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Listeria monocytogenes, *Coxiella burnetii,* and *Bacillus cereus* in fresh cheese illegally marketed without inspection

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Abstract

This study aimed to assess hygienic-sanitary indicators and the presence of pathogenic agents in fresh cheeses produced through informal methods. A total of 51 samples of fresh cheese, which were produced without sanitary inspection and sold informally, were analyzed for the presence of *Listeria monocytogenes*, *Salmonella* spp., *Bacillus cereus*, coagulase-positive *Staphylococcus*, *Escherichia coli*, *Pseudomonas* spp., *Mycobacterium bovis*, *Mycobacterium tuberculosis*, and *Coxiella burnetii* using microbiological and biomolecular analyses. The results revealed the detection of *Listeria monocytogenes* serotype 4b in one sample (1.96%), *Bacillus cereus* in two samples (3.92%), and *Coxiella burnetii* in four samples (7.84%). Furthermore, 28 samples (54.9%) exceeded the allowable limit for *Escherichia coli* counts as per legislation, and 23 samples (45.09%) had coagulase-positive *Staphylococcus* counts above the microbiological limit specified by Normative Instruction (NI) No. 161. The prevalence of noncompliant samples, as indicated by the legislation, is a disconcerting finding that highlights the potential risks to public health associated with the sale of these cheeses. Consequently, the implementation of more stringent monitoring measures regarding the informal trade of fresh cheeses becomes imperative to mitigate the potential contribution to foodborne illness outbreaks.

Keywords: dairy products; foodborne diseases; milk; microbiology; polymerase chain reaction.

Practical Application: Pathogen detection in informal fresh cheeses reveals significant public health risks.

1 INTRODUCTION

In Brazil, approximately 70% of cheese production is attributed to family farming. However, a considerable number of these operations engage in informal trade practices, operating without proper registration and adherence to hygiene and sanitation standards (Vinha et al., 2016). The choice to engage in informal production and trade is often driven by commercial strategies pursued by rural producers seeking greater autonomy and improved financial returns. Nevertheless, many of these farms lack the necessary infrastructure and conditions for appropriate handling and storage of products, compromising the safety of the final goods and posing potential risks to public health (Amaral et al., 2020; Carmo Amorim et al., 2014; Kamimura et al., 2019; Silva et al., 2015; Vinha et al., 2016). The absence of regulation and sanitary control amplifies the likelihood of biological hazards within the cheese production chain, transforming it into a potential reservoir of pathogenic microorganisms that can be transmitted through the final product.

During the acquisition of raw materials, the contamination of milk with *Staphylococcus* spp., originating from bovine mastitis, can occur (Argudín et al., 2010; Le Loir et al., 2003). Additionally, the utilization of raw milk, a common practice in informal cheese production, poses a risk for the transmission of pathogens like *Coxiella burnetii* to humans (Mioni et al., 2020; Rozental et al., 2020). Furthermore, inadequate hygiene conditions throughout the cheese production process can ease contamination by microorganisms from the environment and handlers, such as *Escherichia coli* and *Staphylococcus aureus*, or through cross-contamination involving equipment, utensils, or other food items, including *Bacillus cereus* and *Listeria monocytogenes* (Barancelli et al., 2011; Camargo et al., 2021; Maziero & dos Santos Bersot, 2011).

In order to mitigate these risks, initiatives such as the National Plan for the Control and Eradication of Brucellosis and Tuberculosis (PNCEBT) have been implemented to monitor livestock health and enhance the sanitary standards of milk

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production. Likewise, the Ministry of Agriculture, Cattle and Supplying (MAPA) and the Brazilian Health Regulatory Agency (ANVISA) have established regulations and standards that stipulate microbiological parameters, conduct inspections of food production, and regulate good manufacturing practices, storage, and sale of animal-derived products. These measures contribute to the production of safe goods while ensuring compliance with identity and quality standards (Brasil, 2019, 2022).

The informal trade of animal origin products continues to pose a significant challenge for public health services, as emphasized by previous research (Amaral et al., 2020). Furthermore, factors such as limited awareness regarding the inherent risks associated with consuming these products, cultural influences, and attractive pricing compared to industrialized alternatives contribute to the persistent growth of the informal trade of fresh cheeses (Carmo Amorim et al., 2014).

