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Original Article

Assessment of antibacterial drug residues in milk for consumption in Kosovo

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ARTICLE INFO

Article history:
Received 26 April 2016
Received in revised form
25 June 2016
Accepted 22 July 2016
Available online 5 November 2016

Keywords:
antibacterial residues
detection method
Kosovo
milk
public health

ABSTRACT

The objective of this study was to assess the occurrence of drug residues in the raw milk collected from individual farms and milk collection points during 2009–2010 in six different major regions of Kosovo (Prishtinë, Gjilan, Mitrovicë, Pejë, Gjakovë, Prizren). In the present study, a total of 1734 raw milk samples were collected, and qualitatively screened with two different tests, the Delvotest SP assay and an enzyme-linked receptor-binding assay (SNAP). Overall, 106 (6.11%) out of 1734 samples examined with Delvotest SP contained possible drug residues (5.12% and 7.51% of samples from 2009 and 2010, respectively). All suspect samples were further analyzed by three distinct enzyme-linked receptor-binding assays specific for β-lactams (new β-lactam test), tetracyclines (SNAP tetracycline test), and sulfonamides (SNAP sulfamethazine test). Only the new SNAP β-lactam test detected residues in 40 out of 52 samples in 2009 and 54 out of 54 suspect samples in 2010. A confirmatory method based on liquid chromatography-tandem mass spectrometry was used to confirm the presence of β-lactam drug residues in samples detected by the enzyme-linked receptor-binding assay. Amoxicillin, penicillin G, and cloxacillin were the most frequently detected residues and were in a concentration range between 2.1 μg/kg and 1973 μg/kg. Seventeen of the positive samples exceeded the maximum residue levels for one or more β-lactam drug. The highest number of positive milk samples came from the Pejë Region (58.8%) and Gjakovë Region (23.5%), and the lowest number of positive samples originated from Gjilan (5.88%), with no positive samples detected in two regions, Mitrovicë and Prizren.

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http://dx.doi.org/10.1016/j.jfda.2016.07.007

1. Introduction

Antibiotic residues in milk are of great concern to dairy farmers, milk processors, regulatory agencies, and consumers. In lactating cows, antimicrobial agents are used mostly for the therapy of mastitis but are used to treat other diseases as well. Today, antimicrobial drugs are used to control, prevent, and treat infection, and to enhance animal growth and feed efficiency [1]. Currently, approximately 80% of all food-producing animals receive medication for part or most of their lives [2]. The most likely cause of violative drug residues is the failure to observe proscribed withdrawal times [3–6]. The presence of antimicrobial residues in milk can engender drug hypersensitivity reactions in milk consumers, manifested as dermal reactions, asthma, or anaphylactic shock [7–12]. Antimicrobial drugs can also interfere with the manufacture of dairy products, decrease acid and flavor production associated with butter manufacture, reduce the curdling of milk, and cause improper ripening of cheeses [13,14]. Finally, the use of antibiotics can give rise to an increase in antibiotic resistance of pathogenic bacteria and contribute to a global health crisis [15,16].

In many countries, governmental authorities have established monitoring programs to determine the antibiotic levels in food and set a maximum residue level (MRL) for these drugs. In the European Union, veterinary drug residue monitoring is enforced according to the requirements set down in Council directive 96/23/EC [18] and Commission Decision 97/747/EC [19], and the MRLs were fixed according to Regulation 470/2009/CE [20] and Regulation 37/2010/UE [21]. The Kosovo program of monitoring residues in live animals and animal products has been in place since 2005. Various analytical methods in detecting antibiotic residues in milk have been reported in the literature [17,22]. Microbiological growth inhibition, enzyme-linked immunosorbent assays, and chromatographic methods are the most commonly used [23,24].

Kosovo's dairy sector is one of the key sectors in the development of agriculture and continues to recover after the war in 1999, when at least half of livestock production was depleted. Milk production is widespread throughout Kosovo, with more than 25 dairy processing companies in operation [25]. These dairies produce some 381,896 tons of milk annually, and 58,563.45 tons are imported. The market value of locally produced milk was €35,934,158 and from imports it was €32,463,988 [26]. In Kosovo, there is currently no monitoring of drug residues in milk. Hence, there are no data concerning the presence of antibiotic residues in milk produced and marketed in Kosovo. The present study was therefore designed to assess the presence of antimicrobial drug residues in raw milk marketed at different regions of Kosovo.

