# Determination of Streptomycin Residue in Imported and Locally Produced Honey in Kosovo

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**Research Article**

**Keywords:** Honey, Antibiotics, ELISA, Public Health, Kosovo

**Posted Date:** February 8th, 2022

**DOI:** https://doi.org/10.21203/rs.3.rs-1318821/v1

# Abstract

The objective of this study was to assess the occurrence of streptomycin residues of locally produced and imported honey collected from individual apiaries and retail markets during 2017 from six different regions (Gjilan, Mitrovica, Peja, Prizren, Prishtina, and Ferizaj) in Kosovo. In the present study, 155 honey samples have been qualitatively screened by enzyme-linked receptor-binding assays (ELISA). A descriptive statistic is used to provide the basic features of the sample and measure. In total, 45 (29%) samples were positive, while 110 (71%) were negative. Streptomycin residues were found in a considerable number of local honey samples, 34 out of 131 (25.9%), and imported honey, 11 out of 24 samples (45.8%), respectively. None of the positive samples has exceeded e maximum residue levels (MRL) stated by the European Union (EU) regulations. ELISA analysis demonstrated that streptomycin residues lie between 2.1–9.8 ng/mL. Regarding the regional distribution of the positive samples, most of them were from Ferizaj and Prizren Region. Based on these results, competent authorities should establish and maintain continuous honey monitoring programs to ensure Kosovo consumers', risk-free honey. Besides, there is a pressing need for additional research to accurately assess other aspects of this problem and identify effective corrective actions designed to reduce honey bee contaminants.


# Introduction

Kosovo has suitable conditions for beekeeping development such as climate, relief, and many honey-bearing plants that guarantee the production of good honey and beekeeping products (Panettieri 2013). Beekeeping is a viable business that significantly contributes to increasing and diversifying many rural households' incomes in Kosovo Ministry of Agriculture, Forestry and Rural Development (MAFRD. 2017). Beekeeping provides various benefits, such as income from the sale of bee products, self-employment opportunities, pollination, and biodiversity conservation. Currently, Kosovo has 6,453 beekeepers with 70,664 bee-hives distributed in Kosovo's territory, with average production for bee society 9.55 kg honey or 674 t/yr. In contrast, the import in 2014 was around 140 t/yr Kosovo Agency of Statistics (KAS 2019). In addition to honey, other products are produced, such as pollen, wax, propolis, bees milk, etc. (MAFRD 2015).

Similarly, Kosovo also imports honey, but there is no standard to check the quality of honey being imported. There is no report on the contamination of honey consumed within the country. Beekeepers commonly apply antibiotics to eliminate disease among honeybees. Researchers revealed that residues of antibiotics in honey are originated mostly from improper beekeeping practices and not from the environment (Forsgren et al. 2010; Johanson et al. 2010; Gajda et al. 2013).

Moreover, some pesticides and veterinary drugs are suspected of causing certain types of cancer, teratogenicity, chromosomal abnormalities, and the weakening of humans' immune system (Banerjee. 1999; McEvoy. 2002; Muhammad et al. 2009; Jeong et al. 2010). Some drugs have the potential to produce toxic reactions in consumers directly. Antibiotics can cause cutaneous eruptions, dermatitis, gastro-intestinal symptoms, and anaphylaxis at very low doses (Gehrig and Warshaw 2008). Antibiotic residues consumed along with honey can produce resistance among bacteria in the consumers, and consequently, there is difficulty in treating many infections in humans (Pyun et al. 2008).

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 transfer from food to

humans (Petrović et al. 2008; Asselt et al. 2013). The use of antibiotics in beekeeping is illegal in some European Union (EU) countries. However, there are no maximum residues levels maximum residue levels (MRLs) established for antibiotics in honey according to European Community (EC) regulations, which means that honey containing antibiotics residues are not permitted to be sold (EU 2002). The treatment of honeybees with antibiotics is prohibited in the EU, and there have been significant advances in EU legislation concerning risk assessment. So far, no MRLs have been established for antibiotics and sulfonamides in honey (EU 2010), theoretically meaning that the use of antibiotics by beekeeping is not permitted by European Commission. As stipulated in Annex II of Council Directive, 2001/110/EC (EU 2001), they must, as much as possible, be free from organic or inorganic matter foreign to its composition. EC Directive, 2377/90 with annexes, states that honey should be free from antibiotics contamination (EU 1990), so honey containing these substances cannot be sold in most EU countries, and no MRL of antibiotic residues have been laid down. Some states, like Switzerland, United Kingdom (the UK), 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 lies between 1.0 to 5.0 ng/mL for each antibiotic group.