Given the highlighted impact on public health, the primary objective of this study was to assess the sanitary quality of fresh cheeses produced without sanitary inspection and obtained from the informal trade within the region of Botucatu, São Paulo state.

2 MATERIALS AND METHODS

2.1 Sampling

Between November 2021 and March 2022, a total of 51 fresh cheeses sourced from informal trade were collected for analysis. The collection sites comprised dairy farms (n = 16), commercial establishments (n = 23), and municipal fairs (n = 12) within the Botucatu region of São Paulo state. To ensure proper preservation and transport, the samples were packed in plastic bags and transported in an isothermal box until the time of microbiological analysis.

2.2 Microbiological analyses

2.2.1 Bacillus cereus count

The counting of *B. cereus* was conducted using the ISO 7,932 methodology (ISO, 2004). For this purpose, 25 g of the samples were weighed and diluted in 225 ml of 0.85% saline solution. Serial dilutions were prepared, and the diluted samples were inoculated onto *Bacillus cereus* agar (Kasvi) supplemented with egg yolk. The plates were then incubated at 30 ± 1°C for 48 ± 2 h. Following incubation, five characteristic colonies were selected from each plate and confirmed on blood agar plates through the observation of β-hemolysis. The results were reported as log CFU/g.

2.2.2 Escherichia coli count

The counting of *E. coli* was conducted following the ISO 16649 methodology (ISO, 2001). Initially, 25 g of the samples were weighed and diluted in 225 ml of 0.85% saline solution. Serial dilutions were prepared, and 1 mL aliquots of the diluted samples were inoculated using the pour plate technique. Tryptone bile X-glucuronide agar (Himedia) was added to the plates, which were subsequently incubated at 44 ± 1°C for 18 h. After incubation, colonies exhibiting characteristic features were counted, and the results were reported as log CFU/g.

2.2.3 Coagulase-positive Staphylococcus count

To determine the count of coagulase-positive *Staphylococcus* in the samples, we followed the ISO 6888-1 method (ISO, 2003). Briefly, 25 g of the samples were weighed and subsequently diluted in 225 ml of 0.85% saline solution. Serial dilutions were prepared as required, and 0.1 ml aliquots from the dilutions were inoculated onto the surface of Baird–Parker agar supplemented with egg yolk and potassium tellurite (LB-Laborclin). The agar plates were then incubated at 35 ± 1°C for a period of 48 ± 2 h. For confirmation, a total of five colonies displaying typical characteristics and an additional five atypical colonies were selected from each plate. The selected colonies were subjected to the coagulase test using plasma-EDTA (LB-Laborclin). The resulting coagulase-positive colonies were recorded as log CFU/g.

2.2.4 Pseudomonas spp. count

To determine the count of *Pseudomonas* spp., we employed the ISO 13720 methodology (ISO, 2010) with modifications. Initially, 25 g of the samples were weighed and subsequently diluted in 225 mL of 0.85% Saline Solution. Serial dilutions were prepared, and 0.1 ml aliquots from the dilutions were inoculated onto the surface of cetrimide agar (Kasvi) plates. The inoculated plates were then incubated at 35 ± 2°C for a period of 48 ± 2 h. Characteristic colonies were carefully selected from the agar plates and subjected to the oxidase test for confirmation. Only colonies exhibiting positive oxidase activity were considered as confirmed *Pseudomonas* spp. colonies and results were expressed as log CFU/g.