2. Methods

2.1. Samples

A total of 1734 milk samples from individual farms and milk collection points were collected over a 2-year period (April–October 2009 and February–November 2010) from six major regions of Kosovo (Prishtinë, Gjilan, Mitrovicë, Pejë, Gjakovë, Prizren). In 2009, a total of 1015 milk samples were collected, 826 samples from milk collection points and 189 samples from individual farms. In 2010, in total of 719 milk samples were collected, 635 samples from milk collection points and 84 samples from individual farms. All milk samples were stored at 4°C until analysis. For additional investigations, drug-positive milk samples were stored at −20°C for 3 weeks.

2.2. Screening methods

Antimicrobial drug screening tests were performed at the Kosovo Food and Veterinary Agency in Prishtina, Kosovo. The screening tests used were the Delvotest SP assay supplied by DSM (DSM Food Specialities, Dairy Ingredients, Delft, The Netherlands), and enzyme-linked receptor-binding assays (SNAP tests) provided by IDEXX Lab. Inc. (Westbrook, ME, USA). All drug-positive samples detected by the Delvotest SP were checked with enzyme-linked receptor-binding assays specific for β-lactams, tetracycline, and sulfonamides. Positive samples confirmed by SNAP test were further quantitatively analyzed using liquid chromatography-tandem mass spectrometry (LC-MS/MS).

2.2.1. Reagents and standard solutions for screening tests

Penicillin G (PNG) potassium salt and sulfamethazine were obtained from Fluka (St. Louis, MO, USA), Tetracycline hydrochloride was obtained from Sigma (St. Louis, MO, USA). For the preparation of negative control, drug-free milk from cows that had not been treated with an antibiotic for at least 30 days was collected. The milk was collected from the experimental farm of the Agriculture and Veterinary Faculty (Prishtina, Kosovo).

A stock solution of PNG potassium was prepared in a 100-mL volumetric flask, adding 11.17 mg penicillin and distilled water to the target volume. From this solution, 1 mL (100 μg penicillin/mL) was diluted 100-fold with distilled water (i.e., to a final concentration of 1 μg penicillin/mL).

For preparation of drug-spiked milk samples, drug concentrations of $\geq$ 40 μg/L of milk were prepared by adding the appropriate amount of stock solution directly to milk samples. The equivalent volume of milk was removed prior to adding the appropriate volume of stock solution. The amount added was always $<$ 0.5% of the total volume. Penicillin-G potassium was present at a final concentration of 4 μg/L for the positive control sample, whereas tetracycline and sulfamethazine were added to milk to achieve final concentrations of 60 μg/L and 100 μg/L, respectively.

2.2.2. Delvotest SP microbial test

The qualitative analysis of PNG residues in milk was performed using the Delvotest SP assay as described by Suhren and Beukers [27]. This method is based on the susceptibilities of bacteria to different antibiotics. The method was carried out according to the instructions by the manufacturer.

2.2.3. Enzyme-linked receptor-binding assays (SNAP tests)

Positive samples found by Delvotest SP were subjected to further testing with enzyme-linked receptor-binding assays (SNAP tests). The New SNAP Beta-lactam Test Kit, SNAP

tetracycline test, and SNAP sulfamethazine test were used to screen antibiotic residues in milk. The SNAP tests were performed in accordance with the manufacturer's instructions.

2.3. Confirmatory methods

2.3.1. Reagents and analytical standards

High performance liquid chromatography (HPLC)-grade methanol, acetonitrile, *n*-hexane, and acetic acid were obtained from Sigma-Aldrich (St. Louis, MO, USA). Water was HPLC grade and was prepared with a Milli-Q system (Millipore, Bedford, MA, USA). Sodium phosphate buffer (0.05M, pH 7.5) was prepared by dissolving 0.73 g of NaH_2PO_4 dihydrate and 3.61 g of Na_2HPO_4 monohydrate (Sigma-Aldrich) in 500 mL water. Ammonium acetate (0.05M) was prepared by dissolving 1.92 g of ammonium acetate in 500 mL water and adjusted to pH 7.5 by addition of ammonium hydroxide.

