Microbiological Quality and Detection of Antibiotic Residue in Raw and Pasteurized Milk Consumed in the Reconcavo Area of the State of Bahia, Brazil

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Abstract

The aim of this work was to check the presence of total and thermo tolerant coliforms, mesophilous microorganisms, Escherichia coli, as also residue of antimicrobial agents in raw and pasteurized milk. For this purpose, an analysis was conducted on 50 samples of raw milk and 20 of pasteurized milk from 10 municipalities of the Recôncavo da Bahia, Brazil, through the establishment of the number of total coliforms, thermo tolerant and Escherichia coli using the multiple tube technique, and also the establishment of mesophile microorganisms by deep spreading. For the detection of antimicrobial residue, the Delvotest® kit was used. The counts of total coliforms found in raw milk varied between 2.42x10⁸ and 9.02x10⁶ NMP/mL. The mean values varied between 9.43x10⁶ and 9.02x10⁶ NMP/mL to thermo tolerant coliforms. With regard to Escherichia coli means varied between 1.52x10⁶ and 2.20x10⁹ NMP/mL, and for mesophilous microorganisms the means ranged from 7.65x10⁶ and 4.75x10⁹ CFU/mL. In pasteurized milk the counts of total coliforms found 4.16x10³ and 3.66x10⁹ NMP/mL. The mean varied between 4.16x10³ and 3.10x10¹⁰ NMP/mL to thermo tolerant coliforms. With regard to Escherichia coli means varied between <3 and 2.54x10⁶ NMP/mL, and for mesophilous microorganisms the means ranged from 4.59x10⁵ and 3.60x10⁹ CFU/mL. None of the samples presented any evidence of residue of antibiotics.

Keywords: Coliforms; Mesophiles; Antimicrobial; Raw milk; Microbiology

Introduction

Without any other specification, milk can be defined as the product resulting from the complete and continuous milking of healthy cows, well fed and given sufficient rest, under good conditions of hygiene [1].

Due to its high water content, a pH close to neutral and also a diversity of nutrients, milk has become a perfect medium for the growth of several types of microorganisms which could lead to the deterioration of the milk [2-4]. These microorganisms could come from the animal itself, from humans or from the utensils used in the milking room [4,5].

The presence and the multiplication of microorganisms cause changes in the quality of the milk, thereby limiting its durability and bringing harm to the economy and also to public health, which means that the milk needs to be subjected to heat treatment, to eliminate the contaminants before the milk gets to the consumer market.

In the light of the sanitary risks, which are part of the act of ingestion of the milk obtained which is processed under unsatisfactory conditions of hygiene during production, it becomes necessary to apply efficient heat treatment to destroy the microorganisms, which does not produce significant changes, such as the nutritional quality of the product, such as the degradation of fats, protein or carbohydrates as is the case with pasteurization [6].

Both raw milk and pasteurized milk are prone to contamination with residue of anti-microbial agents, through inadequate procedures of handling, especially in treatment of mastitis, and also through the intentional addition of antibiotics to improve the usable life of the product. The consumption of a product with this kind of residue may have a reflection on reactions such as urticaria, dermatitis, asthma and rhinitis, not to mention the fact that some pharmaceutical products, including nitrofuranes and chloramphenicol have a carcinogenic effect in laboratory animals, thus representing a potential risk. The fact is that even pasteurization does not eliminate the residue of antibiotics present in Serra [7], Nero et al. [8], and Villa et al. [9].

The microbiological analysis of the milk provides useful information that reflects the conditions under which this was obtained, processed and stored [10]. Through these, there is the supply of bacterium counts that serve as indicators of the general health of the herd, the sanitation measures in place at the farm, the handling of the milk, and also the storage temperatures [11].

The microbiological quality of raw milk has already been studied by several different authors and even so the consumption of this product is still a common occurrence, especially in the Recôncavo region of the...
State of Bahia [4]. The dairy activities in this region are represented mainly by small to medium rural dairy producers who have milk as one of their sources of income for survival. Many of them lack the technical assistance which could allow the implementation of techniques to improve the quality and quantity of their production of milk.

The purpose of this work was to analyze the presence of mesophile microorganisms, total and thermo tolerant coliforms, and also to detect residue of antimicrobials in raw milk and also Type C pasteurized milk in the Recôncavo region of the State of Bahia, Brazil.

