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Investigation of Beta-lactam Group Antibiotics Residue in Raw Cow Milk in Sivas Province

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ABSTRACT

In this study, beta-lactam group antibiotic residues were investigated in raw cow's milk. For this purpose, a total of 86 raw milk samples were used as material. Samples were collected periodically from farms in various outlets and surrounding villages in the province of Sivas. Beta-lactam group antibiotic levels in raw cow's milk were determined by ELISA method with commercial test kit. According to the analysis results, beta-lactam levels were between 0.35-0.70 ng/mL in 64 (74.4%) and between 0.71-3.7 ng/mL in 22 samples (25.6%) determined of 86 raw cow milk samples. The residue levels detected in the samples are in accordance with the legal limits declared by the European Union (EU) commission and Turkish Food Codex Communique.

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Introduction

Beta-lactam antibiotics are widely used in veterinary medicine for the treatment of infectious diseases, especially mastitis. Penicillin's, monobactams, cephalosporins, carbapenems and beta-lactamase inhibitors (clavams) are included in beta-lactam antibiotics. Beta-lactams are broad-spectrum antibiotics that have bactericidal effects on Gram-negative and Gram-positive bacteria. Antibiotics in this group act by inhibiting bacterial cell wall synthesis Darwish et al., 2013; Serraino et al., 2013; Jayalakshmi et al., 2017; Bacanlı and Başaran, 2019).

Uncontrolled and incorrect using antibiotics in animals with a value of food causes residues in the products obtained from these animals, significant health problems in humans and economic losses. In dairy animals, the consumption of milk before the legal waiting period following the last antibiotic application poses a potential danger to the consumer, especially infants and children. Conditions such as the type of antibiotic used, dose, route of administration, duration of use, disease status in the udder, milking interval and frequency may affect the washout period. Since the application of antibiotics directly to the udder causes more residues to be observed in the milk taken, it is inconvenient to consume the milk taken from the treated animals within 72-96 hours (Üçüncü, 2013; Coşkun and Şanlı, 2016; Kaya, 2018; Yarsan, 2018; Yılmaz et al., 2018).

It has been reported that antibiotic residues can cause development of bacterial resistance, carcinogenic, teratogenic, and mutagenic effects beside the deterioration of intestinal flora, allergic reactions, suppression of the immune system, anaphylactic shock, nervous disorders. Beta-lactam antibiotics, especially penicillin and cephalosporins, can cause allergic reactions such as skin rashes, allergic dermatitis, and anaphylaxis (Nisha, 2008; Darwish et al., 2013, Tayar and Yarsan, 2014; Yarsan, 2018; Yılmaz et al., 2018; Irkin and Batu, 2019; Sachi et al., 2019; Ture et al., 2019; Küçükbüğrü and Acaröz, 2020; Yavuz et al., 2020).

Milk containing antibiotic residues causes production errors by preventing the reproduction of bacteria and starter cultures that are effective in the fermentation of products such as yogurt, cheese, and butter in the dairy industry. It is reported that processes applied to milk such as pasteurization can destroy the antibiotic to a certain extent, but not completely (Darwish et al., 2013; Üçüncü, 2013; Yılmaz et al., 2018; Irkin and Batu, 2019; Yavuz et al., 2020).

The European Union (EU) commission determined the Maximum Residue Limit (MRL) of penicillin-derived drugs in milk of all food-produced species at 4 ppb for ampicillin, amoxicillin and benzylpenicillin: 30 ppb for cloxacillin, dicloxacillin, nafcillin and oxacillin (EU, 2010).

In Turkey, legal regulations regarding antibiotic residues in animal foods are determined in the "Regulation on Classification of Pharmacological Active Substances that May Be Found in Animal Foods and Maximum Residue Limits". In the regulation, the same MRL values are given for penicillin-derived drugs (ampicillin, amoxicillin, benzylpenicillin, cloxacillin, dicloxacillin, nafcillin, oxacillin) in milk (all food-derived species) in line with EU legislation (TGK, 2017).

In this study was conducted to investigate the beta-lactam group antibiotic residues in cow's milk offered for sale at various sales points and farms in the surrounding villages in Sivas, and to evaluate the data in terms of compliance with standards and public health.

Material and Method

Material

In this study, beta-lactam group antibiotic residues were investigated in raw cow's milk. For this purpose, a total of 86 raw milk samples were used as material. Samples were collected from farms in various outlets and surrounding villages in the province of Sivas as periodically. Samples taken into sterile tubes under aseptic conditions were brought to the laboratory by applying cold chain and analyzed on the same day. Milk samples were kept in the refrigerator (+4).

Method

Beta-lactam group antibiotic levels in raw cow's milk were determined by ELISA (Enzyme Linked Immunosorbent Assay) method. Commercial test kits (RANDOX) were used in the analysis. The procedures were performed in accordance with the kit procedure. Arithmetic mean, standard error, percentage, minimum, maximum values of beta-lactam antibiotics detected in the samples were analyzed in the statistical program as SPSS 22.00 (SPSS, 2014).

