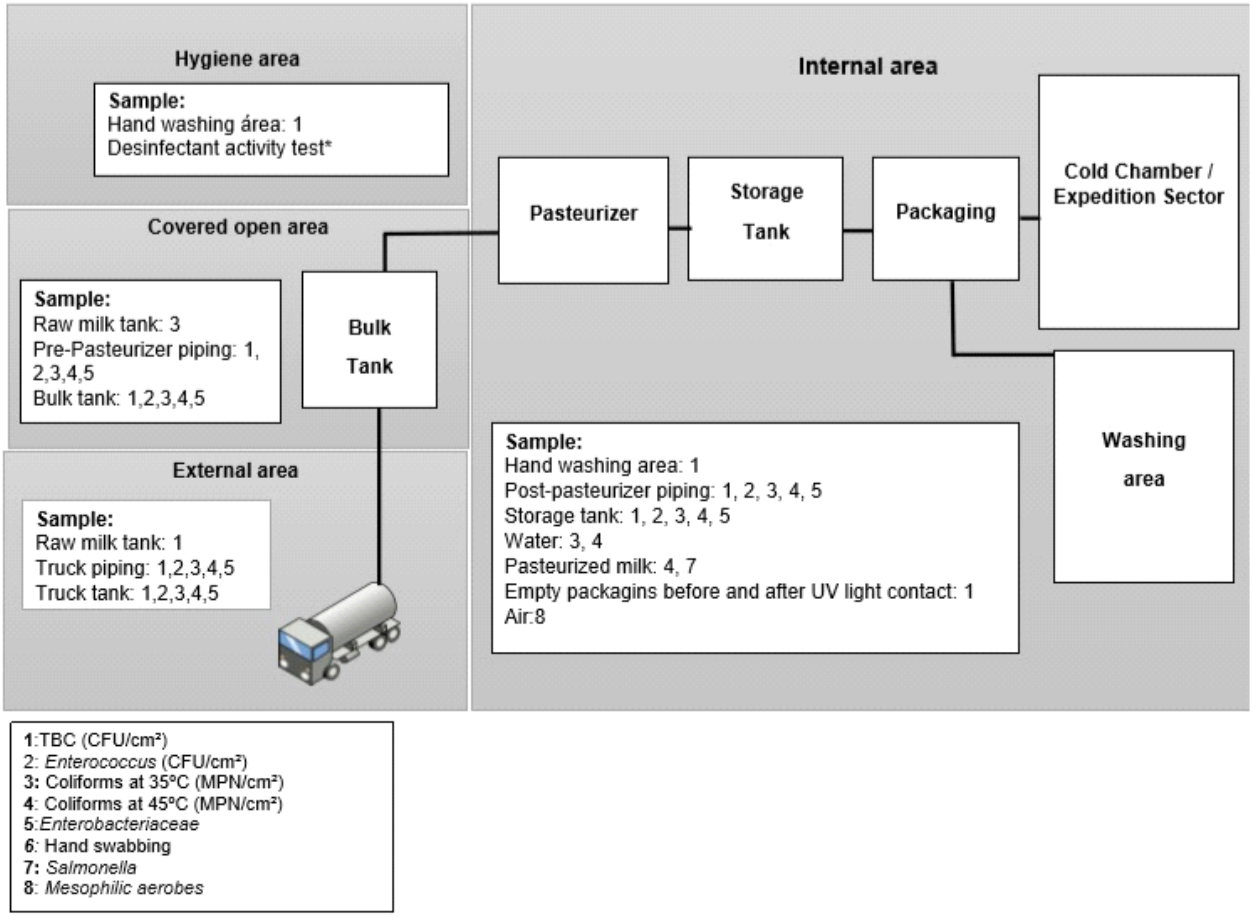


**Table 3** – Total bacterial count (TBC), *Enterococcus*, Coliforms at 35°C and 45°C and *Enterobacteriaceae* in different equipments in the dairy industry.