Analytical standard-grade (VETRANAL) amoxicillin trihydrate (purity 99.3%), ampicillin trihydrate (purity 99.7%,), PNG sodium salt (purity 99.4%,), cloxacillin sodium salt monohydrate (purity 98.9%), and dicloxacillin sodium salt monohydrate (purity 99.4%) were purchased from Sigma-Aldrich. Individual stock solutions of amoxicillin (AMX), ampicillin (AMP), PNG, cloxacillin (CXA) and dicloxacillin (DCX) were prepared to achieve a final concentration of 1000 μg/mL by dissolving each exactly weighed drug (10 mg) in 10 mL of water/acetonitrile (80:20, v/v; AMX and AMP) or water/acetonitrile (50:50, v/v; PNG, CXA, DCX). All solutions were stored at −20°C in amber glass containers.

Intermediate standard mixture solutions containing 10 μg/mL for AMX, AMP, PNG, and 75 μg/mL for CXA and DCX were prepared by mixing the appropriate amount of each stock standard solution in water/acetonitrile (50:50, v/v) with storage at 4°C.

Working standard mixture solutions containing 0.1 μg/mL for AMX, AMP, PNG or 0.75 μg/mL for CXA and DCX were prepared daily by diluting the intermediate standard solution with water/acetonitrile (50:50, v/v). These solutions were used for spiking negative milk samples and for constructing calibration curves.

2.3.2. Sample preparation and LC-MS/MS conditions

All milk samples determined to be positive for drug residues were stored at −80°C until analysis. The sample preparation was a modification of the procedure described by Holstage et al [28]. Briefly, residues from 5-g milk samples were extracted with 10 mL of acetonitrile by mechanical shaking for 10 minutes. The organic phase was separated from solid residue by centrifugation at 4000*g* for 5 minutes at 4°C. The supernatant was transferred in a second 15-mL centrifuge tube, and the extraction was repeated a second time by adding 10 mL of acetonitrile in the centrifuge tube with the precipitate. After shaking and centrifugation at 4000*g* for 5 minutes, the acetonitrile fractions were combined and evaporated to 0.5 mL volume under an air stream at 50°C using a TurboVap evaporator (Zymarck, Hopkinton, MA, USA). A volume of 4 mL phosphate buffer (0.05M at pH 7.5) was added to each sample, and the extract was defatted with 5 mL *n*-hexane. After centrifugation at 4000*g* for 5 minutes, the upper organic layer was eliminated and the aqueous phase was purified through STRATA-X SPE cartridges (60 mg, 3 mL) obtained from Phenomenex (Torrance, CA, USA). Each cartridge was previously activated with 2 mL methanol, 2 mL distilled water, and 2 mL phosphate buffer (0.05M at pH 7.5).

The loaded cartridges were washed with 3 mL phosphate buffer (0.05M at pH 7.5) and 1 mL distilled water, then eluted with 5 mL acetonitrile. The eluate was dried under an air stream at 50°C, and the residue was redissolved with 500 μL ammonium acetate (0.05M at pH 7.5) in acetonitrile (90:10, v/v). The samples were sonicated for 10 minutes, centrifuged at 14,000*g* for 15 minutes and subsequently transferred into LC vials for LC-MS/MS analysis.

All analyses were performed on a liquid chromatographic system (LC) Accela 600 (Thermo Fischer Scientific, San Jose, CA, USA) provided with a quaternary solvent delivery system, a column heater module, and a sampling cooling device, coupled to an LTQ ion trap from Thermo Fischer Scientific. The chromatographic separation was achieved with a Kinetex C18 column (inner diameter, 2.1 mm; length, 100 mm; particle size, 2.6 μm; Phenomenex Ltd.). The mass analyzer was set in the full scan monitoring mode. The analytical conditions are summarized in Table 1.

2.3.3. Method validation

The confirmatory method for β-lactams was validated to be in compliance with the Commission Decision 657/2002/EC [29].

Table 1 – Chromatographic conditions (timing and percentages of linear gradients used) and MS/MS acquisition conditions for β-lactams.