Preparation of Samples for Analysis

The total of 5 mL of the homogenized milk sample was taken into a test tube and incubated for 30 min at 4°C. The milk samples were then centrifuged at 2000 rpm for 15 min. After centrifugation, 0.5 mL of the milk serum remaining under the fat layer was transferred to an eppendorf tube and analyzed.

Table 1. Beta-lactam levels and % distribution in raw cow milk

Beta-lactam (ng/mL)	n	%	Min.	Max.	Mean± SE
0.35-0.70	64	74.4	0.35	0.70	0.48±0.01
0.71-3.7	22	25.6	0.71	2.37	0.90±0.08
Total	86	100	0.35	2.37	0.59±0.03

Test Procedure

Standards (0.0, 0.37, 0.73, 3.7, 7.3, and 36.6 ng/mL) and samples amount of 50 µL were added to each well into the microplate. The amount of 75 µL of conjugate was added to it and incubated for 60 minutes at room temperature in the dark. After this period, the wells were washed 6 times with diluted washing solution. After washing, the amount of 125 µL of substrate was added to each well. The microplate covered and incubated for 20 minutes at room temperature in the dark. At the end of this period, 100 µL of stop solution was added to all the wells and the transformation of blue color to yellow was observed. Finally, the absorbances of the standards and samples were measured in an ELISA device at a wavelength of 450 nm. For the calculation of the absorbance values obtained, a calibration curve was created and the absorbance of the samples versus the beta-lactam levels were calculated in ng/mL.

Results

The levels and % distribution of beta-lactam group antibiotics in raw cow's milk are given in Table 1.

According to the analysis results, beta-lactam levels were between 0.35-0.70 ng/mL in 64 (74.4%) and between 0.71-3.7 ng/mL in 22 samples (25.6%) determined of 86 raw cow milk samples.

Discussion

Milk and milk products consumption are among the important elements in a healthy and balanced diet, providing appropriate growth and development. It is the first food for mammals and provides the necessary energy and nutrients, which are very important for the formation of bone mass. In addition, as in childhood, milk has an important place in the human diet in adulthood (Pereira, 2014). Medicines such as hormones and antibiotics are widely used to treat diseases in animals. More antibiotics are needed to prevent various diseases that also occur in animals. However, it is very important to reliably detect the residues of antibiotics used in animal foods (Lee et al., 2007). Resistance to different antibiotic categories is more common in strains derived from farm animals than in strains derived from domestic animals. β-lactam resistance is more common in domestic mammals (Butaye et al., 2001). The incidence of beta-lactam-resistant infections is increasing due to the production of various enzymes by organisms (Shaikh et al., 2015).

Al-Zuheir (2012) found antibiotic residues in 7 samples (20.6%) in the analysis of 34 raw milk samples collected from farms in Palestine with rapid screening test kit. Of these samples, 4 were positive for beta-lactam and 3 for tetracycline. Zheng et al. (2013) analyzed of 199 raw milk samples taken from collection tanks in China via competitive ELISA, they found beta-lactam residues in 0.5%, quinolones in 47.2% and sulfonamide residues in 20.1%. Beta-lactam level was found above the legal limit in 1 sample. Ibraimi et al. (2013) found beta-lactam residues in 64 samples (50.4%) in the analysis of 127 milk samples collected from farms in Kosovo via ELISA method. The residue level was found above the limit value (2-80 µg/ml) in 15 samples (11.6%). Ghanavi et al. (2013)

found beta-lactam residues (penicillin G) in 236 samples (23.8%) in the analysis of 992 raw milk samples collected from farms in Iran. They were tested by using rapid detection test kit. The residue level was found to be 1.2 µg/mL. Mokhtari et al. (2013), in the analysis of 79 milk samples collected from different cities in Iran, found beta-lactam residues in 32.9% of the samples.

It was determined that various antibiotic residues were detected in the milk samples examined and the residue level was above the legal limit in many samples in studies conducted in countries. In this study, beta lactam group antibiotics were detected in 86 raw milk samples, although it was found to follow the legal limits reported by the European Union Commission and Turkish Food Codex Communiqué.

