








## Antimicrobial detection from antimicrobial residues in raw milk from dairy farms in western Santa Catarina

### *Detecção de resíduos de antimicrobianos no leite cru de propriedades leiteiras no oeste de Santa Catarina*

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#### Abstract

Milk is a food rich in nutrients, which makes it widely consumed in Brazil. Its quality is vital for the safety of consumers. Antimicrobial residues may be present in milk if proper management is not performed in administering veterinary drugs to treat dairy cattle, thus representing a risk to human health and generating losses in manufacturing dairy products. Therefore, this study aimed to evaluate the presence of antimicrobials in in natura milk samples from rural properties located western Santa Catarina State using Delvotest® T. Milk samples were collected from October to December 2021. Of the 55 samples collected, 9.1% tested positive for antimicrobial residues, pointing to the need for greater control in the process of antimicrobial administration and control of the waiting period and periodic laboratory analysis. The study allowed one to detect antimicrobial contamination in the sampled milk and emphasized the importance of monitoring and controlling the use of these antimicrobials in rural properties to ensure food safety.

**Keywords:** antimicrobials; milk; food safety; quality control.

**Resumo**

O leite é um alimento rico em nutrientes e por isso é muito consumido no país. Sua qualidade é de extrema importância para a segurança dos consumidores. Resíduos de antimicrobianos podem estar presentes no leite se não realizado um manejo adequado na administração de medicamentos veterinários para o tratamento de gado leiteiro, representando dessa forma um risco à saúde humana e gerando perdas na industrialização de produtos lácteos. Este estudo teve como objetivo avaliar a presença de antimicrobianos em amostras de leite *in natura* proveniente de propriedades rurais localizadas no Oeste de Santa Catarina através do Delvotest® T. A coleta das amostras de leite foi realizada no período de outubro a dezembro de 2021. Das 55 amostras coletadas, 9,1% apresentaram resultado positivo para resíduos de antimicrobianos, apontando a necessidade de maior controle no processo de administração dos antimicrobianos, bem como controle do tempo de carência e realização de análises laboratoriais periódicas. O estudo permitiu a detecção de contaminação de antimicrobianos no leite amostrado e enfatiza a importância da monitoria e controle do uso destes antimicrobianos em propriedades rurais afim de garantir a segurança dos alimentos.

**Palavras-chave:** antimicrobianos; leite; segurança de alimentos; controle de qualidade.

## 1. INTRODUCTION

Milk is one of the products that leads the ranking of the most consumed animal products worldwide due to its nutritional quality (SUH, 2022). It is a product that comes from complete uninterrupted milking under hygienic conditions of healthy and well-fed cattle. The high demand for milk and dairy products driven by population growth has increased concern about the quality and safety of dairy products because, during the production process, there may be contamination by antimicrobial residues resulting from the use of veterinary drugs (CARVALHO et al., 2020; SUH, 2022).

Thus, evaluating the presence of antimicrobial residues in milk is a requirement as described in Normative Instruction No. 60 of November 26, 2018 (BRASIL, 2018), given that the antimicrobial residues in milk can present risks to human health and cause adverse effects on the intestinal flora. This may impair its local protective action and interfere in the industrial process of derivatives, in laboratory analyses routinely used in dairies, including phosphatase, peroxidase, and reductase tests, and being highly resistant to thermal treatments, both at high and low temperatures, making their production unviable and, consequently, also causing serious economic losses (SILVA, et al., 2014; RAHMAN; HASSAN; CHOWDHURY, 2021).

The contamination by chemical substances in milk, such as the presence of antimicrobials, is considered adulteration and makes it unfit for consumption. The primary source of antimicrobial residues in milk is the inadequate handling of these substances, which during their waiting periods, are eliminated by milk. The most commonly found

are those of the  $\beta$ -lactam group, which is the most used in treating diseases in dairy herds (ALVES et al., 2016). Thus, when the presence of antimicrobial residues is detected in the milk, it must be discarded (SILVA, 2009).