Time (min)	Percentage acetic acid 0.005%	Percentage ACN with 0.005% acetic acid
0.0	100	0
8.00	10	90
9.00	10	90
10.00	100	0
14.00	100	0

Flow rate (mL/min)	0.2
Injection volume (μL)	10
Autosampler temperature (°C)	4
Column temperature (°C)	30

Acquisition conditions

Analyte	Precursor ion (*m/z*)	Collision energy (%)	Product ion (*m/z*)
AMOXY (ESI +)	366	15	305, 234, 211,
	349	18	208,[a] 160, 114
AMPI (ESI +)	350	15	333, 305, 191,
			174, 160,[a] 106
PEN G (ESI −)	333	20	289, 192[a]
CLOXA (ESI −)	434	15	390,[a] 293
DICLOXA (ESI −)	468	15	424,[a] 327

Ionization conditions for positive and negative mode

Sheath gas flow (arbitrary unit)	30
Auxiliary gas flow (arbitrary unit)	15
Capillary temperature (°C)	275

ACN = acetonitrile; AMOXY = amoxicillin; AMPI = ampicillin; CLOXA = cloxacillin; DICLOXA = dicloxacillin; ESI = electrospray ionization; MS/MS = tandem mass spectrometry; PEN G = penicillin G.
[a] Product ion used for quantification.

To avoid possible variability in the instrument response owing to matrix effects, all analytes were quantified by calibration curves prepared daily by processing blank milk samples and spiking the final evaporated extract with a mixture of drugs at four concentration levels including zero (blank). An external standard calibration was used for the quantification of all analytes.

The linearity of the methods was tested in milk over the range of 0.5–10 μg/kg for AMX, AMP, and PNG, and over the range of 2.5–90 μg/kg for CXA and DCX. All the calibration curves tested were characterized by excellent linearity—verified by lack-of-fit tests—and by satisfactory correlation coefficients (r^2) greater than 0.98. Specificity was verified by the lack of chromatographic interference at the retention time of the analytes of interest on a minimum number of 20 blank milk samples.

Because no certified reference materials were available for β-lactams, to determine trueness and precision, recovery and repeatability were evaluated by means of spiked blank samples around the MRL of each analyte. Blank milk samples were spiked prior to the beginning of the extraction procedure with the analytes under investigation: three concentrations levels were chosen (0.5 × MRL, 1 × MRL, 1.5 × MRL), and six replicates were carried out for each spiking level and repeated on 3 different days for a total of 72 samples. The precision of the method was evaluated by calculating the relative standard deviation (RSD %) in intraday repeatability conditions (r %, RSD calculated from the six replicates for each spiking level) and in intralaboratory reproducibility conditions (R %, RSD calculated from the 18 replicates for each spiking level over 3 days). Trueness was calculated by dividing the mean measured value by the fortification level and multiplying by 100 to express the result as a percentage.

The results, which are shown in Table 2, reveal that all RSD % values, for intraday repeatability (r %) and intralaboratory reproducibility (R %) ranging from 3.5% to 14.8% and from 6.8% to 13.8%, respectively, meet the requirement of the Commission Decision 2002/657/EC at all fortification levels. The trueness, expressed as the relative recovery, ranged from 88.3% to 108.6% for AMX, AMP, and PNG, and from 76.5% to 109.7% for CXA and DCX, which are in agreement with the limits (from ≥1.0 μg/kg to 10 μg/kg, 70–110%, and ≥10 μg/kg, and 80–110%) established by Commission Decision 2002/657/EC.

CCα and CCß were determined by analyzing 20 blank samples fortified at their corresponding permitted limit. CCα was calculated as the mean measured concentration at the MRL of each compound plus 1.64 times the standard deviation (SD) of intraday precision at this concentration; CCß was

Table 2 – Validation results for the confirmation of β-lactam residues in milk: results of trueness (recovery in %), intraday repeatability (r %), and intralaboratory reproducibility (R %) in spiked milk samples.

