

Detection of antimicrobial residues in informal raw milk in the central-west of Minas Gerais

Detecção de resíduos de antimicrobianos em leite cru informal no centro-oeste de Minas Gerais

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ABSTRACT

The presence of antimicrobial residues in informal milk, sold in inland cities, a situation commonly found in Brazilian cities, since the inspection of this type of product is incipient. As a case study, the municipalities of Arcos, Lagoa da Prata and Santo Antônio do Monte, in the center-west of Minas Gerais, were considered. Two collections were carried out in 22 informal sales points in 2020. To detect antimicrobial residues, the Trisensor® test was used, in which the presence of three groups of antimicrobials can be observed: beta-lactams, tetracyclines and sulfonamides. After performing the analyses, negative results were observed for the groups of tetracyclines and sulfonamides. However, when evaluating the beta-lactam group, positive results were observed: in a total of 44 samples, 54.5% were positive for the presence of beta-lactams (24 samples). The high incidence of antimicrobial residues in the analyzed samples (54.5%) confirms the need for surveillance of the product that is commercialized, and it is extremely important to adopt strict measures by inspection bodies since the presence of residues of antimicrobials in raw milk can, directly and indirectly, affect health.

KEYWORDS: beta-lactams; milk quality; one health; bacterial resistance.

RESUMO

O objetivo do presente estudo consiste em avaliar a presença de resíduos de antimicrobianos em leite informal, comercializados em cidades interioranas, situação comumente encontrada nas cidades brasileiras, visto que a fiscalização sobre este tipo de produto é incipiente. Como estudo de caso, foram considerados os municípios de Arcos, Lagoa da Prata e Santo Antônio do Monte, no centro-oeste de Minas Gerais. Foram realizadas duas coletas em 22 pontos de venda informal em 2020. Para detecção dos resíduos de antimicrobianos foi utilizado o teste Trisensor®, onde pode ser observada a presença de três grupos de antimicrobianos: beta-lactâmicos, tetraciclinas e sulfonamidas. Após a realização das análises, observou-se resultados negativos para os grupos das tetraciclinas e sulfonamidas. Entretanto, ao se avaliar o grupo dos beta-lactâmicos, observou-se resultados positivos significativos, onde em um total de 44 amostras, 54,5% foram positivas para a presença de beta-lactâmicos (24 amostras). A alta incidência de resíduos de antimicrobianos nas amostras analisadas (54,5%), confirma a necessidade da vigilância acerca do produto que é comercializado, sendo de extrema importância a adoção de medidas rigorosas por parte dos órgãos fiscalizadores, pois a presença de resíduos de antimicrobianos no leite cru pode afetar direta e indiretamente a saúde.

PALAVRAS-CHAVE: beta-lactâmicos; qualidade do leite; saúde única; resistência bacteriana.

For many years, milk has been an important food for the human diet and is considered a healthy drink, in addition to being an affordable nutritional source, being important for supporting the body's functions (CALLEFE & LANGONI 2015). When this milk does not undergo any type of heat treatment, it is called raw milk and can be acquired informally (MOTTA et al. 2015).

Several factors can cause changes in the composition of milk, among them, antimicrobial residues can be mentioned. These are considered chemical contaminants arising mainly from mastitis treatment (SILVA et al. 2016), inflammation of the mammary gland that represents a major impact on milk production (COSER et al. 2012), especially when there's no control over the disposal period indicated for each drug. This is usually associated with milk sold clandestinely (SILVA et al. 2016). Antimicrobial residues in milk are directly linked

to disordered clinical use and the disrespect of producers for the necessary grace period (LANGONI et al. 2017).

The presence of antimicrobial residues in milk can cause undesirable effects to consumers, such as allergic reactions and selection of opportunistic agents from the intestinal microbiota to residual antibiotics in milk in industry, antimicrobial residue can cause loss of yield and change in the quality of the final product (SILVA et al. 2015). It's prohibited to send milk with residues of veterinary products (BRASIL 2021), in addition to being mandatory that industries carry out tests for at least two different groups of antimicrobials at each receipt (BRASIL 2018a). The destination of milk with antimicrobial residues could be the manufacture of inedible products (BRASIL 2021), such as soap and industrial casein.