Streptomycin (an antibiotic) is a protein synthesis inhibitor, and despite its toxicity, it is widely used in veterinary medicine for the treatment of aerobic gram-negative bacteria infections (Oliveira et al. 2009; Horie et al. 2004). Streptomycin is commonly used in apiculture for the prophylactic treatment or control of bacterial brood diseases such as European foulbrood and American foulbrood disease (Victoria et al. 2007; Pena et al. 2005). High concentrations of Streptomycin may produce ototoxicity and nephrotoxic effects. However, regular consumption of Streptomycin at low concentrations in foods may also cause allergies, destroy intestinal flora, and cause resistance to certain microorganisms (Cara et al. 2013; Gačić et al. 2015).

# Material And Methods

### *Samples*

One hundred fifty-five (155) honey samples were collected from different markets (local and imported honey from neighboring countries) and beekeepers in some regions of Kosovo between June and September 2017 (Figure 1).

Sampling was performed according to EU requirements (EU 2006a).

The local honey (131) was provided by the beekeepers of several regions in Kosovo, while imported honey samples (24) were collected from retail markets. All honey samples were labeled either according to their botanical and geographical origin, as suggested by the beekeepers. The samples were stored at room temperature in the dark until analysis. One honey sample was checked to be free of any of the targeted antibiotics, and it was used as blank honey for the calibration curve.

### *Antibiotic analysis by ELISA*

The determination of Streptomycin was done by Enzyme-Linked Immunosorbent Assay (ELISA) method using I'screen STREPTO test kit (Tecna S. r. l., Trieste, Italy). Analyses were performed according to the test kit's instructions. The procedure is based on the binding of free antibiotics from samples and standard solutions to the anti-streptomycin antibodies during the first incubation. Any unbound substance is removed in a washing

step. A second incubation is performed with a streptomycin-horseradish peroxidase (streptomycin-HRP) conjugate, which covers all the antibody's remaining free binding sites. The bound enzyme activity is determined by adding a fixed amount of a chromogenic substrate. The enzyme converts the colorless chromogen into a blue product during the third incubation. The addition of the stop reagent leads to a color change from blue to yellow. The absorbance is measured by a microplate reader (Bio-Tek, USA) at 450 nm. The color development is inversely proportional to the streptomycin concentration in the sample. The concentration of Streptomycin 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 nanograms per milliliters (ng/mL) (Table 1).

Analytical sessions were compliant with assay specifications: Mean B0 absorbance; Binding for the zero standard, maximum binding well (B/B0) 50% value; calculating and evaluating (CV%) value related to standard duplicates mean.

In our study, the recovery of streptomycin in spiked honey samples was found to be 84% (CV = 3.12), 92% (CV = 1.23) and 98% (CV = 1.03) for spiking concentration of 20, 80, 100 ng/mL, respectively. All experiments were made in triplicate.

### *Sample preparation*

Streptomycin screening tests were performed at the National Institute of Public Health of Kosovo. The assessments of Streptomycin amounts were conducted using the enzyme immunoassay I'screen STREPTO (Code AB650) 0.0, 0.25, 1.5, 20 ng/mL. The limit of detection (LOD) for Streptomycin is two 2 ng/mL.

Honey samples were prepared according to the manufacturer's instructions, 1 g of homogeneous honey sample were weighted, then 19 ml of dilution buffer 1x is added, honey was shaken or vortexed until it is completely dissolved. The sample is filtered with Whatman N°1, and the dilution factor was 20. Samples were tested immediately after preparation.