2.2.5 Detection of Listeria monocytogenes

The detection of *L. monocytogenes* followed the ISO 11290-1 methodology (ISO, 2017a) with modifications. A total of 25 g of the samples were weighed and subsequently enriched using 225 mL of *Listeria* enrichment broth (Difco) followed by incubation at 30 ± 1°C for 24 h. After incubation, 0.1 ml of the enriched sample was transferred to Fraser broth and further incubated at 37 ± 1°C for 24–48 h. From the tubes with esculin hydrolysis, seeding was performed on chromogenic *Listeria* agar (Oxoid) and Oxford *Listeria* agar (Oxoid) plates, which were then incubated at 37 ± 1°C for 48 h. Characteristic colonies were selected, purified on tryptic soy agar (Oxoid) with 0.6% yeast extract (Difco), and subjected to confirmation and biochemical identification, including the assessment of β-hemolysis production, motility, and fermentation of carbohydrates such as xylose, rhamnose, and mannitol. The results were expressed as the presence or absence in 25 g of the sample. Molecular confirmation was performed using polymerase chain reaction (PCR) following the method described by Doumith et al. (2004). Additionally, the virulence profile was determined by detecting the genes *inlA*, *inlB*, *inlC*, *hlyA*, and *actA*, as proposed by Liu et al. (2007), Paziak-Domańska et al. (1999), and Suárez et al. (2001).

2.2.6 Detection of Salmonella spp.

The detection of *Salmonella* spp. was conducted in accordance with ISO 6579 (ISO, 2017b). A total of 25 g of the samples were weighed and enriched using 225 mL of buffered peptone water (Oxoid), followed by incubation at 37 ± 1°C for 18 ± 2 h. After incubation, 1 ml of the enriched broth was transferred to Muller-Kauffmann tetrathionate broth (Oxoid), and 0.1 ml was transferred to Rappaport-Vassilidis soya broth (Oxoid), both of which were incubated at 41.5 ± 1°C for 24 ± 3 h. Subsequently, aliquots from each incubated enrichment broth were streaked onto xylose Lysine deoxycholate agar (Oxoid) and bismuth sulfite agar (BS) (Difco) plates, which were then incubated at 37 ± 1°C for 24 ± 3 h. Characteristic colonies were selected and subjected to a battery of biochemical tests, including lysine, H2S production, sugar fermentation, citrate utilization, urea, methyl red, indole production, and motility. *Salmonella* spp. isolates were further confirmed through serological testing based on somatic and flagellar antigens. The presence or absence of the pathogen in 25 g of the sample was recorded as the final result.

2.2.7 Molecular detection of Coxiella burnetii

For the molecular detection of *Coxiella burnetii*, 10 g of cheese samples were weighed in a 50 ml Falcon® tube, 10 ml of 1X phosphate-buffered saline solution was added, and the samples were then macerated by vortexing. The DNeasy Blood and Tissue Kit (Qiagen®) was used to process the samples according to the manufacturer's protocol for tissue samples. Amplification of *C. burnetii* was performed using the protocol described by Vaidya et al. (2010), utilizing the primers Trans 3 (5'-GTAACGATGCGCAGGCGAT-3') and Trans 4 (5'-CCACCGCTTCGCTCGCTA-3') (Hoover et al., 1992). The RT-qPCR protocol comprised a total volume of 25 µL, containing 32 nM of each primer, 12.5 µL of SYBR Green™ Dye GoTaq™ qPCR Master Mix (Promega), 6.7 µL of nuclease-free PCR-grade water, and 5 µl of DNA template. The qPCR assay was conducted using a Real-Time Applied Biosystems™ Step-One instrument (ABI 7500 Fast model). The amplification process involved an initial denaturation step at 95°C for 10 min, followed by 40 cycles of denaturation at 94°C for 30 s, annealing at 61°C for 30 s, and extension at 72°C for 1 min. Fluorescence signals were recorded at the endpoint of each cycle, and a melting curve analysis was performed by heating the samples to 95°C for 30 s, followed by a rate of 2°C/min following an initial step at 61°C for 30 s. As a positive control, DNA from a strain of *C. burnetii* isolated from ticks (Pacheco et al., 2013) was utilized. The confirmation of *C. burnetii* isolates was conducted through electrophoresis, with positive samples having a fragment size of 243 bp.

2.2.8 Mycobacterium bovis and Mycobacterium tuberculosis molecular detection

Preparation of cheese samples for extraction involved weighing 10 g of each sample in a sterile Stomacher® bag, to which 10 ml of sterile saline solution was added. The contents were manually homogenized to dissolve the sample. For DNA extraction from the cheese samples, 200 µL of the diluted supernatant from each sample was processed using the MagMAX™ CORE Nucleic Acid Purification Kit (ThermoFisher®) following the manufacturer's instructions. The amplification of samples for the detection of *M. bovis* and *M. tuberculosis* followed the methodology described by Flamínio (2019). All qPCR reactions were performed using a 7500 Fast Real-Time PCR System with the 7500 Software v.2.3 (Applied Biosystems®). The GoTaq® qPCR Master Mix (Promega, USA) was used for amplification. The concentrations and temperatures were determined according to the manufacturer's instructions.