| Sample | Microorganisms | | | | |
|---|---|---|---|---|---|
| | **TBC (CFU/cm²)** | ***Enterococcus* (CFU/cm²)** | **Coliforms at 35ºC (MPN/cm²)** | **Coliforms at 45°C (MPN/cm²)** | ***Enterobacteriaceae* (CFU/cm²)** |
| **Truck tank** | $4.6x10^3$ | $8.0x10^1$ | $1.4x10^2$ | $3.33x10^1$ | $3.0x10^2$ |
| **Truck piping** | $6.5x10^4$ | $2.0x10^2$ | $1.9x10^3$ | <3.0 | $2.5x10^3$ |
| **Bulk tank** | $6.3x10^4$ | $8.0x10^2$ | $1.1x10^2$ | <3.0 | $6.3x10^1$ |
| **Pre-pasteurizer piping** | $3.9x10^4$ | $6.7x10^4$ | $6.3x10^2$ | <3.0 | $1.5x10^3$ |
| **Post-pasteurizer piping** | $4.5x10^1$ | $6.7x10^1$ | <3.0 | <3.0 | $1.7x10^0$ |
| **Storage tank** | $4.1x10^1$ | $2.6x10^0$ | <3.0 | <3.0 | $<10^1$ |

3 samples were collected and the analyses were performed with 2 replicates analyses to TBC, *Enterococcus* and *Enterobactericeae*, to Coliforms at 35°C and 45°C were performed with 3 replicates. Source: The authors.

**Table 4** - Total bacterial count (TBC), Coliforms at 35°C and 45°C, *Enterobacteriaceae* and *Salmonella* in raw milk from the truck and the bulk tank and pasteurized milk.

| Sample | Microorganisms | | | | |
|---|---|---|---|---|---|
| | **TBC (CFU/mL)** | **Coliforms at 35ºC (MPN/mL)** | **Coliforms at 45°C (MPN/mL)** | ***Enterobacteriaceae* (CFU/mL)** | ***Salmonella* (CFU/mL)** |
| **Raw milk (truck)** | $1.7x10^7$ | $8.9x10^2$ | <3.0 | $2.0x10^5$ | Ausent |
| **Raw milk (bulk tank)** | $3.5x10^5$ | $7.6x10^2$ | <3.0 | $1.1x10^5$ | Ausent |
| **Pasteurized milk** | $1.3x10^4$ | <3.0 | <3.0 | 0.00 | Ausent |

3 samples were collected and the analyses were performed with 2 replicates and the MPN with 3 replicate for milk and 3 replicates for water. Source: The authors.

It should be noted that in Europe, for raw milk, the standards *Enterobactericeae* and Coliformes at 45°C are used to evaluate the hygienic sanitary conditions of the product (BAYLIS et al., 2011). When evaluating the results, the *Enterobactericeae* group was found in all the samples evaluated (Table 4), and there were no counts for Coliforms at 45°C in any of them, being much more sensitive than the standard currently used in Brazil. This demonstrates the need to modify the standards used, since it is much more sensitive as an indicator of bacteria, including non-lactose fermenters (BAYLIS et al., 2011).

Neither the group *Enterobacteriaceae* nor *Enterococcus* is accomplished in any legislation in Brazil and therefore more studies should be carried out as to contribute to new official standards. The taxonomic family *Enterobacteriaceae* is an alternative group of indicators used widely, especially in Europe, in place of coliforms because they cover a broader range of hygiene indicators in the food industry (HERVERT et al., 2016).

Two concentrations of the sanitizer used by the company (Peracetyc EQ®) were tested: the one recommended by the manufacturer (0.3%) and the one normally used in the dairy industry (0.02%). Although not being the correct procedure and despite not following the manufacturer's recommendations, the assay proved the sanitizer was effective for both concentrations tested.

*Staphylococcus aureus* was isolated from the hands of both workers and results were $7.50x10^2$ and $5.00x10^3$ CFU/hand, which is considered high, since this pathogen is a public health issue worldwide, due to high incidence in foodborne disease (KADARIYA et al., 2014), as well as the transmission of virulence factors. The staff performed tasks in the production line, in the external area, in receiving the raw material and in the laboratory. This behavior is in disagreement with GMP, because of the risk of contamination of the production line. The training of the manipulators is highly recommended in order to reduce this problem.