The guarantee of food safety is given the control of different types of contaminants and residues that may be present in production practices. Food quality and safety have been receiving more attention from the world population, especially regarding microbiological and chemical hazards.

In milk, the chemical residues of antimicrobials represent one of the most found substances in Brazil and other countries (NERO et al., 2007). Various researchers have reported the importance of studying the presence of antimicrobials in milk, such as Suh (2022), who reported the need for indicators of potential metabolites and metabolic mechanisms associated with dairy product quality factors. Rahman, Hassan, and Chowdhury (2021) detected racycline and amoxicillin in individual milk samples in Bangladesh. Joubrane et al. (2022) detected antimicrobial residues of oxytetracycline, tetracycline, ciprofloxacin, sulfamethazine, and doxycycline at high levels of pathogenic bacteria and antimicrobial resistance. Thus, the importance of conducting studies to verify milk quality in Brazil is justified.

In order to promote the safety of food of animal origin produced in Brazil through a risk management tool, the Ministry of Agriculture, Livestock, and Supply (MAPA) coordinates the actions of the National Plan for the Control of Residues and Contaminants (PNCRC/Animal) (BRASIL,1999). The tests verify compliance with the maximum limits of chemical residues in animal products

applicable in Brazil, which are established by the National Health Surveillance Agency (ANVISA) based on international references (WHO, 2018; BRASIL, 2019).

To control antimicrobial residues, various tests are used by the industry; one example that stands out is the Delvotest® T, which is based on a colorimetric method capable of screening milk for the presence of antimicrobials, which uses *Bacillus stearothermophilus* var. *calidolactis* as a test organism (SATS et al., 2014). Delvotest® T is a broad-spectrum test that identifies the widest range of antimicrobials according to European maximum residue levels (HUFTON, 2017).

Given the above, this study aimed to use the Delvotest® T kit to analyze the conditions of antimicrobial presence and absence in *in natura* milk samples from individual producers and the joint tank of a dairy located in western Santa Catarina State (southern Brazil).

## 2. MATERIAL AND METHODS

### 2.1 Sample collection

From October to December 2021, fresh milk samples were collected weekly and identified with a number for each dairy farm. The samples were collected directly from the cooling tanks of each farmer and a sample from the tank containing the mixture of all the farmers' milk. The samples were collected in sterile bottles and kept cooled between 2 and 8 °C for later analysis in the laboratory of the Faculdade de Tecnologia Senai (Chapecó-SC).

### 2.2 Analysis methodology

Delvotest® T is a broad-spectrum antimicrobial test capable of identifying the presence of several classes (Table 1).

Table 1 - Antimicrobial class and active substances detectable in the Delvotest® T test.

ANTIMICROBIAL	ACTIVE SUBSTANCE	MRL (µg kg <sup>-1</sup> )	ANTIMICROBIAL	ACTIVE SUBSTANCE	MRL (µg kg <sup>-1</sup> )
Penicillins	Amoxicillin	4	Diamino pyrimidine derivatives	Trimetropin	50
	Ampicillin	4	Lactamase inhibitors	Clavulanic acid	200
	Benzilpecilin	4	Polymyxins	Colistin	50
Cephalosporins	Cephalexin	100	Ansamisins	Rifaximin	60
	Cefazolin	50	Quinolones	Enrofloxacin	100
	Cefapirin	60		Enrofloxacin	100
Sulfonamides	Sulfadiazine	100	Aminoglycosides	Marbofloxacin	75
	Sulfadoxine	100		Gentamicin	100
Sulfones	Dapsone	*	Aminoglycosides	Kanamycin	150
Tetracyclines	Chlortetracycline	100		Neomycin	1500
	Oxytetracycline	100		Lincosamides	Lincomycin
Macrolides	Spiramycin	200	Pirlimycin		100
		Tylosin	50		

MRL: maximum residue limit, commission regulation (EU) No. 37/2010, and amendments as of September 15, 2011.