3 RESULTS AND DISCUSSION

Among the counts of indicator microorganisms, coagulase-positive *Staphylococcus*, *E. coli*, and *Pseudomonas* spp. had the highest averages (Table 1). *C. burnetii* and *L. monocytogenes* were identified in 7.84 and 1.96% of the samples, respectively. No positive samples were detected for *Salmonella* spp., *M. bovis*, and *M. tuberculosis* (Table 1).

In Figure 1, the results obtained in this study are presented, which were compared to the microbiological standards established by ANVISA's IN No. 161/2022 for cheeses with moisture content ≥ 46%. For the limits of *B. cereus*, parameters described for dairy desserts were used (Brasil, 2022). For *Pseudomonas* spp., the study by Champagne et al. (1994) was used since there are no official parameters for any food category.

Out of all the analyzed cheeses, only four (7.84%) samples complied with all the adopted parameters, all of which came from rural properties. Among the remaining samples, 26 (50.98%) did not comply with at least one evaluated parameter, 20 (39.21%) did not comply with two parameters, and one sample (1.96%) did not comply with three parameters.

The presence of *L. monocytogenes* in one of the samples (1.96%) from a commercial establishment demonstrates the noncompliance of the product being made available for consumption and the consequent risk to consumer health. Vinha et al. (2016) found *L. monocytogenes* in 1 (1.30%) out of the 77 analyzed cheeses in their study, while Apolinário et al. (2014) detected the microorganism in 9.6% of the cheeses in their work.

In the present study, the isolated *L. monocytogenes* belonged to serogroup IV (4b), which is the least prevalent in food contamination. However, it is commonly associated with the occurrence of the invasive form of listeriosis, presenting high mortality rates in risk groups (Barancelli et al., 2011). This isolate had genes related to virulence, such as the cell internalization and cell-to-cell spread factor (*inlA*, *inlB*, *inlC*), listeriolysin synthesis (*hlyA*), and actin polymerization (*actA*) in the host, highlighting the epidemiological importance related to this microorganism. Additionally, the isolate carried genes related to biofilm formation and adaptations to stressful conditions (*prfA*, *agrBCDA*). When expressed by the bacterium, these genes assist in the internalization of *L. monocytogenes* (*inlA*, *inlB*) into the host cell wall, propagation to adjacent cells (*inlC* and *actA*), production of listeriolysin for its maintenance within the host cell as well as its multiplication (*hlyA*), and environmental resistance (*prfA*, *agrBCDA*) involving environmental adversities (Liu et al., 2007; Paziak-Domańska et al., 1999; Suárez et al., 2001). The evaluated isolate exhibited important genes related to biofilm formation characteristics, indicating a potential

Table 1. Average counts (LOG) of indicator microorganisms for hygienic-sanitary quality and the presence of pathogens in non-inspected fresh cheese samples (n = 51) informally marketed in the Botucatu region, SP.

Microorganism	Average	Minimum	Maximum	SD	Parameters
Log CFU/g					
Coagulase-positive *Staphylococcus*	2.79	-0.18	7.01	2.39	3.00[(1)]
E. coli	2.59	0.00	5.60	1.74	3.00[(1)]
B. cereus	0.14	0.00	3.96	0.72	2.7[(2)]
Pseudomonas ssp.	2.18	0.00	7.64	2.98	6.34[(3)]
Presence or absence in 25 g					
L. monocytogenes		1/51 (1.96%)			Absence*[(1)]
Salmonella ssp.		0/51 (0.00%)			Absence[(1)]
Molecular detection in 10 g					
Mycobacterium bovis		0/51 (0.00%)			Absence
Mycobacterium tuberculosis		0/51 (0.00%)			Absence
Coxiella burnetii		4/51 (7.84%)			Absence

[(1)]IN 161 (Brasil, 2022) for cheeses with moisture ≥ 46%; [(2)]IN 161 (Brasil, 2022) for *B. cereus* in dairy desserts; [(3)]Champagne et al. (1994); *Absence in 25 g; SD: standard deviation.

