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Occurrence of antibiotic residues in pasteurized bovine milk under Federal Inspection Service control in Pernambuco, Brazil

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Abstract

Antibiotic residues are considered the main chemical contaminants in milk, posing risks to consumers such as allergies, bacterial resistance, and anaphylactic shock in susceptible individuals. This study aimed to assess the occurrence of antibiotic residues from the classes of tetracyclines, sulfonamides, quinolones, fluoroquinolones, and β-lactams in the state of Pernambuco, Brazil. All brands were previously certified by the Federal Inspection Service (Serviço de Inspeção Federal — SIF) and produced in Pernambuco. A total of 19 (nineteen) were analyzed. The methodology applied was liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). Among the 21 samples analyzed, Penicillin G was detected in 4.76% (1/21) of the samples, at a non-quantifiable level. The results indicated that, overall, milk samples under federal inspection met the parameters established by current legislation. Nevertheless, continuous monitoring during milk procurement by dairy industries remains necessary. Further research on the occurrence of antibiotic residues in the region is also recommended.

Keywords: antimicrobials; contamination; food inspection; dairy; penicillin.

Practical Application: LC-MS/MS shows low antibiotic residues in federally inspected milk in Pernambuco.

1 INTRODUCTION

Milk is a widely consumed in Brazil, either in its natural form or processed into derivatives such as cheese, yogurt, and butter. It is associated with health due to its high nutritional value (Ribeiro Júnior et al., 2020). Rich in calcium, high-quality proteins, essential vitamins, and minerals, milk contributes to bone development, maintenance of muscle mass, and strengthening of the immune system (Eberle et al., 2023).

Among the parameters assessed to ensure milk quality is the presence of residues, including antibiotics. Milk contaminated with antimicrobial residues is considered unfit for human consumption due to its potential health hazards. These residues may induce allergic or toxic reactions in susceptible individuals, especially those associated with β-lactam antibiotics, such as penicillins and streptomycin. Moreover, the presence of these compounds can adversely affect the quality and safety of processed dairy products, posing additional concerns for public health and food industry standards (Lobato & Santos, 2019).

The induction of bacterial resistance, commonly associated with the use of tetracyclines, as well as hepatic and renal toxicity linked to gentamicin use, are significant public health concerns (Karunarathna et al., 2024). Furthermore, chronic exposure to subtherapeutic levels of these residues may result in long-term adverse effects, including the development of antimicrobial resistance in consumers (Cansan et al., 2023).

Brazil has made significant advances in the development of sector-specific legislation, implementing programs designed to assess consumer exposure to antibiotic residues in food products of animal origin (Brasil, 2018a; 2018b). The Ministry of Health, through the National Health Surveillance Agency (ANVISA), is responsible for establishing Maximum Residue Limits (MRLs) in food (Brasil, 2022). Regarding veterinary pharmaceuticals, the National Plan for the Control of Residues and Contaminants (PNCRB), established under Normative Instruction No. 42 by the Ministry of Agriculture, Livestock and Supply (MAPA), is applied (Brasil, 2001).

Several qualitative and quantitative assays are available for detecting veterinary drug residues in milk. These methods can be classified according to their underlying principles or mechanisms of action: bacterial growth inhibition, immunological, enzymatic, and high-performance liquid chromatography (HPLC) (Carvalho et al., 2020).

The predominant occurrence of antimicrobial residues in milk is largely associated with indiscriminate antibiotic use, inadequate dosing, and noncompliance with recommended with withdrawal periods. The presence of antibiotics in milk and dairy products poses risks to both public health and product quality (Silva et al., 2023).

Nonetheless, the presence of these residues remains evident in both commercially available milk and dairy products.

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Countries around the world have increasingly recognized the importance of monitoring the chemical quality of food to safeguard consumer health, implementing specific regulations and certification systems. The presence of harmful chemical substances has become one of the main consumer trends in recent years. More informed than ever, consumers actively seek information regarding the origin of the food they purchase, its production methods, and whether these are aligned with social and environmental values (Gazolla et al., 2017).

Given the relevance of this issue to public health, this study aimed to assess the occurrence of antibiotic residues within the dairy production chain in the state of Pernambuco, Brazil.

