Commonly screened antibiotics in raw milk from dairy plants under State Inspection in Rio Grande do Sul, Brazil

Antibióticos comumente investigados no leite cru em laticínios sujeitos a Inspeção Estadual no Rio Grande do Sul, Brasil

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Abstract
This study aimed at identifying the commonly screened antibiotics during the dairy processing routine in raw-milk receiving points in plants inspected by the official services in the state of Rio Grande do Sul (RS), from January 2014 to February 2015. Among the 36 participating industries, the most commonly screened antibiotics were beta lactams (100%) and tetracyclines (69%). The antibiotics screened at the milk receiving point were chosen because of the practicality and speed in performing the screening (67%), rather than specific knowledge on which antibiotics the milk suppliers used. (22%).
Keywords: Antibiotic. Dairy microbiology. Food safety. Quality.

Resumo
Este estudo teve por objetivo identificar os antibióticos comumente investigados durante a rotina de processamento do leite cru recebido em laticínios sob Inspeção Estadual no Rio Grande do Sul (RS), entre janeiro de 2014 e fevereiro de 2015. Entre as 36 indústrias participantes, os antibióticos mais comumente investigados foram beta-lactâmicos (100%) e tetraciclinas (69%). A seleção por quais antibióticos investigar no recebimento do leite foi influenciada pela praticidade e rapidez na execução da análise (67%), em detrimento do conhecimento específico sobre quais antibióticos eram utilizados pelos produtores de leite (22%).
Palavras-chave: Antibiótico. Microbiologia do leite. Segurança alimentar. Qualidade.

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Introduction

Milk and dairy products are very important worldwide, mainly due to their socio-economic and nutritional role (KANEKANIAN, 2014). Regarding the latter, it is worth mentioning not only the complete and balanced composition of its nutrients of high biological value, but also the countless health benefits that are consequence of milk consumption, which is recommended for a wide range of age groups (PEREIRA, 2014). For this reason, it is important to minimize the contamination risk of these products, including those that may occur as a consequence of using veterinary drugs in animal production, such as antibiotics. The maximum residue limit (MRL) is the maximum residual concentration of a veterinary drug permitted in food of animal origin, and values for antibiotics are established by the Ministry of Agriculture, Livestock, and Food Supply of Brazil (BRASIL, 1999; MAURICIO; LINS, 2012). The health risks from the ingestion of antibiotics in milk are associated with hypersensitivity, anaphylactic shock, increased antibiotic resistance, genotoxicity, aplastic anemia, intestinal microbiota imbalance, and secondary collateral effects, depending on the specific residues present in milk (BEYENE, 2016). Thus, MRLs are regarded as a monitoring tool to reduce human health risks associated with the use of antibiotics or other veterinary drugs in animal husbandry (BOURIN; CLÉNET, 2014).

Brazil is currently the fifth milk producer of the world, and Rio Grande do Sul, the southernmost state of Brazil,

is responsible for about 15% of the national production (USDA, 2015). In Rio Grande do Sul, the Secretariat of Agriculture, Livestock, and Irrigation (SEAPI), through the Department of Inspection of Products of Animal Origin (DIPOA) is competent to perform official sanitary surveillance in industrial facilities that perform inter-municipal trade (BRASIL, 1989). DIPOA operates under the scope of the Federal Normative Ruling No. 62 – MAPA (BRASIL, 2011) and the State Resolution No. 0001/15 (RIO GRANDE DO SUL, 2015), which predict the daily quality control of refrigerated raw milk in industrial facilities, through screening for antibiotic residues and other microbial growth inhibitors, according to the MRLs recommended by the Brazilian National Plan for Control of Residues (BRASIL, 1999; MAURICIO; LINS, 2012).

Nevertheless, due to lack of specific guidelines of the above-mentioned legislation, the decision of which antibiotic groups will be tested at reception of the raw milk in the industrial plants during the daily evaluation routine remains the companies'. Qualitative, semi-quantitative, or quantitative tests may be used for the detection of antibiotics in milk. Qualitative tests are considered screening tests, giving positive or negative results, whether or not a certain drug is above the detection limit of the test, but does not allow quantification (WANG et al., 2012). Among the available methods for the detection of antibiotics in milk, it is observed that the qualitative tests are widely used in dairy plants (TRONCO, 2010). In view of these facts, synchrony between production farms and industrial plants should occur, so that the antimicrobials used in animal production should be at a minimum – the same as those screened at the industry level – in the milk received for human consumption.