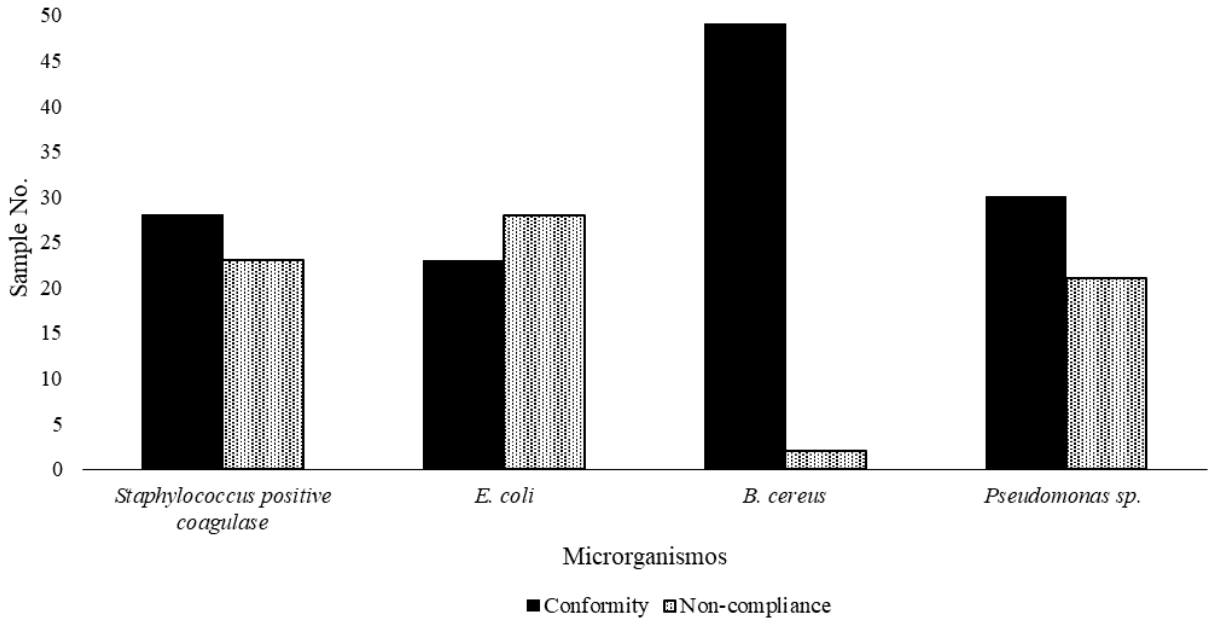

Figure 1. Number of samples in compliance and noncompliance with legal standards of IN 161 (Brasil, 2022) and Champagne et al. (1994).

factor for its persistence in the handling environment, favoring cross-contamination between products and surfaces (Farber & Peterkin, 1991; Mead et al., 1999).

The detection of *C. burnetii* (n = 4; 7.84%) was an important finding as it is a zoonotic agent, and its presence in milk and dairy products is uncommon (Gale et al., 2015). These findings are consistent with those of Mioni et al. (2020) and Rozental et al. (2020) as both studies detected a prevalence of 9.43 and 3.57% of the pathogen in aged cheeses and raw milk, respectively, demonstrating the importance of such foods as potential sources of infection for humans. Although the main route of transmission of *C. burnetii* is through inhalation of contaminated aerosols, outbreaks of Q fever through the ingestion of raw milk and consumption of contaminated cheeses have been reported, suggesting that the risk of consuming unpasteurized contaminated dairy products should not be neglected (Gale et al., 2015; Signs et al., 2012).

The detection of *C. burnetii* in the analyzed cheeses in this study demonstrates the presence of contaminated herds in the sampled region. As it is an airborne pathogen capable of spreading over long distances carried by the wind, there is a possibility of Q fever outbreaks occurring in nearby cities to the infected properties, posing a potential risk to public health (Mioni et al., 2020; Tissot-Dupont et al., 2004).