Source: Adapted from OOGHE (2012)

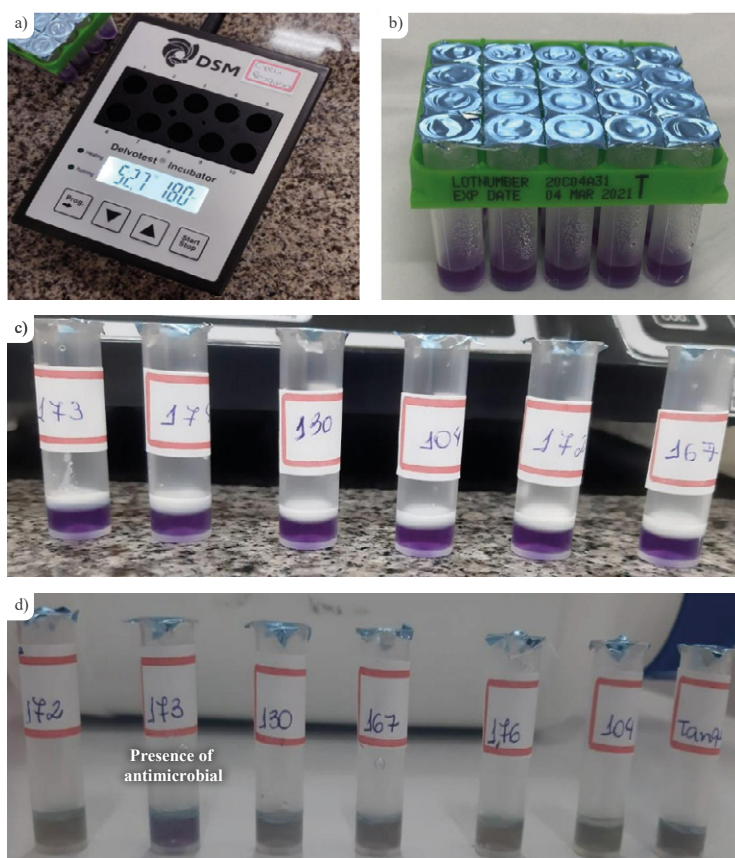
Delvotest® T is supplied with individual tubes filled with agar medium. The agar is solid inoculated with a standardized number of *Bacillus stearothermophilus* var. *calidolactis* spores and the nutrients necessary for growth purposes and trimethoprim antifolate. The medium turns violet by the action of the pH indicator bromocresol purple. About 100 µL of raw milk should be added to the agar surface for incubation at 64 °C for 3 h. Suppose there is no inhibiting substance in the milk sample or at a concentration below the limit of detection (LOD), then, in that case, spores of the bacillus spores germinate, develop, and the acid produced in the fermentation changes the purple color of the bromocresol indicator in the medium to yellow. Nonetheless, if inhibitory substances are present in the sam-

ple under test, germination and growth of the bacillus spores are inhibited. No fermentation occurs, leading to no acid production and no change of the indicator bromocresol purple. The formation of the color can be read visually (BION, 2015).

### 3. RESULTS AND DISCUSSION

The 55 samples collected were analyzed from October to December 2021. To perform the assays, the Delvotest® T incubator (Figure 1a) was used, as well as the ampoules with the reaction medium (Figure 1b), in which the raw milk samples were incubated at 64 °C for 3 h (Figure 1c). After the incubation period, the results were read and interpreted (Figure 1d).

Figure 1 - a) Delvotest T- DSM® Incubator. b) Vials for incubation of raw milk. c) Vials with the addition of 100 µL of raw milk for incubation. d) Result obtained after incubation for antimicrobial residues.



Source: From the authors (2021)

In Figure 1d, one can observe the positive results for the presence of antimicrobials in sample 173 due to the purple coloration, indicating an inhibiting substance. The other samples changed their coloration to yellow because the bacillus spores germinated and produced acid, changing the indicator's color. The results obtained during the months of testing were systematized, as listed in Table 1.