LC-MS/MS

Intra- & interday repeatability for the determination of β-lactams, in fortified milk samples

Compound	Spike level (μg/kg)	Day 1 ($n = 6$)			Day 2 ($n = 6$)			Day 3 ($n = 6$)			Interday ($n = 18$)		
		Mean found (μg/kg)	RSD (%)	Recovery (%)	Mean found (μg/kg)	RSD (%)	Recovery (%)	Mean found (μg/kg)	RSD (%)	Recovery (%)	Mean found (μg/kg)	RSD (%)	Recovery (%)
AMOXY	2.0	1.8	13.6	88.3	2.0	6.6	101.5	2.0	11.8	100.1	1.9	11.9	96.6
	4.0	3.9	12.5	97.3	3.9	6.6	97.8	4.3	11.5	107.9	4.0	11.1	101.0
	6.0	5.8	7.9	96.1	5.9	7.6	98.6	6.0	10.8	100.0	5.9	8.6	98.2
	CCα	4.9 μg/kg											
	CCβ	5.7 μg/kg											
AMPI	2.0	1.8	13.8	90.5	1.9	5.8	96.1	2.1	7.0	103.5	1.9	10.4	96.7
	4.0	3.8	11.9	95.6	4.3	8.6	106.5	3.9	10.9	96.5	4.0	11.1	99.5
	6.0	5.8	8.5	96.8	6.1	11.9	101.4	6.1	10.7	101.2	6.0	10.1	99.8
	CCα	4.7 μg/kg											
	CCβ	5.5 μg/kg											
PEN G	2.0	1.9	10.2	93.0	2.2	11.1	108.6	2.0	15.1	99.4	2.0	13.3	100.3
	4.0	3.8	14.8	95.5	4.1	11.4	103.1	4.0	10.1	100.6	4.0	11.9	99.8
	6.0	5.4	13.3	89.6	6.1	9.7	100.9	6.0	7.1	99.8	5.8	11.0	96.8
	CCα	5.5 μg/kg											
	CCβ	7.0 μg/kg											
CLOXA	15	11.5	9.1	76.5	15.5	5.5	103.1	15.1	9.3	100.7	14.2	13.8	94.9
	30	24.2	14.4	80.6	28.8	3.5	96.2	29.8	10.9	99.2	27.6	13.2	92.0
	45	41.5	8.7	92.2	45.7	6.3	101.5	45.1	6.3	100.2	44.1	7.9	97.9
	CCα	35.8 μg/kg											
	CCβ	41.6 μg/kg											
DICLOXA	15	16.5	11.8	109.7	14.8	11.6	98.9	15.1	5.8	100.4	15.4	10.7	103.0
	30	31.8	12.2	105.9	30.3	8.5	100.9	29.9	7.1	99.6	30.6	9.5	102.1
	45	45.1	7.3	100.3	43.0	8.6	96.6	45.1	4.1	100.1	44.4	6.8	98.7
	CCα	35.3 μg/kg											
	CCβ	40.6 μg/kg											

AMOXY = amoxicillin; AMPI = ampicillin; CLOXA = cloxacillin; DICLOXA = dicloxacillin; LC-MS/MS = liquid chromatography-tandem mass spectrometry; PEN G = penicillin G; RSD = relative standard deviation.

calculated as CCα plus 1.64 times the SD of intraday repeatability at CCα [29,30].

3. Results

3.1. Results by screening methods

The qualitative detection of antibiotic residues by the Delvotest SP screening test applied to 1734 raw milk samples collected over 2 years (2009–2010) led to the identification of 106 positive samples (6.11%), and 1628 negative samples (93.9%). In 2009, 52 out of 1015 samples were drug-positive (5.12%), and in 2010, 54 out of 719 samples were positive (7.51%). The total number of drug-positive samples found in the study is shown in Table 3. Enzyme-linked receptor-binding assay (SNAP) test was used to check the positive samples, thus using the new β-lactam test for the determination of antibiotic residues in positive samples—40/52 in 2009 and 54/54 in 2010 samples were found to be positive; no positive samples were detected by SNAP tetracycline test and SNAP sulfamethazine test (see Table 4).

In terms of the regional distribution of milk samples containing drug residues, the highest numbers of positive milk samples were obtained from the Pejë (58.8%) and Gjakovë (23.5%) regions of Kosovo, whereas relatively low numbers of positive milk samples were from Gjilan (5.88%), and no drug-positive samples were detected in Mitrovicë or Prizren (Figure 1).

3.2. Confirmation of qualitative results

Fifty-five out of 106 drug-positive samples were analyzed by LC-MS/MS, and β-lactam antimicrobial drug residues were detected in 32 samples. The concentrations of β-lactams (AMX, AMP, PNG, CXA) in these samples ranged from 2.1 μg/kg to 1973 μg/kg, as shown in Table 5. Eighteen samples out of 55 would be considered noncompliant because the concentrations of one or more β-lactam residues exceeded the CCα of 5 for AMX, 7 for PNG, 2 for CXA, 1 for AMP, 1 for both AMP and CXA, 1 for both AMP and PNG, and 1 for the combination of AMP, CXA, and PNG. Seven samples were deemed compliant with respect to the established CCα, and six additional milk samples contained only trace amounts of β-lactam residues that were not quantified because they were below the limit of quantification.

