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Determination of antibiotic residues in imported and locally produced honey in Kosovo

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Abstract

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Antibiotic residues in food products pose significant health risks to consumers. Among the hazardous antibiotics, chloramphenicol and tetracycline are of particular concern. To assess the potential public health risks associated with the presence of these residues in honey, an evaluation is necessary. Our study examined honey samples from six key regions of Kosovo: Prizren, Pejë, Gjakovë, Mitrovica, and Gjilan. A total of 200 honey samples were qualitatively analyzed using enzyme-linked receptor assays (I'screen TECNA, Italy). Among these, 99 samples (49.5%) contained tetracycline at concentrations ranging from 3.8 to 15.8 µg/kg, while 101 samples (50.5%) had levels below the detection limit (<3.75 µg/kg). No traces of chloramphenicol were detected in any of the samples. Our findings indicate the presence of tetracycline residues in honey, underscoring the need for immediate action. We urge relevant institutions to consider these results and implement awareness programs to educate farmers on the risks associated with excessive antibiotic use.

Keywords: honey; tetracycline; chloramphenicol; ELISA; public health; Kosovo

Introduction

Honey is a natural product with high nutritional value and is a very important food for humans; it is rich in carbohydrates, proteins, enzymes, amino acids, vitamins, minerals, etc. (Bogdanov et al., 2008). Among others, honey has different properties such as antibacterial, antioxidant, anti-inflammatory, etc. (Stagos et al., 2018; Sipahi et al., 2017). Considering all the positive aspects, the trend of honey consumption per capita in economically developed countries is increasing; in the EU, the consumption of honey per capita is 0.3–1.8 kg/year (Bogdanov et al., 2008). The Republic of Kosovo is also part of the group of countries where the consumption of honey per capita is increasing (MAFRD Geen raport 2023). In recent years, the use of various chemicals such as antibiotics in the fight against various diseases of bees has resulted in the contamination of honey with these kinds of contaminants (Mahmudi et al., 2014; El-Nahhal, 2020; Kumar et al., 2020). The long-term effects of these contaminants cause health problems in humans (Al-Waili et al., 2012). In beekeeping, antibiotics such as tetracycline, chloramphenicol, tylosin, etc., have found use in combating some bee diseases such as European / American foulbrood disease and varroa mites (Bonta et al., 2007; Reybroeck et al., 2012).

Various researchers investigating antibiotic residues in honey have identified the presence of multiple antibiotics, including tetracyclines, chloramphenicol, streptomy-

cin, erythromycin, and cephalosporins (Zhao et al., 2017; Galyautdinova et al., 2020; Bonerba et al., 2021; Rama et al., 2022). The presence of such residues in honey can lead to an allergic reaction following the ingestion of contaminated honey (Asselt et al., 2013). Moreover, the low dosages of antibiotics used for growth promotion or inappropriate antibiotic prophylaxis in food animals (including bees) for long periods could result in antibiotic-resistant bacteria that can be passed from food to humans (Petrović et al., 2008; Asselt et al., 2013). The transmission of these resistance factors to the human population could be harmful and could drastically reduce the effectiveness of antibiotics against various human diseases (Pyun et al., 2008). The use of antibiotics in beekeeping is illegal in some EU countries. However, there is no Maximum Residue Limits (MRLs) established for antibiotics in honey according to European Community regulations, which means that honey containing antibiotic residues are not permitted to be sold (EC /657/2002). The treatment of honeybees with antibiotics is prohibited in the European Union (EU), and there have been significant advances in European Commission (EC) legislation concerning risk assessment. So far, no MRLs (Maximum Residue Levels) have been established for antibiotics and sulfonamides in honey (EC/37/2010), theoretically meaning that the use of antibiotics in beekeeping is not permitted in the EC as stipulated in Annex II of the European Commission (2001). Honey must, as much as possible, be free from organic or inorganic matter foreign to its composition. EC/ 2377/37/90 of the EC with annexes states that honey should be free of antibiotic contamination, so honey containing these substances cannot be sold in most EC (European Comission) countries, and no maximum residue levels (MRL) of antibiotic residues have been laid down. Some countries, like Switzerland, the UK (United Kingdom), and Belgium, have established action limits (level of antibiotics in honey beyond which the sample is deemed non-compliant) for antibiotics in honey, which generally lie between 0.01 and 0.05 µg/kg for each antibiotic group. Tetracycline is a broad-spectrum antibiotic, and its use began very early in veterinary medicine and beekeeping, intending to fight many bacterial diseases. (Reybroeck et al., 2012). Tetracycline is a very dangerous antibiotic for human health. If it is constantly taken in an uncontrolled form, it can cause health problems in people such as allergies, damage to the body microflora, antibiotic resistance, carcinogenic properties, damage to vital organs, etc. (Thanasarakhan et al., 2011). Based on European Commission regulations (EC/470/2009; EC/37/2010), the presence of tetracycline in honey is not allowed. Regardless of this regulation, some European countries and other countries have drafted the allowable limit for the existence of tetracycline in honey for example: In France, 15 µg / kg, in Switzerland and Belgium, 20 µg / kg; and in Great Britain, 50 µg / kg (Monica et al., 2012). Chloramphenicol is a very dangerous antibiotic for human health due to its low elimination rate and prolonged persistence in foods of animal origin (Bai et al., 2013). Therefore, the use of this antibiotic by livestock producers, including honey producers, has been prohibited by law in the European Commission since 1994 (EC/1430/94).