1.1 Relevance of the work

Monitoring antibiotic residues in milk is fundamental for protecting public health and meeting national and international legal requirements. This study provides unprecedented data on the occurrence of these residues in pasteurized milk produced in Pernambuco under federal inspection, using a highly sensitive analytical method (LC-MS/MS). The results contribute to evaluating the effectiveness of official inspections, guide control programs in the milk production chain, and support public policies aimed at food safety.

2 METHODOLOGY

Seven brands of federally certified milk produced in Pernambuco were analyzed, totaling 21 samples collected from three different batches. Due to limited resources at the institutional laboratory, samples were frozen then sent to the National Agricultural Laboratory (LANAGRO/RS), specifically to the Laboratory for the Analysis of Pesticide and Veterinary Drug Residues, where the methodology used was liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) (Figure 1).

Upon arrival, the samples were identified, stored appropriately, and prepared for analysis. A 500 µL aliquot of each sample was pipetted into Eppendorf tubes, previously homogenized at room temperature. For the positive control, three

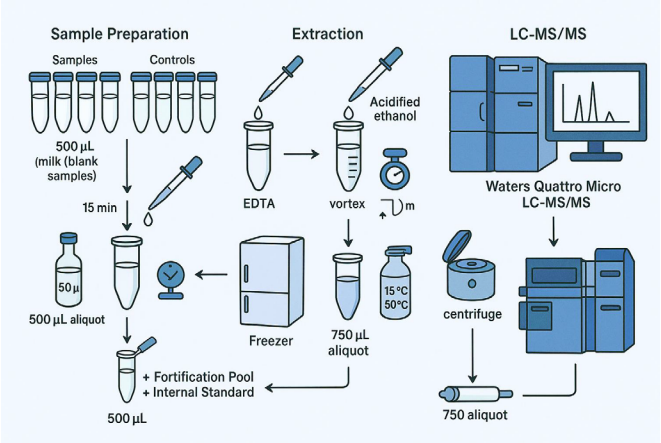


Figure 1. Schematic representation of sample preparation, extraction and LC-MS/MS analysis of milk samples for antibiotic residue detection.

samples were spiked with a solution containing all target compounds at concentrations corresponding to the detection capability (CCβ): 0.5x, 1.0x, and 1.5x the Maximum Residue Limit (MRL).

Following spiking, the samples were incubated in the dark for 15 minutes before processing. 500 µL of milk (blank samples) was placed into Eppendorf tubes (1.5–2.0 mL), followed by the addition of 25, 50, and 75 µL of the Fortification Pool solution and 23 µL of the Internal Standard Pool. Samples were homogenized and subjected to extraction.

For the extraction, approximately 20 µL of 150 mM EDTA was added, and the tubes were vortexed to ensure homogenization. Subsequently, 600 µL of acidified ethanol (4% acetic acid) was added to each tube, followed by vortexing for one minute and shaking for 10 minutes. The samples were then kept in the freezer for 30 minutes and centrifuged at 12,000 rpm for approximately 30 minutes at 50°C.

A 750 µL aliquot of the extract was transferred to 1.5 mL vials, and 250 µL of mobile phase (water with 0.1% formic acid and acetonitrile with 0.1% formic acid, 98:2) was added. Tubes were vortexed for one minute.

For the preparation of controls at the CCβ level, 500 µL of milk (blank samples) was allocated into three Eppendorf tubes. To each tube, 25 µL of the Fortification Pool solution and 23 µL of the Internal Standard (IS) Pool were added. The homogenization and extraction procedures were then repeated. For the preparation of the Tissue Standard (TS), following the extraction of the samples, 25 µL of the Fortification Pool solution and 25 µL of the Tissue Internal (TI) Pool solution were added to the tubes reserved as tissue standards.

An aliquot of 750 µL was withdrawn from the extract and 250 µL of the mobile phase was added, then the vials were properly sealed for analysis. For detection, a Waters Quattro Micro LC-MS/MS system was used, with a C18 column and an Electrospray ionization source in positive mode (ESI+). The data were recorded on a computer integrated with the system.

Data analysis was performed using descriptive statistics, including relative and absolute frequencies, means, and standard deviations. To evaluate the association between the results of the investigated parameters and sampling times, a univariate analysis was performed using the Chi-square test (Sampaio, 1998). The statistical calculations were executed using the SPSS for Windows software, version 19.0 — Statistical Package for the Social Sciences.