### *Statistical analysis*

Statistical analyses were performed using the Statistical Package for Social Sciences Statistical Package for the Social Sciences (SPSS, USA) software, version 21. The t-test and analysis of variance (ANOVA) were used to assess Streptomycin's differences 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

There was a moderate incidence rate of Streptomycin in the local honey with 34 (25.9%) out of 131 samples positive, while 11 (45.8%) out of 24 imported honey samples were found positive on the Kosovo market as seen in Table 2.

The mean locally produced honey was 2.709 ± 0.227 ng/mL and imported honey 2.850 ± 0.559 ng/mL, respectively.

Most of the positive samples were found in the Ferizaj region (9/42.8%) with a concentration level of 2.24 - 9.78 and Prizren (13/33%) with a concentration level of 2.2 - 6.82, respectively. In foreign (imported) honey, present on

the Kosovo market, Streptomycin's frequency was high. Thus 11(45.8%) out of 24 samples were positive, with a concentration level of 2.2 - 9.8; none of the positive samples were above MRL (Table 3).

The descriptive statistics for the differences in streptomycin concentration in bee honey for the six regions (Gjilan, Mitrovica, Peja, Prizren, Prishtina, and Ferizaj) and imported bee honey determined in this study are shown in Table 4. In a pairwise comparison of the concentration of Streptomycin in bee honey between six regions of Kosovo and imported bee honey, we observed that Prishtina had the highest concentration, followed by Ferizaj, Imported, Prizren, Mitrovica, Peja, and Gjilan. The interval plots presented in Figure 2 offer better visualization of the differences in streptomycin concentrations among the regions.

# Discussion

In our study, Streptomycin was determined in 25.9% of locally produced honey and 45.8% of imported honey samples. These results are in parallel with the findings of some previous reports (Ortelli et al. 2004; Baggio et al. 2004; Saridaki-Papakonstadinou et al. 2006; Dugalić-Vrndić et al. 2011; Mahmoudi et al. 2014; Galarin et al. 2015) reported a lower incidence or not detected the antibiotics in honey.

Reybroeck (2003) monitored 248 samples of locally produced and imported honey on the Belgian market for residues of antibiotics in 2000-2001. Streptomycin was detected in 4 (248), tetracycline 2 (72), and sulfonamides in 3 (72) samples. In imported honey, Streptomycin was detected in 51 (102), tetracyclines in 29 (98), sulfonamides in 31 (98), and chloramphenicol 40 (85) samples. Ortelli et al. (2004) analyzed 75 samples in Switzerland (34 originated from Asian countries), of which 13 samples (17%) contained chloramphenicol residues. In Greece, Saridaki-Papakonstadinou et al. (2006) conducted a survey in which 251 honey samples produced across were analyzed by liquid chromatography to detect tetracycline-derived residues; 29% of the samples had tetracycline residues. The majority of samples contained residues from 18.0-55.0 ng/mL of honey, while some others had residues in excess of 100.0 ng/mL. Vidal et al. (2009) reported the presence of erythromycin residues in 3 out of 16 samples of honey in Spain by using ultra-performance liquid chromatography-tandem mass spectrometry ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS). Gunes et al. (2008) have analyzed 50 honey samples for the possible presence of erythromycin residues in honey collected from the hives in Turkey. The samples were analyzed by liquid chromatography-mass spectrometry using electrospray ionization in the positive ion mode liquid Chromatography-Electrospray Ionization-Mass Spectrometry (LC-ESI-MS), and 4 (8%) of the honey samples were contaminated with erythromycin residues. In Italy, Baggio et al. (2004) have investigated 88 imported honey samples, and 32 out of 88 samples were positive on Streptomycin. Sabatini, Carpana et al. (2003) analyzed Italian honey for the presence of antibiotics whereas they found 2.7% of tetracycline and 4.5 oxytetracycline residues. Mahmoudi et al. (2014) investigated oxytetracycline residue in 145 honey samples collected from Ardabil provinces, the Northwest region of Iran, by using ELISA and HPLC methods. The ELISA assay showed that 34 out of 145 samples were positive while the minimum and maximum oxytetracycline residue levels were 5.32 and 369.1 ng/mL, respectively. There are few reports of antibiotics in honey in neighboring countries as Serbia and Bosnia and Herzegovina. Thus, Dugalić- Vrndić et al. (2005) determined the antibiotics and sulphonamides residues in 100 honey samples from Belgrade market and supermarket. In total, 18 samples were found positive. In contrast, Dugalić-Vrndić et al. (2011) tested 65 honey samples and found residues of antibiotics and sulfonamide in 8 samples. Apić et al. (2015) examined 193 honey samples in Vojvodina. The presence of antibiotic residues in honey resulted in 5 out of 193 tested honey samples. Mujić et al. (2011) researched the

presence of antibiotic residues in 46 honey samples, whereby no concentrations of antibiotics and sulfonamides were found.