To date, no published information is available on antibiotic-contaminated honey in the Republic of Kosovo based on our research. To the best of our knowledge, these findings represent the first reported data on tetracycline and chloramphenicol residues in honey in Kosovo. Previous research on the presence of streptomycin in honey confirmed contamination with this antibiotic (Rama et al., 2022). The aim of this study was to analyze tetracycline and chloramphenicol residues in honey collected from various regions of Kosovo.

Materials and Methods

Sampling

A total of 200 honey samples were collected in 2023 from both imported and locally produced honey, sourced from individual apiaries and retail markets across six regions in the Republic of Kosovo: Prizren (34), Peja (30), Mitrovica (25), Prishtina (28), Ferizaj (28), and Gjilan (25) (Figure 1). Additionally, 30 imported samples from neighboring countries were included. The samples were stored at 4°C in sealed glass containers until analysis. The testing was conducted at the National Institute of Public Health of Kosovo and the Food and Veterinary Agency of Kosovo.

ELISA assay

Enzyme-linked receptor-binding assays (ELISA) method was used to determine the antibiotic residues. ELISA technique was performed according to the manufacturer's instructions using the I'screen TECNA, Italy, test kit, AB710/AB711 for tetracycline, SM390, and AB630 for chloramphenicol. For tetracycline, 1 g of honey sample was tacken and pretreated with 4 mL of dilution buffer 1X, and then was mixed on a vortex for 5 minutes. The supernatant was filtered through filter paper Whatman 1. pH was adjusted to 7.4 with 0.5 N NaOH. The screening was done in a 450 nM ELISA reader (Awareness Technology, USA). For chloramphenicol, 2 g of honey sample was placed in a test tube, 4 ml of distilled water was added, and the diluted sample was placed in a thermostat at 60 °C for several minutes. After that, 4 mL of ethyl acetate was added and vortexed for 10