Only two samples (3.92%) were above the established limits for *B. cereus*, with counts of 3.96 and 3.34 log CFU/g, both from commercial establishments (Table 1 and Figure 1). The observed count of *B. cereus* in this study was lower than the minimum limit described in the scientific literature capable of causing clinical illness and deleterious effects on food degradation (Doyle et al., 2013; Forsythe, 2013). However, the low count of *B. cereus* in the analyzed cheeses does not diminish the potential risk of consuming this product as it is not an immediate consumption food and can remain stored in the refrigerator for a few days.

Due to its psychrotrophic characteristics, *B. cereus* can multiply in this low-temperature environment.

The absence of legal parameters for *B. cereus* counts in cheese is concerning as the isolation of the microorganism was not unique to our study. Kabuki et al. (2008) found counts ranging from 10^3 to 10^5 CFU/g of *B. cereus* in cheeses from the commerce in Campinas/SP, with 22% of these cheeses presenting counts exceeding 10^5 CFU/g, which is considered infective and potentially enterotoxigenic dose.

Regarding coagulase-positive *Staphylococcus*, counts ranging from -0.18 to 7.01 log CFU/g were observed, with 23 samples (38%) not meeting the allowed limits for cheese according to Brazilian legislation (Table 1 and Figure 1). The high count of coagulase-positive *Staphylococcus* in the cheese samples suggests hygiene failures in the production process or poor sanitary quality of the raw materials (Kamimura et al., 2019). However, it is not possible to distinguish the source of contamination due to the unpasteurized raw material, which could be the issue if the contamination focus originated from bovine mastitis. Melo et al. (2013) reiterated that high populations of *Staphylococcus* spp. have inhibitory potential against more sensitive bacteria in competition for the microbiota, which could also explain the high count of these bacteria in the analyzed samples.

Food with coagulase-positive *Staphylococcus* counts above 10^5 CFU/g may have the potential for enterotoxin production and can cause intoxication (Nia et al., 2016). In the study, it was observed that 13 samples (25.49%) had counts of coagulase-positive *Staphylococcus* above this value, posing a potential risk for enterotoxin production. However, due to their heat resistance, staphylococcal enterotoxins may be present even when coagulase-positive *Staphylococcus* is no longer viable in the food or even when food contamination occurs through coagulase-negative *Staphylococcus* strains, which also have the potential for enterotoxin production and are more prevalent in cases of cow mastitis (Freitas Guimarães et al., 2013; Le Loir et al., 2003).

For *E. coli*, 28 samples (54.9%) were found to be outside the standards established by IN nº 161 (Brasil, 2022), with counts ranging from 0.0 to 5.6 log CFU/g (Table 1 and Figure 1). The presence of high counts of *E. coli* among the samples, regardless of the collection sites, may indicate possible fecal contamination and consequent poor hygienic-sanitary quality in cheese production. Another relevant factor for this result is the fact that many producers/traders do not have suitable facilities for production, storage, and commercialization of products (Vinha et al., 2016). Pathogenic *E. coli* strains are among the main microorganisms involved in foodborne diseases, but many strains of *E. coli* inhabit the intestines of humans and animals in a commensal manner. Isolation of the agent may not distinguish commensal strains from pathogenic ones; therefore, it may not necessarily be related to the occurrence of foodborne diseases (Ramos et al., 2020).

The counts of *Pseudomonas* spp. ranged from 0.0 to 7.64 log CFU/g, with an average count of 2.18 log CFU/g, originating from the three types of evaluated locations. The literature on cheese contamination by *P. aeruginosa* is scarce and conflicting, with studies reporting both the absence and presence of the bacteria (Okuno et al., 2021). In addition to this fact, there is a lack of legal standards for *Pseudomonas* spp. counts in Brazil, which makes it difficult to determine references for its evaluation. The results of the analysis of this spoilage bacteria in our study were based on the reports of Champagne et al. (1994), who described *Pseudomonas* spp. counts above 6 log CFU/g as capable of producing proteases and lipases to the point in which food spoilage is noticeable and undesirable to the consumer. It is believed that the impact of *Pseudomonas* spp. on human health may be underestimated as the microorganism has been associated with foodborne outbreaks even at low prevalence, with potential multidrug resistance (Capodifoglio et al., 2016; Paula et al., 2021; Quintieri et al., 2019). As an opportunistic agent in terms of human health interest, it would be important to determine the infective dose capable of causing clinical symptoms.

