Study of *Staphylococcus aureus* in raw and pasteurized milk consumed in the Reconcavo area of the State of Bahia, Brazil

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Abstract

This study is to verify the presence of *Staphylococcus aureus* in milk consumed in Reconcavo Baiano, Brazil for which 50 samples of raw milk and 20 samples of pasteurized milk were analyzed from 10 municipalities of this region (Cabeceiras do Paraguaçu, Cachoeira, Conceição do Almeida, Cruz das Almas, Dom Macedo Costa, Maragogipe, São Sebastião do Passé, Saubara, Santo Amaro and Santo Antônio de Jesus). The *Staphylococcus aureus* was isolated in Baird Parker agar where typical and atypical colonies were selected and submitted to coagulase and complementary tests. Out of 50 samples of raw milk studied, 34 showed contamination by *Staphylococcus aureus*, corresponding to 68% of the samples being contaminated. In the pasteurized milk, 6 samples were contaminated with this microorganism, corresponding to 30% of the 20 samples. The presence of this pathogenic microorganism indicates a potential health hazard to those who consume milk from this region.

Keywords: Public health; Mastitis; Contamination

Introduction

The presence of *Staphylococcus aureus* in raw milk generally comes from cows with mastitis, from handlers or from deficient hygiene. When found in milk, high levels of contamination can be reached quickly under favorable conditions. Its presence in foods can be a risk to human health, causing a public health problem, as these bacteria produce toxins that can cause toxic food infections [1].

The capacity to coagulate plasma, the principal characteristic of the *Staphylococcus aureus*, is highly correlated to the capacity to produce enterotoxins harmful to the tissues of the contaminated host [2].

The presence of *Staphylococcus aureus* shows up unsanitary conditions in the cattle herd and counts above 10³ UFC in milk increase the risk of staphylococcal toxin production more resistant to the heat processes of pasteurization [3]. Normally the production of enterotoxin is found at temperatures of 40°C to 45°C, although Smith et al. [4] detected production of toxins at temperatures of 10°C to 46°C.

Various conditions favor the growth of *Staphylococcus aureus* and the production of enterotoxins such as the temperature, activity of water, concentrations of salts and pH, and even the competitiveness of the micro-flora [5].

Milk can be contaminated by *Staphylococcus aureus* when there is infection of the mammary gland or by bad hygiene habits, such as coughing or sneezing and not washing hands when handling milk storage equipment, during or after milking, and in this case, human activity is responsible for the contamination, as this bacteria colonizes the nasal pathways in human beings [2,6].

For Jay [7] the micro biota found on the hands and on the uniform of food handlers, especially of milk, is a reflection of hygiene habits as the single most important factor in the contamination of milk. As food contamination by human hands cannot be completely controlled, suitable refrigeration at temperatures below 5°C is one of the ways of preventing *Staphylococcus aureus* contamination and consequently the formation of staphylococcal toxin [3].

The production of good quality milk is a challenge that can be overcome provided that basic care is taken at the source of production [8]. Education of handlers and principally of consumers on the dangers of consuming bad quality raw and pasteurized milk is needed.

The population of Reconvavo da Bahia, Brazil does not have this level of awareness on the dangers of consuming low quality products, as little is invested in basic education, and a large part of the population sticks to rural habits such as the consumption of raw milk. On the rural properties in this region, cattle raising is predominantly traditional, still using techniques that are very rudimentary in their way of farming and of milking.

The importance of microorganisms in the milk means that their microbial contamination index can be used to judge the quality, as well as the sanitary conditions of its production and the health of the herd [9].

Microbiological analyses of foods are important to maintain the population informed as to the sanitary level, principally of milk, preventing *Staphylococcus aureus* contamination and consequently the formation of staphylococcal toxin [3].

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as well as shedding light on public health problems arising from the consumption of products of animal origin without any prior health inspection [10].

Thus, this study is to verify the presence of Staphylococcus aureus in raw and pasteurized milk consumed in Reconcavo da Bahia, Brazil.

Material and Methods

Collection of samples

Fifty samples of raw milk and 20 of type C pasteurized milk were collected from 10 municipalities of the Reconcavo in Bahia (Cachoeira, Conceição do Almeida, Cruz das Almas, Dom Macedo Costa, Maragogipe, São Sebastião do Passe, Saubara, Santo Antônio de Jesus). The milk was obtained from points of sale and was bought without supplying any information that it was for research purposes, so as not to influence the result of the experiment. The samples collected were transferred to sterile recipients and transported in an isothermal box to the Animal Microbiology and Parasitology Laboratory of the Federal University of Reconcavo da Bahia, where the research into Staphylococcus aureus was carried out.