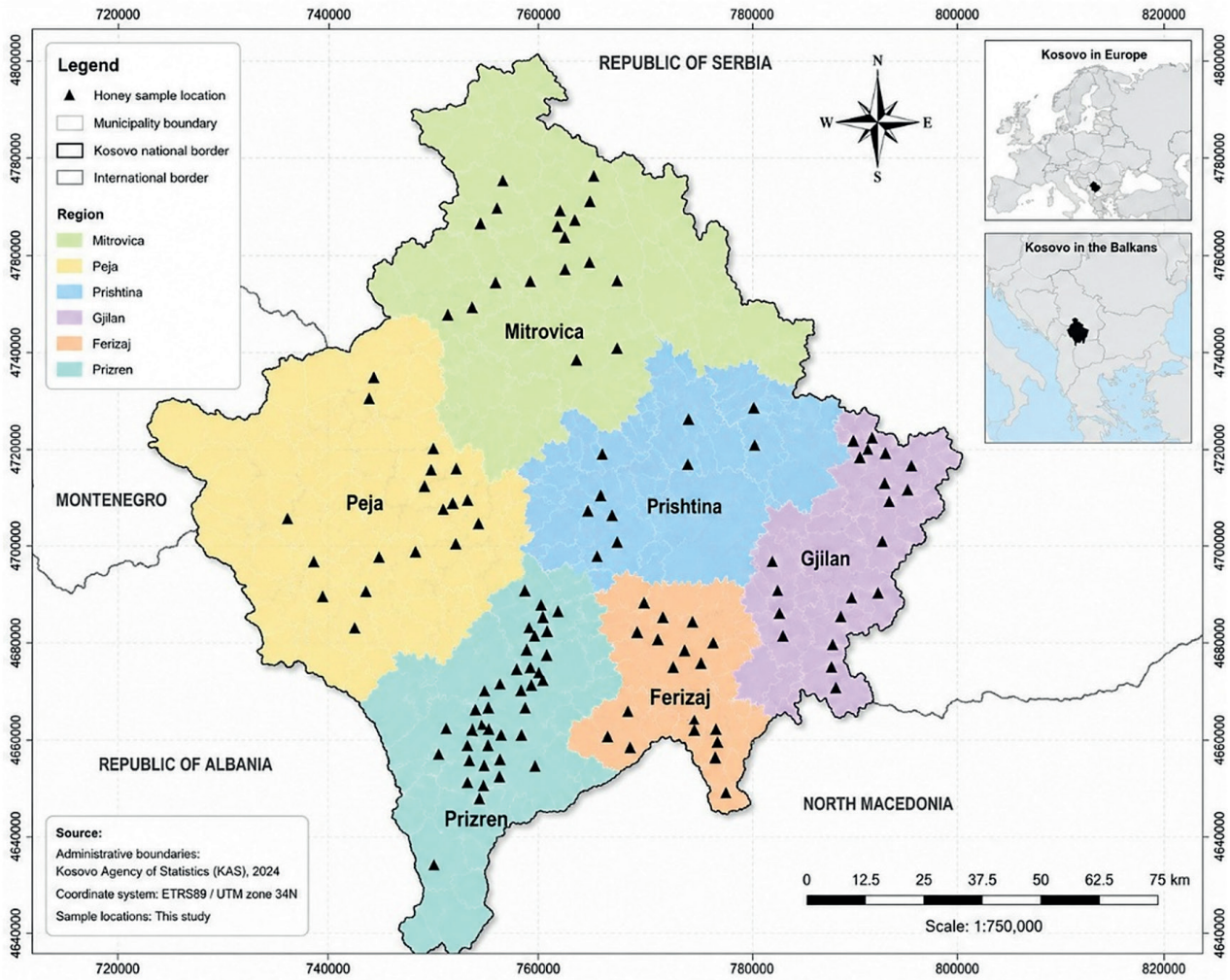


Fig. 1. Sampling sites in six different regions of Kosovo: Gjilan, Mitrovica, Peja, Prizren, Prishtina, and Ferizaj
Source: Administrative boundaries: Kosovo Agency of Statistics (KAS), 2024
Coordinate system: ETRS89/UTM zone 34N
Sample locations: This study

minutes. After centrifugation (10 min, 2000 g), 2 mL of ethyl acetate was pipetted (corresponding to the upper layer, which should be clear; if necessary, re-centrifuge) into a glass tube and evaporation was carried out at 50 °C under a stream of nitrogen and air. The residue was dispersed by vortexing in 0.5 mL of 1x dilution buffer. In the test, portions of 50 mL were used. The dilution factor was 0.5. Control results were obtained using a 450 nm ELISA reader (Awareness Technology, USA). The concentration of tetracycline was calculated from the calibration curve, which was obtained using six standards with the following concentrations: 0, 0.1, 0.25, 1, 5, and 20 µg/kg (Table 1).

Table 1. Obtained reference values of I 'screen TETRA

Standards	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6
Conct. (ng/mL)	0.0	0.1	0.25	1	5	20
B/B	100.0	81.0	70.1	47.0	23.2	13.1

Source: Authors' own elaboration

Statistical analysis

Statistical analyses were performed using the Statistical Package for Social Sciences (SPSS, USA) software, version 21. The t-test and analysis of variance (ANOVA) were used to assess differences in tetracycline concentration in honey concentration between the six regions and imported honey samples. The level of significance for the differences was set at $P < 0.05$; $P < 0.01$; $P < 0.001$.