Among the analyzed samples, no contamination by *Salmonella* spp. was observed, which is in accordance with Brazilian legislation stating that the pathogen should be absent in 25 g of the cheese sample with moisture ≥ 46%. According to Apolinário et al. (2014), the association between lactic acid bacteria and coliforms promotes acidification of the environment, consequently inhibiting other bacteria that are not resistant to acidic pH, such as *Salmonella* spp. The study corroborates the results obtained by Amaral et al. (2020) and Silva et al. (2015), who also did not isolate *Salmonella* spp. in the cheeses analyzed in their research.

The absence of detection of *M. bovis* and *M. tuberculosis* in the samples of this study does not exempt the consumption of these products from presenting hazards to the consumer as the lack of milk pasteurization is a high-risk factor for the persistence of such pathogens, as well as for *C. burnetii*. Contradicting our findings, Cezar et al. (2016) and Harris et al. (2007) isolated the pathogen in fresh and rennet cheeses, thereby demonstrating the inherent risk in consuming food produced with unpasteurized milk from herds without sanitary control for such diseases.

The number of noncompliant cheeses for *Staphylococcus* coagulase-positive, *Pseudomonas* spp., and *B. cereus* was lower compared to cheeses that met the official microbiological parameters. However, this does not mean that the product is suitable for consumption as a high percentage of samples were found to be above the established microbiological limits, posing a risk to public health. Additionally, the high counts of *E. coli* and the presence of *L. monocytogenes* and *C. burnetii* indicate a potential threat to public health. Moreover, the two samples of *B. cereus* had high counts compared to the parameters for other dairy products specified in the regulatory legislation (Brasil, 2022).

The high counts of *Staphylococcus* coagulase-positive and *E. coli* indicate poor hygienic and sanitary quality, whether due to the lack of good agricultural production practices, improper cleaning of milking equipment, animal health issues, or poor handling practices in cheese production and incorrect storage. The presence of *B. cereus* may indicate cross-contamination and likely the absence of good manufacturing practices, while the presence of *L. monocytogenes* suggests failures in the production process of these cheeses.

These results are concerning because the lower cost of informal fresh cheese compared to processed products often plays a decisive role in consumers' purchasing decisions. Coupled with the lack of information about the risks associated with consuming this type of product, it puts the population's health in a vulnerable position. As observed in the study by Carmo Amorim et al. (2014), the average purchase value of informal cheeses can be an important factor influencing consumers' decisions, combined with a lack of knowledge about the differences between formal and informal products, as well as a preference for the taste of informal cheese reported by participants in the study.

The absence of self-control programs in the artisanal manufacturing process, as well as a lack of training in good handling and storage practices, inadequate sanitary inspection, and the unrestricted trade of informal food products throughout the country, poses a risk to public health. This not only exposes the population to the occurrence of foodborne disease outbreaks but also hinders the identification, tracing, and recall of these products by manufacturers to prevent the emergence of new outbreaks. An example of the importance of all these factors combined with good collaboration between official agencies and the industry can be seen in the work of Nüesch-Inderbinen et al. (2021), which described a listeriosis outbreak that occurred in Switzerland in 2020. The industry itself, through routine analyses, was able to identify the contamination of Brie cheese and correlate it with the occurrence of foodborne illness cases. The Swiss public health service demonstrated agility by conducting tracing and confiscating the products manufactured by the company that were still available in retail, thereby preventing further infections by *L. monocytogenes*.

4 CONCLUSION

The significant proportion of samples failing to meet microbiological standards, as prescribed by legislation, in this study underscores the compromised sanitary quality of unregulated fresh cheese being informally sold in the Botucatu/SP region. This poses a serious public health concern, underscoring the urgent need for robust sanitary inspection measures to curtail the distribution of such products and mitigate the risk of potential outbreaks associated with the consumption of these foods.

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