Thus, the present study aimed to evaluate the presence of antimicrobial residues in informal milk sold in the center-west of Minas Gerais, a situation that is commonly found in Brazilian cities, since the inspection of this type of product is incipient.

In natura milk samples were collected in the municipalities of Arcos, Lagoa da Prata and Santo Antônio do Monte, in the center-west of Minas Gerais. The collections were made in two stages to repeat the battery of tests to detect antimicrobial residues. The first battery of samples was carried out in September 2020, where 22 material collections were carried out for further analysis, samples that were acquired from different suppliers.

After collection, the samples were kept and transported in sterile containers and stored in an isothermal box with recyclable ice to the Microbiology Laboratory of the Centro Universitário de Formiga (UNIFOR-MG), where tests were carried out to detect antimicrobial residues. The second collection and analysis battery was carried out in October 2020, where 22 samples of in nature milk were collected again from the same suppliers as the previous battery, so that the tests for detecting antimicrobial residues could be repeated.

To carry out the detection of antimicrobial residues in nature milk, the Trisensor® test (Unisensor) was used. It is an enzyme-linked immunosorbent test that is based on receptors in the reagent strip format for the simultaneous detection of all compounds from three of the main families of antimicrobials that cause residues in milk, beta-lactams (such as penicillins and cephalosporins), tetracyclines and sulfonamides (PILON & DUARTE 2010). To carry out the tests, the milk sample was collected with a 200µl pipette, adding this content to the test microtube containing the reagent, and then the content was mixed until obtaining a homogeneous sample.

The sample was then incubated in a water bath at 40 °C for three minutes and after this period, the reactive strip was inserted into the sample, which was again incubated in the water bath for another three minutes. At the end of the time, the reactive strips were removed from the container for interpretation of the results according to the manufacturer, with the aid of the figure provided by the test.

After collecting and performing the analyses, negative results were observed for the group of tetracyclines and sulfonamides in all samples collected. However, as in the work by GUIMARÃES et al. (2019), when evaluating the group of beta-lactams, positive results were observed with regard to the presence of residues in milk where, in a total of 44 samples collected, 24 samples (54.5%) were positive for beta-lactams.

In the first battery of analyses, of the 22 samples analyzed, 20 of them (90.9%) were positive for beta-lactams. In the second battery of analyses, 22 samples were collected again and four of them (18.2%) were positive for beta-lactams (Table 1).

Presence of these residues in milk can cause hypersensitivity reactions, possible anaphylactic shock, and selection of more resistant strains. In addition, they can hinder industries in the use of raw materials, due to the changes they can cause in the manufacture of yogurts, cheeses, fermented dairy drinks and butter (SILVA et al. 2015), and it's necessary to remember that antimicrobials aren't eliminated during processing.

For the most part, screening tests are used for the detection of beta-lactams, since it's the group most used to perform the treatment of mastitis (SANTOS & FONSECA 2019). In a study by NETTO et al. (2005), in the southern region of Brazil, it was found that beta-lactams are the most used group for the treatment of infections in dairy cows, with a percentage of 38.22%. Also, in a research carried out by YAMAKI et al. (2004), in the region of Castilla, Spain, who detected 1.7% of milk samples contaminated by antimicrobials, 29.8% of which belong to the beta-lactam group. Among the antibiotics used in the dairy herd, beta-lactams are the class that most causes hypersensitivity reactions in allergic people (GUIMARÃES et al. 2019).

The second battery of analyzes obtained results similar to the study carried out by COSTA et al. (2017), who analyzed 71 milk samples and obtained 12.7% of their samples positive for beta-lactams, which indicates a common incidence in the use of antibiotics in different regions of the country. A study by

KOSGEY et al. (2018), in which informal milk in Kenya was analyzed regarding antimicrobial residue presence also found alarming percentages (23.8%) of gentamycin and tetracyclines combined.

Table 1. Results of the analysis of in natura milk sold informally in the center-west of Minas Gerais regarding the presence of antimicrobial residues.