Results

In this study, a total of 200 honey samples were collected from individual apiaries and retail markets in six different districts of the Republic of Kosovo, including both imported and local honey. Table 2 reveals that tetracycline was detected in 49.5% (99 samples) of the analyzed honey, with concentrations ranging from 3.8 to 15.8 µg/kg, while the remaining 50.5% (101 samples) had levels below the detection limit (< 3.75 µg/kg). The regional distribution of tetracycline in honey across Kosovo (Prizren, Peja, Mitrovica, Prishtina, Ferizaj, and Gjilan) and imported honey is detailed in Table 3. A pairwise comparative analysis showed the following descending order of tetracycline concentrations: Prizren > Gjilan > Ferizaj > Mitrovica > Imported > Peja> Prishtina. Table 4 provides statistical comparisons, including mean differences, standard error, T-values, and P-values of tetracycline levels between locally sourced honey (from the six regions) and imported honey.

The highest mean difference in tetracycline levels was observed between honey samples from Prizren (r1) and Peja (r2), with a value of 3.78, while the lowest difference was found between Prishtina (r4) and imported honey (0.03). Regarding standard error (SE), the largest value occurred between Mitrovica (r3) and Prishtina (r4) (1.48), whereas the smallest SE was recorded between r3 and r6 (0.27). The highest T-value was obtained in the comparison between Prizren (r1) and Peja (r2), while the lowest T-value was seen between Prizren (r1) and imported honey. For P-values, the highest (least significant) value appeared in the r5 – r7 comparison, whereas the lowest (most significant) was observed in the r1–r2 comparison.

Discussion

To date, no studies have investigated the presence of tetracycline and chloramphenicol in honey produced in or imported to Kosovo. However, similar research in other regions provides comparative insights. Korkmaz et al. (2017) detected tetracycline in 59% of 59 Turkish honey samples using ELISA. Similarly, Sema et al. (2020) found tetracycline in 73.3% of samples in another Turkish study. These rates align closely with our findings, possibly due to the widespread use of Turkish veterinary pharmaceuticals in Kosovo.

In Romania, Monica et al. (2012) reported tetracycline in 50 % of honey samples. Saridaki-Papakonstadinou et al. (2006) identified tetracycline in 29% of 251 Greek honey samples, likely reflecting similar beekeeping practices in the Balkans. Reybroeck (2003) found minimal antibiotic residues in Belgian honey (tetracycline in 2 of 72 samples; no chloramphenicol). In Switzerland, Ortelli et al. (2004) detected chloramphenicol in 17% of samples, primarily of Asian origin.

Mahmudi et al. (2014) reported tetracycline (14.07%) and chloramphenicol (8.8%) in Iranian honey. Kumar et al. (2020) identified oxytetracycline (15.3%) and erythromycin (5.3%) in Indian honey via liquid chromatography. Santana

Table 3. Distributions by regions and contamination level of tetracycline

Regions	Total samples	Contamination samples with tetracycline's (%)	Contamination level (µg/kg) tetracycline's
Local			
Gjilan	25	14 (56.0)	3.8–9.43
Mitrovica	25	12 (48.0)	4.0–13.61
Peja	30	13 (43.3)	4.5–10,50
Prizren	34	23 (67.6)	5.0–15,58
Prishtina	28	9 (31.0)	3.9–8.0
Ferizaj	28	14 (50.0)	4.6–10.24
Imported	30	14 (46.6)	4.0–10.10
Total	200	99	3.8–15.58

Source: Authors' own elaboration

Table 2. Antibiotic residues detected in commercial honey samples collected from some regions of Kosovo and imported honey

Antibiotics	n/N [a] (% positive samples)	Mean ± SD[b]	Minimum quantified (µg/kg)	Maximum quantified (µg/kg)	Tolerance limits (µg/kg)[c]
Tetracycline	99/200 (49.5%)	5.35±0.33	3.8	15.8	5.0
Chloramphenicol	Not detected	-	-	-	0.3

[a]n = number of positive samples and, N total number of samples

[b]mean ± standard deviation samples.