Thus, this work aimed at investigating which antibiotics are commonly screened in daily routines, and why these are the selected antimicrobials tested at raw-milk receiving points in industrial plants, under State inspection, in Rio Grande do Sul (RS), from January 2014 to February 2015.

Materials and Methods

An exploratory descriptive study was performed, from January 2014 to February 2015, and the data collection was performed through a questionnaire, comprising 4 questions: (1) Classification of the plant; (2) Group of antibiotics screened at the raw-milk reception point; (3) Reason for the company's selection of a specific test for the detection of antibiotics; and (4) for how long has the company performed the antibiotic screening described above in item (2). This questionnaire was applied to 36 dairy companies, which agreed to participate in this work, of the 57 plants in activity and registered in the DIPOA, distributed among the 7 meso-regions of the State of Rio Grande do Sul, representing a 63% response rate from the searched plants.

The data obtained were tabulated in a structured spreadsheet, being quantified and analyzed according to the number of mentions for each response. Figure 1 shows the distribution of the companies under inspection by the Official State Veterinary Services and those that participated in this study, within the 7 meso-regions.

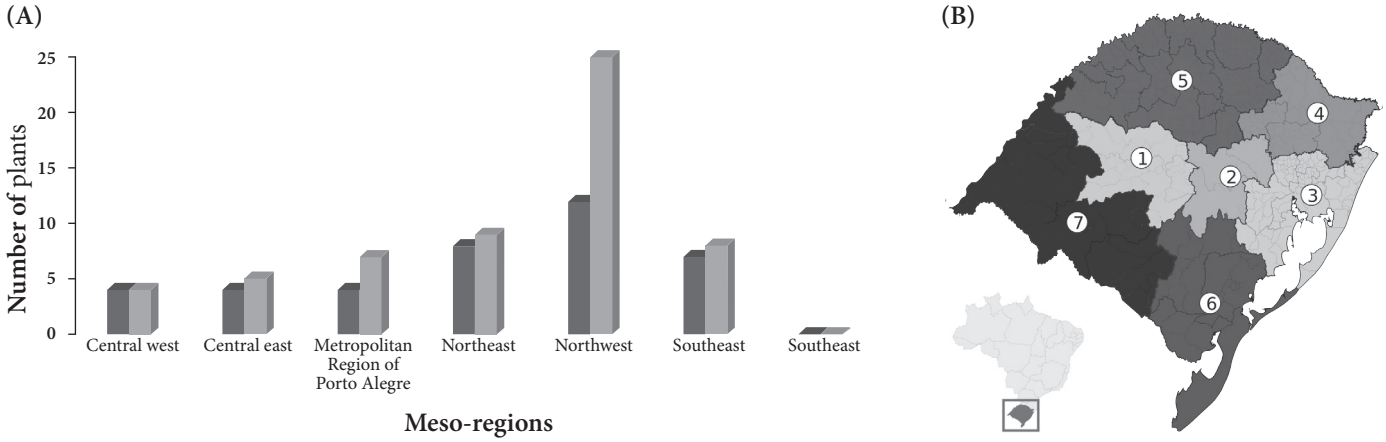

Figure 1 – (A) Distribution of registered and active dairy plants at DIPOA/SEAPI, from January 2014 to February 2015, among the 7 meso-regions of Rio Grande do Sul state. Black bars correspond to the participating plants and grey bars to all the existing plants. (B) Region under study in Brazil, which includes the state of Rio Grande do Sul (in black, inset map). Subdivision of the above-mentioned meso-regions defined by the Brazilian Institute of Geography and Statistics: (1) Southwest, (2) Central West, (3) Northwest, (4) Northeast, (5) Central East, (6) Metropolitan region of Porto Alegre, and (7) Southeast

In the dairy plants under study, we observed the use of qualitative antibiotic detection kits (screening tests), which varied the detection principle and the manufacturer laboratory, according to the choice of each plant. The antibiotic detection tests that were identified during the present study were manufactured by the following laboratories: CHR Hansen Holding A/S (Denmark), Idexx Laboratories Inc. (USA), Neogen Corporation (USA), Unisensor Diagnostics Engineering (Belgium), and Zeulab S. L. (Spain).

Results and Discussion

The analysis was based on the classification of the 36 dairy plants participating in this survey, which were under inspection by the Official State Veterinary Services in the State of Rio Grande do Sul, and subsequently, results were analyzed from the data obtained using the questionnaire.