Staphylococcus aureus research

From 1 mL of each sample of raw milk, series of dilutions were prepared (10⁻¹ to 10⁻⁸) of raw milk and (10⁻² to 10⁻⁶) of pasteurized milk, using a diluting 9 mL of saline peptone solution at 0.1%. Then 0.1 mL of the 10⁻¹, 10⁻², 10⁻³ dilutions were inoculated on the surface of Baird-Parker agar and spread with a Drigalski spatula. The incubation was done at a temperature of 35°C ±2°C for 30 to 48 hours. Five typical colonies were selected (shiny black with an opaque ring, surrounded by a clear halo) for seeding in tubes containing BHI and the incubation was done at a temperature of 35°C ±2°C for 24 hours [11].

Coagulase test

From each tube cultivation in BHI, 0.3 mL was transferred to sterile tubes containing 0.5 mL of rabbit plasma. The incubation was done at a temperature of 35°C ±2°C for 6 hours. The presence of coagulates was verified, taking into consideration the following criteria:

- Reaction 1+: small disorganized coagulation.
- Reaction 2+: small organized coagulation.
- Reaction 3+: large organized coagulation.
- Reaction 4+: coagulation of all the contents of the tube which does not come unstuck when inverted.

The presence of Staphylococcus aureus was confirmed when the coagulation reaction was of the type 3+ and 4+ [11].

Statistical analysis

The SAS statistical program was used for the statistical analysis of variance, and the test Tukey to the level of 5% probability was used to compare the averages [12].

Results and Discussion

Out of 50 samples of raw milk studied, 34 of them were contaminated by Staphylococcus aureus, corresponding to 68 % of samples contaminated, with averages varying between 6.3x10² to 2.8x10⁸ UFC/mL, as shown in Table 1.

Out of the 34 contaminated samples of raw milk, 6 had levels of Staphylococcus aureus corresponding to 10⁷ UFC/mL; 14 had levels of 10⁶ UFC/mL; 7 counts of 10⁵ UFC/mL; 6 samples of 10⁴ UFC/mL and 1 sample of 10³ UFC/mL. These values can be seen in Figure 1.

The Brazilian legislation in effect, IN 51 and RDC no.12 [13], sets no maximum or minimum parameters for detection of Staphylococcus aureus. Counts of Staphylococcus aureus above 10⁷ increase the probability of production of staphylococcal toxins that are resistant to boiling carried out in the homes when buying raw milk, and to the pasteurization processes [14]. Considering that most of the samples had numbers of bacteria above 10⁷ UFC/mL, the milk consumed in Recôncavo is a serious risk to the health of the population.

Chapaval et al. [15] found production of staphylococcal enterotoxins in milk when stored at temperatures of 37°C to 42°C or when exposed to variations in temperature. Considering that the average temperatures recorded in Recôncavo in the summer are around 37°C and that the in natura milk is often sold at ambient temperature, the probability of production of staphylococcal enterotoxin is likely.

Pasteurized milk may also be predisposed to the likely production of toxin cited by Chapaval et al. [15] because in various situations, the shop owners switch off the chillers at night to save electricity, leaving the product exposed to variations in temperature.

The presence of Staphylococcus aureus also shows up deficient sanitary conditions of the cattle herd given that Staphylococcus aureus is predominant as the cause to most cases of mastitis.

The in natura milk results from Recôncavo are similar to those of Freitas et al. [16], when raw milk was analyzed in Belem, finding that 71.43% of the samples were contaminated with Staphylococcus aureus, varying from < 1x10⁹ to 1.25x10⁸ UFC/mL [1].

Badini et al. [17], in samples of in natura milk in the municipalities of Botucatu and São Manuel detected that 50% of the samples were contaminated with Staphylococcus aureus. Stamford et al. [18] found that 77% of milk sold in the state of Pernambuco had enterotoxigenic Staphylococcus.

Quintana et al. [1], analyzing raw milk in Morrinhos, in the state of Goias, found that 28.5% of the samples had values of S. aureus above 10⁶ UFC/mL. Santana et al. [19] found 18.8% of positivity with numbers above 10⁵ UFC/mL in 101 samples from Londrina and Pelotas.

Of the pasteurized milk, 6 samples showed contamination with this microorganism, corresponding to 30% out of 20 samples. These data...
are similar to those of Freitas et al. [16], who found 30.5% positivity in 23 samples investigated, with counts varying from $<1 \times 10^4$ UFC/mL to uncountable.