Materials and Methods

Collection of samples

A total of 70 samples were analyzed, including raw and pasteurized milk from 10 municipalities in the Recôncavo region of the State of Bahia (Cabaceiras do Paraguacu, Cachoeira, Conceição do Almeida, Cruz das Almas, Dom Macedo Costa, Maragogipe, São Sebastião do Passé, Saubara, Santo Amaro, Santo Antônio de Jesus) between May 2010 and February 2011.

Microbiological analyses

The microbiological analyses were based on the methodology as recommended by the Brazilian Ministry for Agriculture, Fisheries and Supplies [12] to establish the most probable number (MPN) of total and thermo tolerant coliforms, *Escherichia coli* and also the count of mesophile microorganisms (CFU - Colony Forming Unit).

For the establishment of the MPN of total and thermo tolerant coliforms, three dilutions of each sample were submitted to presumptive and confirmative testing, using the multiple tube technique. In the presumptive test, the diluted samples were inoculated in tubes containing a Lauryl Sulphate Tryptose Broth (LSB), being incubated at a temperature of 36 ± 2°C, for 48 hours. From the tubes that turned out to be positive in LSB, then there was a confirmative test for thermo tolerant coliforms, with the transfer of an extract to tubes with a Brilliant Green-Lactose-Bile Broth at 2% and incubated at 36 ± 2°C for 48 h. To confirm the presence of thermo tolerant coliforms, with the transfer of an extract to tubes that turned out to be positive in LSB, then there was a confirmative test incubated at a temperature of 36 ± 2°C, for 48 hours. From the tubes presumptive and confirmative testing, using the multiple tube technique. In the presumptive test, the diluted samples were inoculated in tubes containing a Lauryl Sulphate Tryptose Broth (LSB), being incubated at a temperature of 36 ± 2°C, for 48 hours. From the tubes that turned out to be positive in LSB, then there was a confirmative test for thermo tolerant coliforms, with the transfer of an extract to tubes with a Brilliant Green-Lactose-Bile Broth at 2% and incubated at 36 ± 2°C for 48 h. To confirm the presence of thermo tolerant coliforms, with the transfer of an extract to tubes that turned out to be positive in LSB, then there was a confirmative test incubated at a temperature of 36 ± 2°C, for 48 hours. From the tubes presumptive and confirmative testing, using the multiple tube technique. In the presumptive test, the diluted samples were inoculated in tubes containing a Lauryl Sulphate Tryptose Broth (LSB), being incubated at a temperature of 36 ± 2°C, for 48 hours. From the tubes that turned out to be positive in LSB, then there was a confirmative test for thermo tolerant coliforms, with the transfer of an extract to tubes with a Brilliant Green-Lactose-Bile Broth at 2% and incubated at 36 ± 2°C for 48 h. To confirm the presence of thermo tolerant coliforms, with the transfer of an extract to tubes that turned out to be positive in LSB, then there was a confirmative test incubated at a temperature of 36 ± 2°C, for 48 hours. From the tubes presumptive and confirmative testing, using the multiple tube technique. In the presumptive test, the diluted samples were inoculated in tubes containing a Lauryl Sulphate Tryptose Broth (LSB), being incubated at a temperature of 36 ± 2°C, for 48 hours. From the tubes that turned out to be positive in LSB, then there was a confirmative test for thermo tolerant coliforms, with the transfer of an extract to tubes with a Brilliant Green-Lactose-Bile Broth at 2% and incubated at 36 ± 2°C for 48 h. To confirm the presence of thermo tolerant coliforms, with the transfer of an extract to tubes that turned out to be positive in LSB, then there was a confirmative test incubated at a temperature of 36 ± 2°C, for 48 hours. From the tubes presumptive and confirmative testing, using the multiple tube technique. In the presumptive test, the diluted samples were inoculated in tubes containing a Lauryl Sulphate Tryptose Broth (LSB), being incubated at a temperature of 36 ± 2°C, for 48 hours. From the tubes that turned out to be positive in LSB, then there was a confirmative test for thermo tolerant coliforms, with the transfer of an extract to tubes with a Brilliant Green-Lactose-Bile Broth at 2% and incubated at 36 ± 2°C for 48 h. To confirm the presence of thermo tolerant coliforms, with the transfer of an extract to tubes that turned out to be positive in LSB, then there was a confirmative test incubated at a temperature of 36 ± 2°C, for 48 hours.

In all the 20 samples of pasteurized milk, there was also the detection of contamination with total coliforms, thermo tolerant coliforms and mesophiles. The results obtained through microbiological analyses of the pasteurized milk can be seen in Table 2.