The classification of the participating facilities is depicted in Figure 2. It was observed that dairy plants (83%) are predominant among the interviewed industrial plants. This type of plant has authorization to receive the milk and perform dairy food processing such as fermented dairy products (RIO GRANDE DO SUL, 1999). In this case, the presence of antibiotic residues in milk, used as raw material to produce cheese and yoghurt, among others, is absolutely unwanted, taking into account the risk of heightened losses to the industry due to technological problems caused by the inhibition of lactic acid bacteria involved in the fermentation process (LAWLEY et al., 2012).

Thus, the participating companies were asked which group of antibiotics were screened at the raw-milk receiving points, from six possible options (beta-lactams, tetracyclines, sulphonamides, aminoglycosides, macrolides, others), offering the option of multiple-choice responses and including groups not cited using the open field. The results are presented in Figure 3. In Figure 3A, data were quantified by repetition, i.e., how many times each group was cited, and in Figure 3B responses were grouped according to multiple-choice responses from the participating companies.

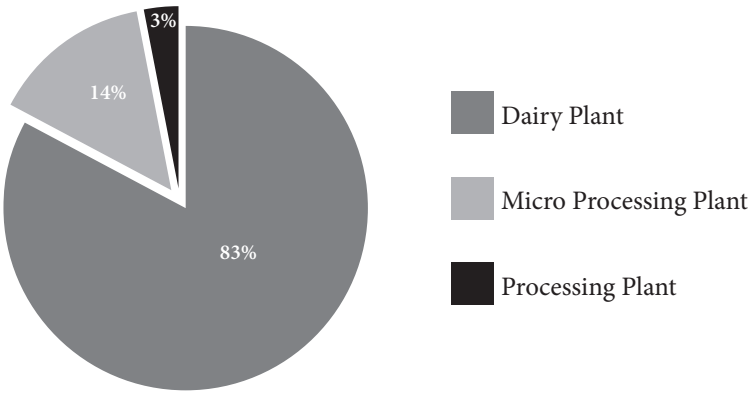

Figure 2 – Distribution of the participating dairy plants under state inspection of the DIPOA/SEAPI, according their industrial classification

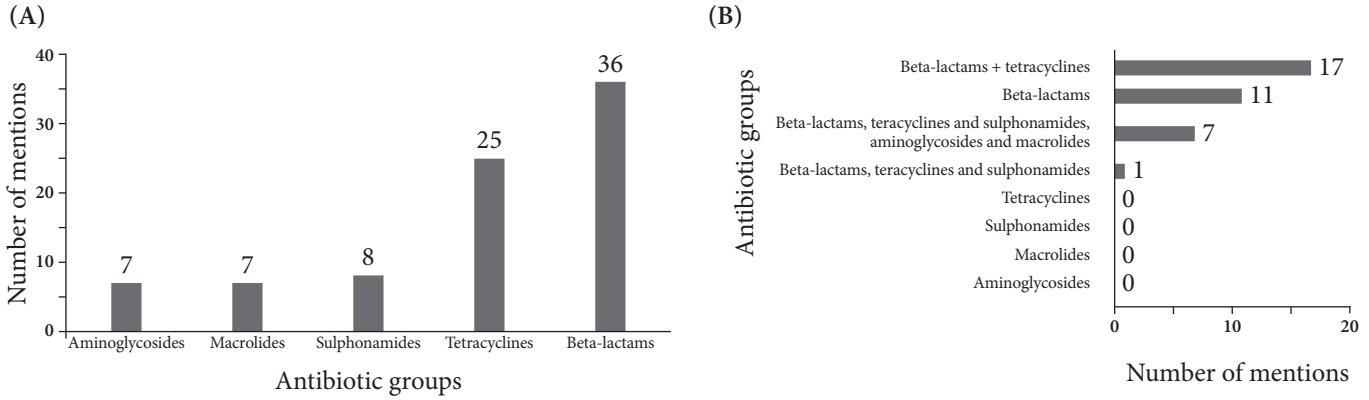

Figure 3 – Antibiotic groups screened in raw milk in dairy plants under state inspection by the DIPOA/SEAPI. Data were quantified (A) by repetition of citation or (B) according to simultaneity of screening by the antibiotic detection test

It can be observed that the beta-lactams and tetracyclines were the most frequently screened antibiotics in the raw-milk evaluation routine at the dairy plants under official sanitary surveillance in Rio Grande do Sul. The simultaneous screening of both these antibiotic groups was reported by 17 (47%) of the interviewees. On the other hand, when considering repeated citation, beta-lactams were screened by 36 (100%) plants, out of which 11 (31%) screened exclusively this antibiotic group, while tetracyclines were screened by 25 (69%) plants. Among the pathologies that can affect dairy herds, mastitis stands out, and is a common cause for veterinary-drugs utilization in farms, with consequent presence of antibiotic residues in milk (WYDER et al., 2011; BRUNTON et al., 2012). In mastitis treatment, beta-lactams comprise the first choice of antimicrobials used against streptococci and staphylococci susceptible to penicillin (PYÖRÄLÄ, 2009). Tetracyclines are used in systemic therapy, including treatment against coliform infection (CHOPRA; ROBERTS, 2001), which has a high incidence in cows with clinical mastitis in Brazil (OLIVEIRA et al., 2015). Therefore, the dairy plants should have concerns regarding these residues in milk.

