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Evaluation of residues of β-lactam, sulfonamide, tetracycline, quinolone, fluoroquinolone e pyrimidine in raw milk

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Abstract

Food quality and safety have been a concern of the world population, especially in relation to the chemical and microbiological hazards present in food. The presence of antimicrobial residues at levels above the maximum residue limit makes milk unsuitable for human consumption. In this context, the objective of this study was to analyze 184 raw milk samples from the dairy region of Pernambuco state, in order to evaluate the presence of 31 antimicrobial residues from six different groups (*β*-lactam, sulfonamide, tetracycline, quinolone, fluoroquinolone and pyrimidine). The samples were collected from producers who supply the dairys with and no inspection service and the analyses were performed by liquid chromatography coupled to mass spectrometry. In the qualitative analysis of multiresidues, no sample presented violation for the antimicrobials analyzed. In the quantitative analysis of *β*-lactams, only one sample of producers who supply milk to dairys with state inspection service presented a result above the maximum residue limit for cloxacillin (464 μg.L^{-1}). It is concluded that raw milk produced in the dairy region of the state of Pernambuco has a low rate of violation of the values established by the current legislation for antimicrobial residues.

Keywords: mastitis; maximum residue limit; inspection service; antimicrobial residue; LC-MS/MS.

Practical Application: Contribute to the elaboration of public policies, benefiting the producers and consumers.

1 Introduction

Dairy herds are often affected with mastitis, causing losses of financial resources and reduction of the quality of milk and dairy products (Bezman et al., 2015; Down et al., 2017; Machado & Bicalho, 2018). To prevent and combat this disease, antimicrobials are administered, but, during the grace period reported by the manufacturer, milk should be discarded, since antimicrobial residues are present and are not removed in their entirety by processing this raw material in industry (Calbert, 1951; Gajda et al., 2017; Poonia et al., 2017; Rossi et al., 2018; Tempini et al., 2018).

The consumption of milk with antimicrobial residue above the Maximum Residue Limit (MRL) represents a public health problem, since it can cause: allergic reactions, antimicrobial resistance, blood dyscrasias, gastrointestinal disorder, neurological disorder, cancer, among other effects (Titouche et al., 2013; Baynes et al., 2016; Delatour et al., 2018; Du et al., 2019). In addition, the presence of antimicrobials generates problems for the dairy, as they alter the results of analyses, inactivate or delay the activity of starter cultures in the production of cheese, yogurts, compromising the production of acids, resulting in failure of coagulation and maturation, causing modification of the sensory properties of dairy products (Calbert, 1951; Berruga et al., 2016; Gajda et al., 2017).

In Brazil, the control of residues of veterinary medicines in food is carried out through the National Program for the Control of Residues and Contaminants (PNCRC) of the Ministry of Agriculture, Livestock and Supply (MAPA). The PNCRC's regulatory function is to control and monitor these residues in food. For this, the level of tolerance or MRL that refers to the presence of antimicrobials in foods of animal origin has been established (Brasil, 1999).

The objective in this study is to evaluate the presence of 31 residues of antimicrobials of six different groups (*β*-lactam, sulfonamide, tetracycline, quinolone, fluoroquinolone and pyrimidine), in raw milk, produced in the Agreste region, the dairy basin of the state of Pernambuco, and supplied to dairys with the Federal Inspection Service (FIS), State Inspection Service (SIS) and the No Inspection Service (NIS).

2 Materials and methods

The study covered eighteen municipalities representing the dairy region of Pernambuco, which accounts for 83.8% of milk production in the state (Instituto Brasileiro de Geografia e Estatística, 2018). The choice of producers was random and for each dairy we collected only one sample of the same producer, per season of the year, according to rainfall. The amount of raw

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milk samples collected, according to the inspection service and the time of collection is described in Table 1.

Before each collection, the raw milk was homogenized and, with the aid of a stainless steel ladle, approximately 200 mL were collected and placed in sterile vials. The ladles were sanitized with distilled water and alcohol at 70% vv before and after each collection, routinely, to avoid cross-contamination. All vials were properly identified with univocal numbering and with necessary information to ensure traceability, such as: vial number, date and time of collection, sample temperature, dairy, type of inspection service, producer and municipality. All samples were placed in styrofoam boxes with ice cubes immediately after collection.

The samples were subsequently frozen in a freezer and sent to the Laboratory of Analysis of Pesticide Residues and Veterinary Medicines (RPM Laboratory), of the National Agricultural Laboratory of Rio Grande do Sul (LANAGRO-RS) to perform the qualitative analysis of multiresidues and quantitative analysis of residue β-lactams by liquid chromatography coupled to mass spectrometry (LC-MS/MS).

2.1 Qualitative analysis of multiresidues by LC-MS/MS

The qualitative analysis of multiresidues was performed according to the procedure described in the Screening Method for qualitative detection of twenty-five residues of antimicrobials from five different groups (sulfonamides, tetracyclines, quinolones, fluoroquinolones and pyrimidine), using LC-MS/MS, a method validated for milk samples and accredited by INMETRO (Laboratório Nacional Agropecuário, 2012).

2.2 Quantitative analysis of residue β-lactams by LC-MS/MS

All steps of the quantitative analysis to detect and quantify the residues of the six β-lactams (ceftiofur, penicillin G, penicillin V, oxacillin, cloxacillin and dicloxacillin) using LC/MS/MS, were carried out by methodology described by Jank et al. (2012).

The analytical parameters of the analytes analyzed in this method are described in Table 2.