**Table 1: Arithmetical means of microbiological analyses in raw milk consumed in the Recôncavo Region, State of Bahia, Brazil, between May 2010 and February 2011.**

| Municipality                  | Total Coliforms NMP/mL | Thermotolerant Coliforms NMP/mL | *Escherichia coli* NMP/mL | Mesophiles UFC/mL |
|------------------------------|------------------------|---------------------------------|--------------------------|------------------|
| Cabaceiras do Paraguacu      | 3.02x10⁹               | 9.43x10⁸                        | 4.03x10⁹                 | 7.85x10⁸         |
| Cachoeira                    | 2.44x10¹⁰              | 2.42x10⁸                        | 1.23x10⁹                 | 2.31x10⁹         |
| Conceição do Almeida         | 7.52x10⁹               | 7.52x10⁹                        | 2.20x10¹⁶                | 3.14x10¹⁰        |
| Cruz das Almas               | 7.22x10⁹               | 2.75x10⁹                        | 1.60x10⁹                 | 4.26x10⁹         |
| Dom Macedo Costa             | 6.82x10⁹               | 5.04x10⁹                        | 4x10⁹                    | 6.07x10⁹         |
| Maragogipe                   | 4.78x10⁹               | 4.55x10⁹                        | 4.40x10⁹                 | 2.55x10⁹         |
| São Sebastião do Passé       | 6.99x10⁹               | 4.86x10⁹                        | 7.07x10⁹                 | 1.39x10⁹         |
| Saubara                      | 9.02x10⁹               | 9.02x10⁹                        | 2.90x10⁹                 | 4.11x10⁹         |
| Santo Amaro                  | 2.42x10⁹               | 2.42x10⁹                        | 2.20x10⁹                 | 3.33x10⁹         |
| Santo Antônio de Jesus       | 8.81x10⁹               | 8.24x10⁹                        | 1.52x10⁹                 | 4.75x10⁹         |
Moura et al. [19], analyzing samples of pasteurized milk in the city of Quixeramobim, State of Ceará, Brazil, obtained total coliform counts ranging from <3 to > 2.4x10³ NMP/mL, while the counts of thermo tolerant coliforms ranged from <3 to 1.1x10³ NMP/mL, while there was absence of *Escherichia coli*, this being a result different from that found in the pasteurized milk consumed in the Recôncavo region of the State of Bahia.

Comparing raw and pasteurized milk in all the municipalities analyzed (Table 3), there were no statistically significant differences between the average counts of total coliforms, *Escherichia coli* and mesophiles (p > 0.05). In the case of thermo tolerant coliform counts, there were statistically significant differences (p < 0.05), with the raw milk showing greater contamination with this microorganism when compared with pasteurized milk (Table 4).

In Normative Instruction (IN) No. 51 of the Brazilian Ministry for Agriculture, Fisheries and Supplies – MAPA [1], at no moment is there permission for the sale of raw milk directly to the consumer, which means that the sale of this type of milk is prohibited, mainly because it can transmit several different zoo noses and also be responsible for the fearful cases of food poisoning. The same Resolution currently means that the sale of this type of milk is prohibited, mainly because this bacterium suggests fecal contamination, its presence, especially in raw milk, shows precarious conditions of hygiene and sanitation in the process of obtaining such milk, as this micro-organism is the only member of the coliform group that comes exclusively from faeces, meaning that this product should be declared as unfit for consumption [22].

The arithmetic means of total and thermo tolerant coliforms, mesophiles and *Escherichia coli* at high levels as found in pasteurized milk suggest that the process has been inadequately performed, with faults in either the time or the temperature of pasteurization, or possibly some contamination after the process, with faults in packaging, this because the mesophile bacteria and coliforms are easily destroyed by the pasteurization temperature [23].

Data from other countries also suggest contamination of the milk. In the region around Bogotá, in Colombia, the mesophile counts vary between 10⁵ and 88x10⁷ CFU/mL and coliforms between 10⁵ and 61x10⁵ NMP/mL, values which are also high [24]. In Pakistan, the counts of *Escherichia coli* varied from 7.1x10⁹ to 12.6x10⁹ CFU/mL and the mesophile count ranged from 2.1x10⁶ to 6.1x10⁹ CFU/mL [25]. In India, respectively 88.3% and 70% of 60 samples were contaminated by total and thermo tolerant coliforms [26]. These are countries with educational and social aspects similar to those of Brazil and particularly the region we have studied.