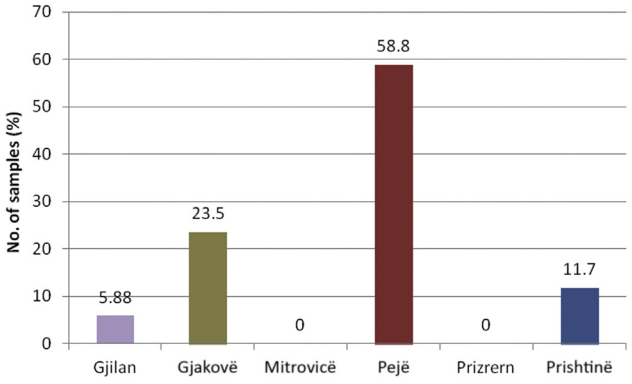

Figure 1 – Distribution of β-lactam positive milk samples in different regions of Kosovo (2009 and 2010).

Table 3 – Raw milk samples screened by the Delvotest SP test to detect the presence of antibiotic residues in 2009 and 2010.

Raw milk	No. of samples	No. of positive samples, *n* (%)
2009	1015	52 (5.12)
2010	718	54 (7.51)
Total	1734	106 (6.11)

4. Discussion

This study confirms that penicillins are the main group of antibiotics detected in milk samples, and these findings are in agreement with the observations reported by several other investigative teams [28,31–38]. This is likely to be a reflection of the frequent use of chemotherapeutic drugs in the therapy and prevention of specific diseases in dairy cattle and the use of intra-mammary infusions containing β-lactams for the treatment of mastitis. A report by Chung et al [23] identified 21 antibiotic contaminated samples out of 269 analyzed milk samples, representing 7.8% of the total. Khaskheli et al [39] analyzed 137 milk samples and, using the qualitative microbial method with *Bacillus subtilis* in plates for detection of β-lactam residues, identified 87 samples (63.5%) as negative and 50 samples (36.5%) as positive. In Romania, a survey was conducted [40], in which 124 milk samples (4.45%) out of a total of 2785 were found to be contaminated with antibiotic residues, with 2531 samples (90.88%) free of antibiotic residues. Nikolić et al [41] analyzed 6161 raw milk samples in Montenegro, of which 7.84% of the samples were drug-positive, and in Croatia [42], a very low percentage (0.69% [42]) or no milk samples [43,44] containing antibiotics were detected above the maximum residue levels (MRLs)

Table 4 – Raw milk samples screened by SNAP test in 2009 and 2010.

Analytical test used	2009			2010		
	No. of samples	No. of negative samples	No. of positive samples	No. of samples	No. of negative samples	No. of positive samples
New SNAP beta lactam test	52	0	40	54	0	54
SNAP tetracycline test	52	52	0	54	54	0
SNAP sulfamethazine test	52	52	0	54	54	0

Table 5 – Confirmatory results for suspect samples analyzed by LC-MS/MS.