[c]Tolerance limits are as per FSSAI (2014)

Source: Authors' own elaboration

Table 4. The differences in tetracycline concentration among the regions included in the study

Regions	Difference between regions	Mean Difference	SE of difference	T-Value	P- Value
r_1 (n = 39)	r2-r1	3.78	1.08	3.50	0.001
	r3-r1	1.22	1.14	1.07	0.286
	r4-r1	3.42	1.29	2.64	0.009
	r5-r1	3.02	1.06	2.85	0.005
	r6-r1	1.17	1.05	1.12	0.265
	r7-r1	3.05	1.02	3.00	0.003
r_2 (n = 20)	r3-r2	2.56	1.29	1.98	0.050
	r4-r2	0.36	1.43	0.25	0.799
	r5-r2	0.76	1.23	0.62	0.538
	r6-r2	2.61	1.21	2.15	0.033
	r7-r2	0.73	1.19	0.61	0.540
r_3 (n = 17)	r4-r3	2.20	1.48	1.48	0.140
	r5-r3	1.80	1.28	1.41	0.161
	r6-r3	0.05	0.27	0.04	0.969
	r7-r3	1.83	1.24	1.47	0.143
r_4 (n = 12)	r5-r4	0.39	1.42	0.28	0.783
	r6-r4	2.24	1.41	1.59	0.113
	r7-r4	0.37	1.39	0.26	0.792
r_5 (n = 21)	r6-r5	1.85	1.20	1.55	0.124
	r7-r5	0.03	1.17	0.02	0.982
r_6 (n = 21)	r7-r6	1.88	1.16	1.62	0.107

Note: Given characters denote: r-regions; r_1 – Prizren; r_2 – Peja; r_3 – Mitrovica; r_4 – Prishtina; r_5 – Ferizaj; r_6 – Gjilan; r_7 – Imported. Significance of variations is denoted as follows: *P < 0.05; **P < 0.01; *** P < 0.001.
Source: Authors' own elaboration

et al. (2018) found tetracycline in 1 of 33 Brazilian samples (35.8 µg/kg). Studies in Serbia (Dugalić-Vrndić et al., 2011; Apić et al., 2015) revealed antibiotic residues in 8 of 65 and 2.59% of 193 samples, respectively. Conversely, Mujić et al. (2011) detected no antibiotics in 46 Bosnian honey samples using microbiological assays.

Kosovo's 49.5 % tetracycline prevalence is significantly higher than in Serbia (2.59 %), Bosnia (0 %), and Greece (29%), but closely matches Turkish studies (59–73.3%). This supports the hypothesis that shared veterinary practices or pharmaceutical imports (e.g., from Turkey) may contribute to elevated residues. Chloramphenicol was not detected in Kosovo, consistent with most European studies (e.g., Belgium, Bosnia). The variability in antibiotic prevalence underscores the influence of regional veterinary practices and regulatory frameworks. Kosovo's results mirror trends in neighboring countries, suggesting shared challenges in antibiotic use in apiculture. The data underscore the impact of regional veterinary practices and highlight opportunities for regulatory harmonization.

Conclusion

This study was conducted in Kosovo to assess the potential presence of antibiotic residues in honey. The findings reveal detectable levels of antibiotics in honey meant for human consumption, raising public health concerns. Although the contamination appears regionally limited, the significant residue levels warrant urgent action from public authorities, veterinarians, beekeepers, and consumers. To address this issue, Kosovo should enforce stricter regulatory measures and establish a well-monitored sampling system. Effective food control mechanisms, including thorough risk assessments, are essential to restoring consumer confidence. Additionally, competent authorities must implement continuous monitoring programs to guarantee the safety of honey and related products for consumers. Further research is necessary to determine the full scope and prevalence of antibiotic contamination in honey across different regions and to develop strategies for reducing residues. Strengthening regional cooperation could help harmonize practices and further reduce antibiotic residues in honey. By adopting appropriate measures, Kosovo can minimize the risks posed by antibiotic residues and ensure the production of safe, high-quality honey. Strengthening regional cooperation, for instance, with Turkey, could help harmonize practices and further reduce antibiotic residues in honey.

Conflicts of interest

The authors declare no potential conflict of interest.

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