Concerning the residue of antimicrobial agents, out of the 20

| Municipality               | Total Coliforms NMP/mL | Thermotolerant Coliforms NMP/mL | *Escherichia coli* NMP/mL | Mesophiles UFC/mL |
|----------------------------|------------------------|--------------------------------|--------------------------|------------------|
| Cruz das Almas             | 3.66x10¹¹              | 3.10x10⁸                      | 2.54x10⁸                 | 3.60x10⁹         |
| São Sebastião do Passé     | 4.45x10⁹              | 4.45x10⁹                     | 2.24x10⁸                 | 2.46x10⁹         |
| Saubara                    | 4.16x10⁷              | 4.16x10⁷                     | <3                       | 4.59x10⁷         |
| Santo Amaro                | 8.82x10⁸              | 5.32x10⁸                     | 2.27x10⁸                 | 2.42x10⁹         |
| Santo Antônio de Jesus     | 2.48x10⁹              | 2.34x10⁷                     | 7.2x10⁷                  | 7.32x10⁹         |

Table 2: Arithmetic means of microbiological analyses in pasteurized milk consumed in the Recôncavo Region, State of Bahia, Brazil, between May 2010 and February 2011.

| Milk            | Total Coliforms NMP/mL | Thermo tolerant Coliforms NMP/mL | *Escherichia coli* NMP/mL | Mesophiles UFC/mL |
|-----------------|------------------------|--------------------------------|--------------------------|------------------|
| Raw             | N                      | Mean 5.41x10¹⁰ a               | Raw                      | 3.48x10¹⁰ a      |
| Pasteurized     | 20                     | 5.53x10¹⁰ a                   | Pasteurized              | 7.16x10⁹ b       |

Table 3: Arithmetic means of total coliforms and thermo tolerant coliforms comparing raw and pasteurized milk in all the municipalities of the Recôncavo region, State of Bahia, Brazil.

| Milk            | *Escherichia coli* NMP/mL | Mesophiles UFC/mL |
|-----------------|--------------------------|------------------|
| Raw             | N                        | Mean             |
| Pasteurized     | 20                       | 1.50x10⁺ a       |

Table 4: Mean counts of *Escherichia coli* and mesophiles comparing raw and pasteurized milk, in all municipalities of the Recôncavo Region, State of Bahia, Brazil.
samples of pasteurized milk and 50 of raw milk that have been analyzed, none showed any residue of antimicrobial agents. These results are indeed satisfactory but surveillance through testing, especially in the case of pasteurized milk, must be constant, so that the population is protected from the exposure to the effects of this residue, which could range from allergies to the selection of resistant strains.

The results of this work were similar to those found by Mendes et al. [27] in Mossoró, State of Rio Grande do Norte, which also did not detect residue of antibiotics in 32 samples of raw milk, using the same Devolvest® kit. Souza [28] did not detect the presence of antibiotic residue either, in milk samples obtained in Sacramento, State of Minas Gerais, Brazil, this being the same result as that found by Mendes et al. [29] studying samples of raw milk in the region around Muriaé, Minas Gerais.

In Uberlândia, Almeida et al. [30] analyzed 158 samples of milk for the presence of antibiotics and found an occurrence rate of 1.89% of samples with residues of beta lactamics.

In the city of Patos, State of Paraíba, Medeiros et al. [31] analyzed 30 samples of raw milk and found a positivity rate of 43% for the presence of antibiotics, while Tetzner et al. [32] found 33.3% of positivity in samples from the Triangle Region of the State of Minas Gerais, Brazil.

For organic milk from the countryside of São Paulo state, in 2.7% of samples there was confirmed presence of antimicrobial residue, which shows the incorrect use of antibiotics in systems for the production of organic products [33].

Conclusions

Based on the microbiological analyses carried out in this work assignment, we can say that the raw and pasteurized milk as consumed in the cities studied shows very poor conditions of sanitation and hygiene, and this is proved by the high presence of micro-organisms of the total coliform group, as also of thermo tolerant coliforms and Escherichia coli showing that the milk is not appropriate for human consumption.

We have not found any samples with residue of antibiotics during the period of research, which is a satisfactory result, but there is a need for constant surveillance through regular checks, to make sure that the population of the Recôncavo area of the State of Bahia is not exposed to the harmful effects on health caused by the consumption of milk contaminated with residue of anti-microbial agents.

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