Sample	AMPI (μg/kg)	AMOXY (μg/kg)	PEN G (μg/kg)	CLOXA (μg/kg)	DICLOXA (μg/kg)
2009					
DP021	nd	nd	C (2.2)	nd	nd
BF037	nd	nd	nd	nd	nd
DP071	nd	nd	nd	nd	nd
BB110	nd	nd	NC (98)	nd	nd
DE115	nd	nd	<LoQ	nd	nd
BB120	nd	nd	nd	nd	nd
EI121	nd	nd	NC (1973)	nd	nd
DO125	nd	nd	nd	nd	nd
GO128	nd	nd	nd	nd	nd
DE138	nd	nd	NC (5.5)	nd	nd
DP146	nd	nd	nd	nd	nd
DE168	nd	NC (7.2)	nd	nd	nd
DP175	nd	nd	C (20)	nd	nd
DE185	nd	C (2.1)	C (4.1)	nd	nd
DE191	nd	NC (14)	nd	nd	nd
DP195	nd	NC (7.6)	nd	nd	nd
DY196	nd	nd	nd	nd	nd
IK196	NC (8.9)	nd	nd	C (20)	nd
FV200	nd	nd	nd	nd	nd
FV201	NC (171)	nd	<LoQ	NC (439)	nd
PM203	nd	nd	nd	nd	nd
BD206	nd	nd	<LoQ	nd	nd
BL209	<LoQ	nd	nd	nd	nd
DO213	nd	nd	nd	nd	nd
GO214	nd	nd	nd	nd	nd
2010					
KA216	<LoQ	nd	nd	<LoQ	nd
GO236	nd	nd	NC (24)	nd	nd
GO244	nd	nd	NC (24)	nd	nd
KA270	nd	nd	nd	nd	nd
KP291	nd	nd	NC (6.4)	nd	nd
DE292	nd	nd	nd	<LoQ	nd
DK293	nd	nd	nd	nd	nd
IK294	<LoQ	nd	C (5.1)	<LoQ	nd
EI294	<LoQ	nd	nd	NC (42)	nd
DY296	nd	nd	nd	nd	nd
DP297	nd	NC (15)	nd	nd	nd
BD298	nd	nd	nd	nd	nd
DO299	nd	nd	nd	NC (49)	nd
BL317	nd	nd	nd	nd	nd
DE318	nd	C (4.6)	nd	nd	nd
DE319	nd	nd	nd	nd	nd
FR320	NC (784)	nd	NC (156)	NC (542)	nd
KP379	nd	nd	NC (6.9)	nd	nd
GA390	nd	nd	nd	<LoQ	nd
DO391	nd	nd	nd	nd	nd
DP392	nd	nd	nd	nd	nd
PM393	nd	nd	nd	<LoQ	nd
FV394	nd	nd	nd	nd	nd
GO426	nd	nd	nd	nd	nd
DP461	C (2.5)	nd	nd	C (32.4)	nd
DP514	nd	NC (43)	nd	nd	nd
GO554	nd	nd	nd	nd	nd
DE587	NC (7.0)	nd	C (15)	nd	nd
KP661	nd	nd	C (5.1)	ND	nd
DK671	nd	nd	nd	nd	nd

AMOXY = amoxicillin; AMPI = ampicillin; C = compliant (sample containing a concentration of β-lactam residues lower or equal to CCα); CLOXA = cloxacillin; DICLOXA = dicloxacillin; LC-MS/MS = liquid chromatography-tandem mass spectrometry; NC = not compliant (sample containing a concentration of β-lactam residues higher than CCα); LoQ = limit of quantification (corresponding to the 1st spiking level used in validation of the LC-MS/MS method: 2 μg/kg for AMP, AMX, PNG; 15 μg/kg for CXA and DCX); nd = not detected; PEN G = penicillin G; RSD = relative standard deviation.

established by European Union and Croatian legislation. Similar results were obtained in raw milk samples from Slovenia [45]. By contrast, tetracyclines (48.9%), sulfonamides (18.4%), and quinolones (6.8%) were found in milk samples from Macedonia, although drug residues were below the maximum residue limits [46].

5. Conclusion

The present investigation is the first performed in Kosovo to evaluate the presence of antibiotic residues in foodstuffs, and in particular, milk and dairy products. Our results indicate that β-lactams are the main class of antimicrobial drugs detected in milk intended for human consumption in Kosovo. The considerable levels of residues detected in raw milk, although regionally limited, are a human health concern that prompts a number of recommendations addressed to public authorities, veterinarians, livestock producers, and consumers. In addition to implementing appropriate regulatory legislation and providing an adequately controlled sampling network, we should be able to provide effective means for food control with appropriate risk assessments that will instill confidence in consumers. Competent authorities should establish and maintain continuous dairy monitoring programs to ensure risk-free milk products to Kosovo consumers. In addition, there is a pressing need for additional research to accurately assess other aspects of this problem and identify effective corrective actions that are designed to reduce milk contaminants.

Conflicts of interest

The authors declare that there are no conflicts of interest.

Acknowledgments

The project was partially funded with grants from Padova University (Fund PVS 2008 and 2009). The authors thank also the Kosovo Food and Veterinary Agency for their local support.

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