Of the 6 contaminated samples of pasteurized milk, 3 had Staphylococcus aureus counts corresponding to $10^3$ UFC/mL; 2 with $10^2$ UFC/mL and 1 sample $10^4$ UFC/mL (Figure 2).

The averages of Staphylococcus aureus from the pasteurized milk varied between $3 \times 10^2$ to $1.4 \times 10^4$ UFC/mL, as shown in Table 2.

Statistically there were no differences between the averages comparing the raw milk and the pasteurized milk, and also between the averages of the municipalities ($p>0.05$). These averages can be seen in Table 3.

The high counts of Staphylococcus aureus in raw milk are even predictable in being a product of unknown origin where one would presume there is no care for the health of the herd, and the cases of subclinical mastitis are not detected or adequately treated. Nevertheless, high counts in pasteurized milk are unacceptable, showing inadequate hygiene habits of operators during processing of the milk, as Staphylococcus aureus are found in the nasal cavity of human beings.

Unlike the data found in this study, Leite et al. [20] did not detect Staphylococcus aureus in the samples analyzed of pasteurized milk in Salvador. Whereas for organic milk from the interior of São Paulo, Ribeiro et al. [21] found that 25.7% of the samples were contaminated with Staphylococcus aureus out of 148 samples.

In most cases, contamination by Staphylococcus aureus in milk is due to animals with mastitis. Studies conducted at the experimental station in Nova Odesa, São Paulo, by Zafolon et al. [22], showed that the prevalence of Staphylococcus aureus was higher by up to 54.4% in rainy periods, and these data can help producers take preventive measures in their handling during these periods.

In 208 samples of milk from cows with mastitis, Fagundes et al. [23] isolated 6.7% of Staphylococcus aureus and 14.3% producers of enterotoxin.

Contamination in milk was detected in other countries, with similar results as the study in question. In Palestine, 48 (36.9%) of samples were positive for Staphylococcus aureus from the total of 130 samples [25]. Farhan and Salk [25] found counts of Staphylococcus aureus varying between $7.1 \times 10^5$ to $12.6 \times 10^5$ UFC/mL in raw milk sold in the region of Lahore, Pakistan, which are significant numbers likely to lead to production of enterotoxin. In Turkey, Ekici et al. [26] found 18.18% of samples contaminated from the total of 66 samples. In the north of Morocco, Bendahon et al. [27] isolated 40% of Staphylococcus aureus in raw milk from 27 samples, and in India, 61.7% positivity was detected in 60 samples of raw milk researched [28].

Park et al. [29] analyzed 30,019 samples of raw milk in Korea and detected 104 samples contaminated with Staphylococcus aureus.
In Norway, Jorgensen et al. [5] isolated 11 positive samples for the producer gene of enterotoxin. D’Amico et al. [30] found 29% of positivity for Staphylococcus aureus in cow’s milk in Vermont, in the United States.

Control measures during milking must be taken to prevent the dissemination of Staphylococcus aureus in the herd as the bacteria have been found in other places besides the animal itself, such as: the feed, the churns for storing the milk and in humans too [31]. Souza [32], studying points of isolation of these bacteria on one of the properties in Sacramento in Minas Gerais found that, 2.7% of Staphylococcus aureus were isolated on the hands of the person doing the milking and in 6.6% of them; the contamination was in the churns.

The mammary gland is the principal place of infection of Staphylococcus aureus when the animal has mastitis, but studies carried out by Capurro et al. [33] identified the skin of the shanks as being a significant reservoir, due to its anatomical position, and other places such as the muzzle, groin and wounds, reinforcing the care to be taken during milking so as not to disseminate the bacteria to other animals, compromising the quality of the milk.

The work of health inspection in municipalities is crucial to raise consumer awareness on the consumption of good-quality milk through educational programs and the inspection of establishments that insist on selling raw milk. Dairy inspection and the adoption of APPCC principles and good fabrication practices can also contribute to a reduction in contamination of pasteurized milk.

Conclusions

The counts of Staphylococcus aureus in milk analyzed indicate the product is improper for human consumption, and the population of Reconcavo in Bahia is vulnerable to toxic infections.

The presence of Staphylococcus aureus in raw and pasteurized milk undermines the importance of preventive measures to ensure the quality of the milk from milking through to the processing in the dairy industry, in order to avoid the occurrence of pathogenic microorganisms and consequently to prevent risks of transport of toxic infections by the consumption of contaminated milk.

Given this imminent risk, in their turn, the competent authorities should adopt severe inspection measures in order to prohibit the informal sale of milk.

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