Cities	Samples		Classes of antimicrobials					
	1st analysis	2st analysis	Tetracyclines		Sulfonamides		Beta-lactams	
			1st analysis	2st analysis	1st analysis	2st analysis	1st analysis	2st analysis
Santo Antônio do Monte	1	1	-	-	-	-	+	+
	2	2	-	-	-	-	+	+
	3	3	-	-	-	-	+	+
	4	4	-	-	-	-	+	+
	5	5	-	-	-	-	+	-
	6	6	-	-	-	-	+	-
	7	7	-	-	-	-	+	-
	8	8	-	-	-	-	+	-
Arcos	9	9	-	-	-	-	-	-
	10	10	-	-	-	-	+	-
	11	11	-	-	-	-	+	-
	12	12	-	-	-	-	+	-
	13	13	-	-	-	-	+	-
	14	14	-	-	-	-	-	-
	15	15	-	-	-	-	+	-
Lagoa da Prata	16	16	-	-	-	-	+	-
	17	17	-	-	-	-	+	-
	18	18	-	-	-	-	+	-
	19	19	-	-	-	-	+	-
	20	20	-	-	-	-	+	-
	21	21	-	-	-	-	+	-
	22	22	-	-	-	-	+	-
Total (n)	22	22	0/22	0/22	0/22	0/22	20/22	4/22
Total (%)	100%	100%	0%	0%	0%	0%	90,9%	18,2%

Caption: Negative (-) and positive (+) to antimicrobial detection.

According to VIDAL-MARTINS et al. (2013), in a survey interviewing 855 consumers about their preference for milk consumption, in São Paulo, SP, unexpected results were obtained, as 31.18% of respondents said they preferred to consume informal milk without supervision, explaining that the preference would be to believe that it's a healthier milk without the addition of preservatives. Also in this research, consumers were asked if milk would be able to transmit diseases, where 65.96% claimed "no" and only 34.03% said "yes". However, when asked about which disease milk could transmit, they scored on foot-and-mouth disease. These data are important because they highlight the total ignorance of consumers about the products they consume and the risks they present to their health (SILVA 2019).

The results of the present study reinforce the need for constant surveillance of the product that reaches the consumer's table; this shows the need to disclose what the population is consuming, in addition to reinforcing the importance to these of the food inspection agencies (SILVA et al. 2015). According to studies by FAGNANI et al. (2019) the provision of knowledge, as a form of awareness, about legislation and diseases transmitted by milk, could reduce the consumption of informal milk. Improving milk quality is an action that must be carried out jointly by inspection services and frequent awareness campaigns for producers and consumers (NASCIMENTO et al. 2001).

Respect for the grace period and the use of medicines only under the veterinarian's prescription, when necessary, are important steps regarding reduction in misuse. Moreover, the correct record of information about the treatments, detailing the period of use of the medicine, the days of disposal and the return of milk to human consumption, are also fundamental preventive measures to reduce the problems caused by the presence of antimicrobial residues in milk (SANTOS & FONSECA 2019).

TREIBER & BERANEK-KNAUBER (2021) point out the importance of an active surveillance in developing countries, such as Brasil, reinforcing monitoring and implementing educational programs to aware producers and consumers about the risks of development of multi-resistant germs and their impact in animal and, mainly, in human health.

According to Normative Instruction (IN) 76, of November 26, 2018, “refrigerated raw milk must not contain residues of veterinary products and contaminants above the maximum limits provided for in complementary standards” (BRASIL 2018b). The Art. 501 of Decree 9,013 of March 29, 2017 adds “it is considered inappropriate for any type of use of raw milk, when in the selection of the raw material, it presents inhibitors of microbial growth” (BRASIL 2017). Thus, the supervisory bodies ensure that products with antimicrobial residues do not reach the consumer's table.

According to a survey of the presence of antimicrobial residues carried out by RODRIGUES et al. (2012), in the region of Campos Gerais, Paraná, 400 milk samples were analyzed over five years after the implementation of payment policies regarding the requirement “absence of inhibitor”. Thus, they obtained the following results: 2005 (35% of positive samples), 2006 (29%), 2007 (29%), 2008 (27%), 2009 (23%), 2010 (12%), indicating that the appearance of residues decreases when incentive programs, educational work and quality programs are implemented.

With the study, we can observe the presence of antimicrobial residues in raw milk commercialized informally in the center-west of Minas Gerais, with beta-lactams being the most prevalent group in the analyzed samples, being present in 54.5% of the 44 samples, probably due to its wide use in mastitis treatments.

Thus, it's evident the need for the adoption of strict measures by the inspection bodies, since the presence of antimicrobial residues in raw milk directly affects the health of those who consume them, in addition to compromising future processing of the raw material.

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