Amongst the antimicrobials used in dairy herds in the State of Paraná, the beta-lactams, followed by aminoglycosides and tetracyclines, were the most frequently used in dairy farms (NETTO et al., 2005), and the presence of tetracycline residues was detected in samples of pasteurized milk (PRADO et al., 2015). Furthermore, a study performed in the metropolitan region of Rio de Janeiro revealed that oxytetracycline, belonging to the tetracyclines group, was the most commonly used in lactation herds by the farmers of that region (SPISSO et al., 2010). In international context, concerns regarding the beta-lactams and tetracyclines are the same as in Brazil. For example, in the USA, the Pasteurized Milk Ordinance (PMO) emphasizes the test for the presence of antibiotic residues from the beta-lactam group, and requires sample collection directly in farms or in industrial facilities for the presence of at least six antimicrobials belonging to this group (specifically penicillins, ampicillin, amoxicillin, cloxacillin, cephapirin, and ceftiofur) (FDA, 2015). In Bosnia, 95% of the detection tests used in dairy plants are for screening beta-lactams, considering the broad use of this group of antibiotics to treat mastitis in the herds (FEJZIC et al., 2014). On the other hand, tetracyclines comprise the most used antimicrobials in Africa and, consequently, those antibiotics were the most commonly found in foods of animal origin (DARWISH et al., 2013). Both beta-lactams and tetracyclines are frequently reported as drugs of choice for the treatment of infections affecting dairy herds and, probably for this reason, these antimicrobials receive more attention from the dairy plants.

Moreover, we investigated the reason and perception of the dairy industry in choosing the test routinely used for screening antibiotics in raw milk. Four alternatives were offered, based on the observations made by official inspection service: price of the detection test, knowledge of the antibiotics used in their mil-supplying farms, guidance of the technician responsible for the dairy plant, and others, allowing multiple-choice responses and the addition of other reasons not cited using the open-field description. Figure 4A depicts the answers that were individually quantified according to how many times each reason was cited, and other reasons that were not listed in the items offered in the questionnaire. Furthermore, in Figure 4B, the responses were grouped according to the multiple-choice responses of each participating plant.

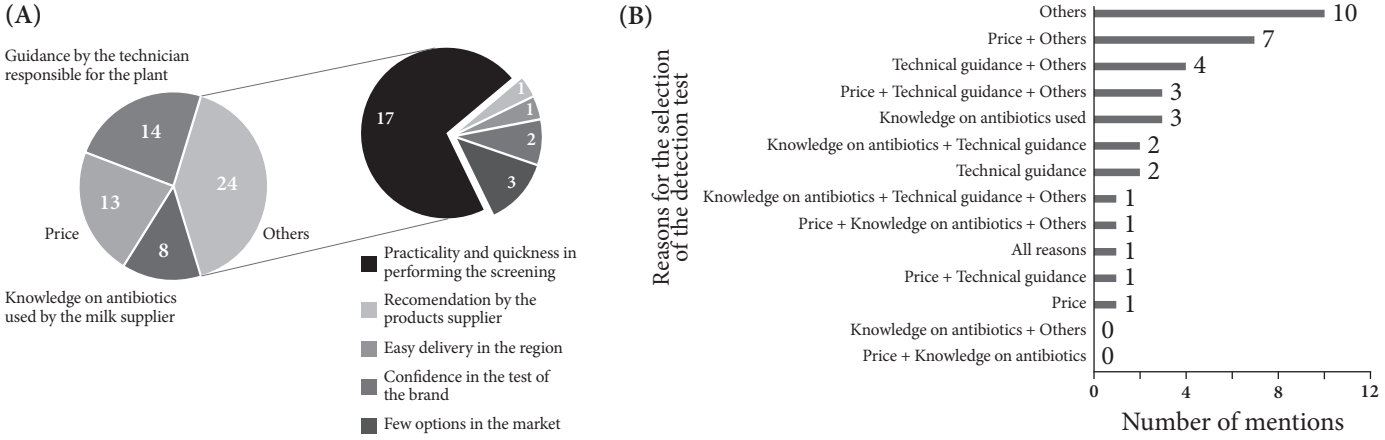

Figure 4 – Reasons for the selection of the antibiotic-detection tests in raw milk in dairy plants under state inspection of DIPOA/SEAPI. Data were quantified (A) by repetition of citation, detailing the other options described, according to the open field or (B) according to multiple-choice responses from each company participating in the study