Abebew et al. (2014) found antibiotic residues in 48 samples (12%) in the analysis of 400 milk samples collected from farms in Ethiopia via HPLC method. Penicillin G levels were found to be between 0-28 µg/L. Shata et al. (2015) found ampicillin residues in 7 samples (53.8%) and penicillin G residues in 2 samples (15.3%) in the analysis of 55 raw milk samples in Egypt via HPLC method. Residue levels (mean) were determined as 902.72 µg/L and 9.54 µg/L, respectively. Chowdhury et al. (2015) in the analysis of 200 milk samples collected from local and commercial dairy cows in Bangladesh by TLC and HPLC method, they found amoxicillin residues in 14% of local milk and 38% of commercial milk. The residue level was determined as 9.84 µg/ml and 56.16 µg/ml, respectively. Ahlberg et al. (2016) analyzed of 480 raw milk samples taken from farms in Kenya by HPLC method. They found antibiotic residues in 9% of the samples. Beta-lactam ratio was determined as 5%, sulfonamide 2.5% and tetracycline 0.6% in the samples. Olatoye et al. (2016) in the analysis of 328 raw milk samples in Nigeria by HPLC, they found penicillin G residues in 41.1% of the samples. The residue level was determined as 15.22 µg/L. Rama et al. (2017) in the analysis of a total of 1734 raw milk samples taken from different farms and outlets in Kosovo, antibiotic residues were detected in 106 samples (6.11%) by enzyme linked receptor binding assays and HPLC method. The residual levels of amoxicillin, penicillin G and cloxacillin were determined between 2.1-1973 µg/kg, respectively. Layada et al. (2016) found antibiotic residues in 127 samples (65.4%) in the analysis of 194 milk samples (raw and fermented) collected from farms and markets in Algeria via LC/MS/MS method. Contamination rate was higher (100%) in raw milk. Worku et al. (2017) reported that in the analysis of 100 milk samples collected from different farms in Ethiopia, they detected penicillin residues in 36% of the samples by microbial inhibition test. Han et al. (2017) in the analysis of 192 raw milk samples collected from different milk stations in China, they detected multiple antibiotic residues in 12 samples (6.25%). Penicillin G ratio was 1.04%, sulfacetamide 0.52%, trimethoprim 3.13% and lincomycin 1.56% by ultraperformance liquid chromatography with tandem mass spectrometry.

When the findings of this study and the studies in the countries where it was conducted are compared, it is seen that the minimum and maximum beta lactam levels in raw milk samples are lower than the levels of the researchers.

Şen (2013) reported that in the analysis of 100 raw milk samples taken from producers in Ankara and its region via HPLC method. They were not found in any of the sample's penicillin residues (cloxacillin and dicloxacillin). In this study, beta lactam group antibiotic residue was found in 86 raw milk samples.

Aycan and İnce (2018) found beta-lactam residues in 42 samples in the analysis of 80 raw milk samples collected from the Afyonkarahisar region via ELISA method. The residue level was found above the limit value declared in the regulation in 7 samples. Khanal et al. (2018) in the analysis of 140 raw milk samples collected from different sources in Nepal (South Asia), they found residue of amoxicillin in 81% (68-802 µg/kg), sulfadimethoxin in 41% (31-69 µg/kg), penicillin G in 27% (13-353 µg/kg) and ampicillin in 12% by HPLC method. Moudgil et al. (2019) in the analysis of 492 raw milk samples taken from farms in India, they detected antibiotic residues (enrofloxacin, oxytetracycline, tetracycline, sulfametazole) in 78 samples (16%). The residue level was found above the limit value in 20 samples (4%) by HPLC method. Rahman et al. (2021) found antibiotic residues in 7% (14 samples) of raw milk in the analysis of 200 raw milk collected from farms and processed packaged milk samples (300 samples in total) taken from markets in Bangladesh using thin-layer chromatography (TLC) and ultra-high-performance liquid chromatography (UHPLC) methods. Gentamicin residues were detected in 4%, amoxicillin in 2%, oxytetracycline in 3.3%, streptomycin in 1.3%, gentamicin and ceftriaxone in 0.6% of positive samples. The mean oxytetracycline level in the samples was 61.29 µg/L, and the amoxicillin level was 124 µg/L. No antibiotic residues were found in processed milk samples. Zeghilet et al. (2022) in the analysis of 109 raw milk from raw milk collectors in Constantine, Algeria, and 13 pasteurized milk samples collected from reconstituted pasteurized milk outlets, they detected antibiotic residues in 13 samples (10.66%). Beta-lactam residues were detected in 12 raw milk (9.84%) and 2 pasteurized milk samples (1.64%), and tetracycline residues in 1 raw milk sample (0.82%) by using rapid detection test kit.

In this study, beta-lactam group antibiotic residues were found in all samples analyzed. However, it was determined that the residue in the milk was below the legal limits. By the way the presence of antibiotic residues in all samples indicates the widespread use of beta-lactam antibiotics. Consumption of milk by people of all ages, especially infants and children; in addition, with economic value in the dairy industry due to its nature as an animal product, the detection of antibiotic residues in milk poses a risk for public health. For this reason, the milk of animals treated with antibiotics should not be offered for consumption until the antibiotic purification process. In this regard, veterinarians should clearly inform the animal owner about the antibiotic they are applying. And unnecessary use of antibiotics should also be avoided.

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