3 Results

In the qualitative analysis of multiresidues, the samples did not present violation, indicating that they did not have the analytes studied in values above the MRL. However, six samples (6.5%), collected in the dry period, were positive for oxytetracycline, meaning that these samples presented concentration of this substance above the CCβ ($CC\beta_{oxytetracycline}$ = 25 μg.L^{-1}). The samples identified as positive by the screening method were analyzed in a specific confirmatory methodology for the tetracycline group. Among the six samples that were positive, three had concentrations above CCβ by confirmatory method, but lower than MRL ($MRL_{tetracycline\ group}$ = 100 μg.L^{-1}), which were: two samples from SIS producers (49.7 μg.L^{-1} and 31.4 μg.L^{-1}) and one sample from NIS producers (26.9 μg.L^{-1}); and three samples showed concentrations lower than the CCβ, which were: a FIS producer sample (11.9 μg.L^{-1}), a SIS producer sample (13.5 μg.L^{-1}) and an NIS producer sample (7.1 μg.L^{-1}).

In the quantitative analysis of residue β-lactams, the samples collected in rain period did not present concentrations above the MRL. However, three samples had penicillin G residues in a concentration lower than the MRL, which were: a producer sample from the FIS (0.2 μg.L^{-1}), a producer sample from the SIS (2.0 μg.L^{-1}) and a producer sample from the NIS (0.4 μg.L^{-1}). In the dry period, a producer sample from the SIS (1.1% of the samples collected in this season or 2.8% of the samples submitted to SIS collected in this season) presented cloxacillin residue in a concentration higher than the MRL (464 μg.L^{-1}). This result represents a concentration greater than 15 times the MRL for cloxacillin, according to Table 3.

Table 1. Number of raw milk samples depending on the inspection service and the season of the year.

Inspection Service	Season of the year	
	Rain period	Dry period
FIS	27	28
SIS	36	36
NIS	29	28
Total	92	92

4 Discussion

The results found may indicate the use of good agricultural practices in dairy production, because if the application of antimicrobial occurs during the period when the cow is not in production and the grace period indicated by the manufacturer of the antimicrobial for milk disposal is respected when application occurs in the period that the animal is lactating, the risk of passage of residues of antimicrobials into milk will be low (Andrew et al., 2009; Rossi et al., 2018). However, the results may also indicate low frequency of antimicrobial treatment and also low occurrence of clinical mastitis, which may be related to the low animal productivity (Kayitsinga et al., 2017).

A condition observed during the collections which may also have contributed to these results was the use of natural therapies by some producers, mainly small producers, in the treatment of mastitis, due to the low purchasing power of this group, this fact was also observed by Kayitsinga et al. (2017). On the other hand, the effect of dilution cannot be ruled out (Rassouli et al., 2014; Novaes et al., 2017), which occurs after the mixture of milk free of antimicrobial residue with contaminated milk, which may also reflect the reality found in these samples.

Table 2. Analytical parameters obtained for β-lactams in milk (μg.L^{-1}).

β-lactams	LOD	LOQ	CCα	CCβ
Ceftiofur	10.0	25.0	120.4	147.9
Penicillin G	0.4	1.0	4.7	5.7
Penicillin V	0.4	1.0	4.7	6.1
Oxacillin	3.0	7.5	36.5	53.7
Cloxacillin	3.0	7.5	35.6	52.8
Dicloxacillin	3.0	7.5	36.3	56.6

LOD - Limit of detection, LOQ - Limit of quantification, CCα - Limit of decision, CCβ -Detection capacity. Source: Jank et al. (2012).

Table 3. Maximum residue limit values for antimicrobial groups studied in raw milk.

Group	Analyte	MRL ($\mu g.L^{-1}$)
β-lactams	Penicillin G, penicillin V	4
	Oxacillin, cloxacillin, dicloxacillin	30
	ceftiofur	100
Sulfonamide	Sulfadimetoxine, sulfaquinoxaline, sulfadiazine, sulfatiazole, sulfapiridine, sulfametoxazole, sulfametazine, sulfaclorpiridazine, sulfizoxazole, sulfadoxine, sulfamerazine	100
Tetracycline	Chlortetracycline, tetracycline, oxytetracycline	100
Quinolone	Oxolinic acid, nalidixic acid	20
	Flumequine	50
Fluoroquinolone	Norfloxacin	10
	Sarafloxacin	20
	Danofloxacin	30
	Ciprofloxacin, enrofloxacin	50
	Difloxacin	100

Source: Jank et al. (2012); LANAGRO-RS/MET RPM 10 02 (Laboratório Nacional Agropecuário, 2012).

The low rate of violation of samples verified in this study is in accordance with the results reported by the PNCRC in the years 2010 to 2017, since of the 3,370 milk samples analyzed only 12 (0.36%) were contaminated (Brasil, 2011, 2012, 2013, 2014, 2015, 2016, 2017, 2018). Similar results were also reported by Bilandžić et al. (2011), Rassouli et al. (2014), Prado et al. (2015). However, higher amounts of samples with antimicrobial residues were found by Aalipour et al. (2015), Layada et al. (2016), Orwa et al. (2017), Rama et al. (2017), Wang et al. (2017), Khanal et al. (2018).

5 Conclusion

This study concludes that raw milk produced in the dairy region of the state of Pernambuco has a low rate of violation of the MRL established by the PNCRC, however, a more constant and effective control of inspection services is necessary in order to ensure greater safety for producers and consumers.

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