In the work of Galarin et al. (2015), 74 honey samples were collected in the Italian market, and nine or (12%) samples were found positive on sulfonamides.

Bonerbaa et al. (2021) examined 98 honey samples for Streptomycin during 2018-2019 in Italy's different regions. The presence of Streptomycin resulted in 33 out of 98 tested honey samples.

In another study, Kokmaz et al. (2017), using the ELISA technique, 59 samples were tested for the possible presence of Streptomycin and sulfonamides. Thus, 35 or (59%) samples were found positive on Streptomycin, with a level of 6-42 ng/ml, and 31 (53%) positive samples on sulfonamide, at a level of 3-32 ng/ml.

# Conclusions

The present investigation is the first research performed in Kosovo to evaluate the presence of antibiotic residues in foodstuffs, and in particular, bee honey. In our validation tests, we spiked honey samples at the level of 5 ppb of Streptomycin, and we obtained a sensitivity of 97.5%. That means that 97, 5% of samples are dosed as positives. Usually, that value has been reported as C Cbeta (EU, 2002; 2010). Moreover, the difference in B/Bo% of spiked samples vs negative samples is 21 B/Bo% points of difference.

Our results indicate the presence of Streptomycin in honey intended for human consumption in Kosovo. The considerable levels of residues detected in honey, although regionally limited, are a human health concern that prompts several recommendations addressed to public authorities, veterinarians, honeybee 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 proper risk assessments that will instill confidence in consumers. Competent authorities should establish and maintain continuous monitoring programs to ensure risk-free honey and its products to Kosovo consumers. Besides, 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 honey contaminants.

# Abbreviations

1. ELISA- Enzyme-linked receptor-binding assays;
2. MRL- Maximum residue levels;
3. ng/mL. - Nanograms per milliliters
4. UE- European Union;
5. MAFRD- Ministry of Agriculture, Forestry and Rural Development;
6. KAS- Kosovo Agency of Statistics;
7. EC- European Commission;
8. UK- United Kingdom;
9. Streptomycin-HRP- Streptomycin-horseradish peroxidase
10. USA- United States of America;

11. CV% - Calculating and evaluating;
12. LOD- The limit of detection;
13. SPSS- Statistical Package for the Social Sciences;
14. ANOVA- Analysis of Variance;
15. UPLC-MS. Ultra-performance liquid chromatography-tandem mass spectrometry;
16. LC-ESI-MS - Liquid Chromatography-Electrospray Ionization-Mass Spectrometry;
17. HPLC- High-performance liquid chromatography;
18. B/Bo%- Binding for the zero standard, maximum binding well;

# Declarations

### - Availability of data and materials

Not applicable

### -Competing interests

Not applicable


### - Funding

This work was financially supported by the Ministry of Education, Science, and Technology of Kosovo as a small research project for science and technology development (2-2109).


### - Authors' contributions

A. R. and I. H. wrote the main manuscript text and figures; I. M. prepared statistical data and A. Z, B. ZH, A. L, D. H, F. L. assisted by laboratory analysis. All authors reviewed the manuscript.