It was found that for 24 (67%) plants, the reasons for the selection of the test for antibiotic detection is based on factors other than those listed in the survey (Figure 4A and 4B). Practicality and speed in performing the test were the characteristics searched for the acquisition of the test (Figure 4A). Depending on the test used, there is a significant variation of the time required for analysis, since it may vary from few minutes to hours. Thus, the use of simplified and quick tests for antibiotic residue detection is of extreme importance for the plants, and are those which are preferred, since they allow the plant to quickly define the destination of the milk batches (NERO et al., 2007). Furthermore, for 14 (39%) plants interviewed the guidance given by the technician responsible for the plant, followed by the price of detection (36%), which may vary by more than 1000% depending on the test used, were the reasons for the choice of the tests in the plants (Figure 4A). In addition, it was shown that knowledge of the antibiotics used by the milk suppliers represented a concern for only 8 (22%) interviewees, demonstrating a lack of synchrony between these two points of the milk-processing chain.

It is important to emphasize that the dairy plants have used imported qualitative tests. Broad-spectrum screening tests have the ability to detect most of the major classes of antibiotics used in veterinary medicine. However, MRLs may vary between countries and therefore certain screening tests would not be able to detect all antibiotic compounds at the specific concentrations required in each country (MAURICIO; LINS, 2012; WANG et al., 2012). Regarding this, the scope of description of certain tests indicates the detection of antibiotics only above the MRL allowed by the national legislation, which can generate false-negative results. The opposite could be also identified, such as the detection of antibiotics even below of the stipulated MRL, which may lead to false-positive results. In addition, the screening tests often analyze an antibiotic group, but MRL values are determined for individual compounds. Therefore, a careful interpretation of the results obtained with qualitative tests is imperative.

From the public health standpoint, detection of concentrations below MRLs by screening tests is interesting because it minimizes the risk of antibiotics reaching the consumer through contaminated milk. However, from the commercial point of view, producers could be penalized and the milk discarded, though the concentrations of antibiotics be within the permitted MRL range (TENÓRIO et al., 2009). Therefore, it is necessary to analyze the suspect and positive samples using confirmatory methods.

Furthermore, the period in which the screening for a specific antibiotic was carried out in each company was also assessed. The results are demonstrated in Figure 5, with the screening of the same antibiotic groups for a period greater than 3 years being performed in 21 (58%) plants. This information suggests that there is no intention of screening other substances than those commonly screened.

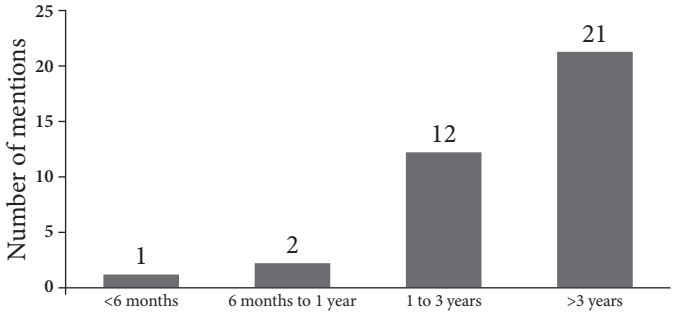

Figure 5 – Period in which the same group of antibiotics was screened in raw milk by the dairy plants under state inspection of DIPOA/SEAPI

Conclusion

According to the data obtained in the present study, we concluded that the antibiotics belonging to beta-lactam and tetracycline groups were commonly screened at raw-milk receiving points in dairy plants under state inspection in Rio Grande do Sul, Brazil. However, in the majority of industries the choice of screening these antimicrobials was not associated with prior knowledge about which antimicrobials were used in the milk-supplier farms. Therefore, it is of the utmost importance to encourage the internal control programs in the dairy plants, in order to prioritize the antimicrobials used in dairy herds that supply

the raw milk, thus preventing failures in the identification of residues with consequent losses to the dairy industry and risk to public health.

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Authors' Disclaimer

The authors are staff members from different organizations, however they alone are responsible for the views expressed in this article and do not necessarily represent the decisions or the stated policy of their organizations.

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