3 RESULTS AND DISCUSSION

Of the twenty-one samples analyzed, only one detected Penicillin G at a non-quantifiable level, representing 4.76% of the samples. However, penicillin hypersensitivity is observed in up to 10% of the world's population, even at low concentrations. Thus, evaluating the long-term effects of low-dose exposure represents a current need. This is also evident when considering the magnitude of the impact of residue ingestion on public health, as it has not yet been fully elucidated.

Silva (2023) evaluated PNCRC data from 2019 to 2023. Of the 5,963 raw milk samples, 1,722 (29%) contained beta-lactams, 1,353 (23%) presented sulfonamides, and 1,107 (19%) exhibited quinolones/fluoroquinolones. Similarly, a study by Pivato (2019), which analyzed 103 samples of commercial milk from supermarkets for macrolides, beta-lactams, sulfonamides, tetracyclines, and lincosamides, identified residues of the same drug classes. One of the main advantages of chromatographic methods over commercial kits is the ability to identify individual compounds, rendering these analytical techniques more specific and selective.

An assessment conducted in Rio Grande do Sul, Brazil, in 2021 investigated the occurrence of antibiotics in refrigerated raw milk. Using the Enzyme-Linked Immunosorbent Assay (ELISA), researchers found samples contaminated with beta-lactam residues, in addition to other drugs commonly used to treat infections (Ströher et al., 2022).

Despite this, the detection of residues below the Maximum Residue Limit (MRL) is a frequent occurrence, which may pose risks to consumer health. According to Silva et al. (2022), it is crucial to consider these risks cautiously, as the effects of ingesting these contaminants may be severe, and some are not yet fully elucidated. This scenario highlights the need for additional studies, including further experiments and long-term follow-ups.

Guimarães et al. (2019) emphasize that pasteurization and ultra-high temperature (UHT) processes are not effective in eliminating all drug residues present in milk. In a study with 90 samples, 73% of pasteurized samples and 46% of UHT samples retained contaminants, demonstrating that residues persist even after treatment.

Pivato (2019) emphasizes the need for caution in the consumption of products containing these substances, especially for pregnant women. The ingestion of milk containing tetracycline, sulfonamide, and streptomycin residues can expose the fetus to teratogenic effects, impacting fetal development and contributing to the increase of microbial resistance.

Researchers across Brazil have evaluated the occurrence of antibiotic residues using different detection methods, demonstrating the presence of antimicrobials in milk samples (Ribeiro, 2021). In the state of Tocantins, residues were detected in 1.74% of raw milk samples using a microbial inhibition assay (Lobato & Santos, 2019).

According to Gottardo et al. (2021), the presence of these residues is often associated with the inappropriate use of antibiotics and non-compliance with the recommended withdrawal period between treatment and milking prior to the release for human consumption.

Andretta (2023) explains that the main factors perpetuating the presence of contaminants in the dairy chain include the indiscriminate use of therapeutic and prophylactic agents, mostly antibiotics. Furthermore, the author states that disregarding the recommended withdrawal period for each medication also contributes to the dissemination of these contaminants. This is a recurring problem on both small and large-scale dairy farms, highlighting the need for broader awareness of good management practices and the responsible use of veterinary drugs to ensure milk quality and public health.

To prevent such drugs from reaching consumers, and thereby avoiding direct or indirect harm, the Federal Inspection Service regularly conducts checks for the control of antibiotics in milk, in accordance with the RIISPOA of 2020 (Regulation for Industrial and Sanitary Inspection of Animal Products). Additionally, it oversees farms and dairy processing facilities to verify compliance with good agricultural practices, thereby preventing contamination by veterinary drugs and ensuring adherence to the prescribed withdrawal periods following their use. In cases of non-compliance, sanctions such as fines, suspension of production or facility operations, cancellation of sanitary registration, and product recalls may be imposed (Brasil, 2020; 2022).

4 CONCLUSION

The results of this study indicate a low incidence of antibiotic residues in milk produced and commercialized in the State of Pernambuco under the supervision of the Ministry of Agriculture, Livestock, and Food Supply (MAPA). This regulatory oversight comprises a set of laws, normative instructions, and ordinances designed to ensure producer compliance and promote awareness regarding dairy cattle management and adherence to withdrawal periods, as well as to encourage public policies based on quality-linked payment systems. The overarching objective is to identify, prevent, and eliminate risks to human health associated with the use of veterinary drugs in food-producing animals. Nonetheless, continuous and systematic monitoring of these substances remains essential.

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