Regarding air quality, results were $9.17x10^1$ CFU.cm$^{-2}$.week$^{-1}$ at the entrance, where handwashing was performed, and $2.25x10^2$ CFU.cm$^{-2}$.week$^{-1}$ in the production area. HICLEY et al. (1992) claim that the number of mesophilic aerobes should not exceed 30 CFU.cm$^{-2}$.week$^{-1}$. SALUSTIANO et al. (2003), also evaluated the air quality of the processing area of a dairy industry using the sedimentation technique and obtained a mean mesophilic aerobic count of 73.6 CFU.cm$^{-2}$.week$^{-1}$. in the milk pasteurization room. The results could be explained by the presence of handlers in the production area, involving them in the high air contamination, since each manipulator is able to spread between 20 and 70 microorganisms/min. Although being a crucial GMP, to regularly perform the air and environment disinfection (RADHA; NATH, 2014), it was not executed.

Hence APHA should be used as a reference and each establishment set its own quality parameters. The water results were 3.73 NMP.mL$^{-1}$ of coliforms at 35ºC and absence of coliforms at 45ºC and *Enterobacteriaceae*, in accordance with legal parameters (BRASIL, 2004a). This result was already expected since the dairy industry performed water chlorination and quality monitoring on a daily basis.

Although these results prove that the milk produced did not pose a risk to consumer health, since the microbiological condition was acceptable, it was possible to prove that the process was at risk. The dairy processing plant revealed a regular situation because there were risks and therefore corrective measures should be adopted.

**Biofilm formation potential assay**

The TBC (>$10^4$) showed possible biofilm formation in the truck piping, in the bulk tank, and in the pre pasteurizer piping (Table 3) (ANDRADE, 2008). Table 5 presents the biofilm formation analysis, where it can be seen that all surfaces had strong biofilm producing microorganisms. Even in sites that did not indicate the possible biofilm development according to the TBC (truck tank, post pasteurizer tubing, and storage tank), revealed potential to be strong biofilm producers. In addition, the fact that *Enterococcus* was found on all surfaces (Table 3) could be considered an indication of the possible presence of biofilms, due to its already known adhesion and aggregation capacity (PORTO et al., 2016).

*Enterococcus* is found in milk and milk products, as well as other types of food (ROSADO, 2017). Milk is one of the main reservoirs of this genus due to its presence in the environment, fecal material (DELPECH et al., 2013), contaminated water, milking equipment and receiving tanks (PORTO et al., 2016). So it is necessary to effectively clean the equipment because biofilms can become ten to thousand times more resistant to the effects of the sanitizers (GRISTINA et al., 1987). Therefore, the correct use of quality control tools is of fundamental importance to obtain safe processed foods.

**Table 5** – Capacity to form biofilms.

| Sample | Optical density (540nm) |
|---|---|
| **Truck tank** | 1,650 |
| **Truck piping** | 1,607 |
| **Bulk tank** | 1,520 |
| **Pre-pasteurizer piping** | 1,391 |
| **Post-pasteurizer piping** | 1,285 |
| **Storage tank** | 0,831 |
| **Control** | 0,200 |

Source: The authors.

**CONCLUSIONS**

It was concluded that the milk processing plant evaluated was at moderate risk, requiring training of employees and revision of GMP. In addition, it may be suggested that the application of the methodology of Tomichi et al. (2005) to determine the 'Indispensable' control points for microbial contamination indicator verification proved to be a satisfactory tool for classifying the condition of a milk processing plant. In addition, the use of *Enterobacteriaceae* as a quality indicator is a feasible and practical technique for replacing the traditional methodology used for coliforms in the verification of GMP and in addition to the coliform group at 45°C for GHP verification. The results showed that TBC and *Enterococcus* could be used as indicators of the presence of biofilm. The *Enterococcus* should be considered for use in

dairy products because of the risks involved in process safety. The investigation of the biofilm formation potential should be used to obtain more precise answers on the risk to the processing plant.

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