Table 2 - Results of the analysis of raw milk samples for antimicrobial residues.

WEEK	TANK	PRODUCER						
		173	176	172	130	104	167	174
1	-	-	-	-	-	-	-	AT
2	-	+	-	-	-	-	-	AT
3	-	+	-	-	-	-	-	AT
4	-	+	-	-	-	-	-	AT
5	-	+	AT	-	-	-	-	-
6	-	-	AT	-	-	-	-	-
7	-	+	AT	-	-	-	-	-
8	-	-	AT	-	-	-	-	AT

**Legend:** (-) Negative result for antimicrobial; (+) Positive result for antimicrobial; (NA) Not applicable.

Source: The authors (2021)

The results of the assays performed for individual producers with sets 176, 172, 130, 104, 167, and 174 were negative for antimicrobials, representing 87.23% of the samples. This is a significant result since the farms are small with little knowledge about the importance of antimicrobial control. Nevertheless, 12.77% of the individual samples detected the presence of antimicrobials in raw milk (Table 1). We also noted that all the samples contaminated with antimicrobials were from the same farm (set 173). However, the method used is qualitative, it does not make sense to state the amount and type of antimicrobial present, although these results highlight the need for periodic controls and analysis.

For the milk tank samples (containing the mixture of all producers), the tests showed that all samples were negative for the presence of antimicrobials (100%). This can be due to the dilution of the positive sample. In this way, the antimicrobials concentration was below

the test's detection limit. It is worth noting that Normative Instruction No. 77, of November 26, 2018, established in Art. 33 that for the detection of residues of products of veterinary use, the analysis must be performed on the milk of the set of tanks or cans of each transport vehicle for at least two groups of antimicrobials and frequently determined in its self-control. The industry performs the tank analysis as the legislation recommends and is only evaluated individually if the tank sample is positive. The negative result shows that the concentration of antimicrobials is below detection levels, so the product can be consumed.

Costa and Lobato (2009) evaluated the incidence of antimicrobial residues in UHT milk marketed in Seropédica, Rio de Janeiro, using the Delvotest®SP-NT commercial kit analysis method. These results are possibly due to lack of more specific and in-depth tests or perhaps lack of monitoring of producers and the industry's production process.



The concern with antimicrobial resistance resulted in the composition of a tripartite agreement, encompassing the concept of One Health, between the World Health Organization (WHO), the Food and Agriculture Organization (FAO), and the World Organization for Animal Health (OIE) with coordinated actions to mitigate risks at the interface public health, animal health, and the environment, recognizing multidimensionality and the need for an intersectoral response that this problem requires (OIE, 2016; SILVA et al., 2020).

In addition, the 2030 Agenda for Sustainable Development expresses the importance of food quality through Sustainable Development Goal number 2: zero hunger and sustainable agriculture. Several goals are foreseen by 2030, such as ending hunger and ensuring access for all people, particularly the poor and people in vulnerable situations, including children, to safe, nutritious, and sufficient food throughout the year. Therefore, this work meets the goals set by the 2030 Agenda as an alternative to improving food quality and safety.

Recently, normative acts were established seeking the adoption of self-control tools on dairy farms, applied to managing good agricultural practices to monitor and control antimicrobial residues in milk. Thus, the producing establishment must perform milk analysis at a frequency determined by its self-control for all groups of antimicrobials for which analytical screening specifications are available (BRASIL, 2018).

## 4. CONCLUSIONS

Data analysis allowed us to conclude that of the samples evaluated for individual producers, 12.77% tested positive for antimicrobials. Nevertheless, the samples tested for mixing tanks showed no antimicrobial presence, possibly due to the dilution effect,

which puts the consumer's health at risk. In this sense, we highlight the need for further studies to identify the risk factors associated with antimicrobial residues in milk that are determinants for adopting control measures in dairy farms. It ratifies the importance of adopting good agricultural practices in milk-producing establishments through a program of self-control of the raw material seeking the monitoring and detection of antimicrobial residues.

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