### - Acknowledgements

Not applicable


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# Tables

Table 1. Obtained reference values of l'screen STREPTO

| | 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 | 83.0 | 70.8 | 47.6 | 23.8 | 13.7 |

Table 2. Occurrence of streptomycin antibiotic in commercial honey in Kosovo during 2017

| **Honey** | No. Of Samples | No. Of positive Samples | No. Of negative Samples | Positive samples Mean ± SD |
|---|---|---|---|---|
| **Local** | 131 | 34 (25.9%) | 97 (74.1%) | 2.709 ± 0.227 |
| **Imported** | 24 | 11 (45.8%) | 13 (54.2%) | 2.850 ± 0.559 |
| **Total** | 155 | 45 (29 %) | 110 (71%) | 2.731 ± 0.210 |

Table 3. Distributions by regions of positive samples and concentration levels (ng/mL) in locally and imported honey during 2017

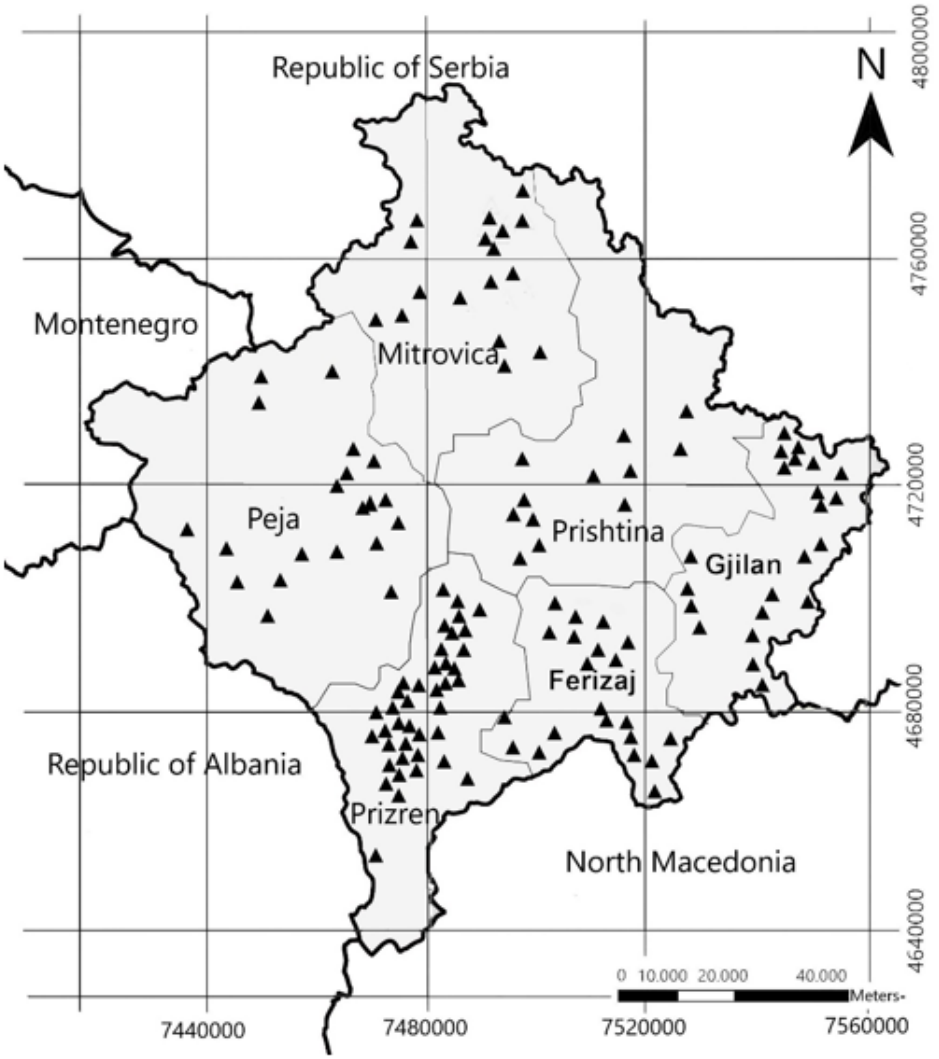
| Regions | Total samples<br>n | Positive samples<br>n (%) | Lower and upper Streptomycin contamination level (ng/mL) |
|---|---|---|---|
| **Local** | | | |
| Gjilan | 22 | 2 (9.1) | 6.92-9.32 |
| Mitrovica | 17 | 3 (17.6) | 2.31-5.78 |
| Peja | 20 | 4 (20) | 2.1-5.3 |
| Prizren | 39 | 13 (33.3) | 2.2-6.82 |
| Prishtina | 12 | 3 (25) | 4.38-8.78 |
| Ferizaj | 21 | 9 (42.8) | 2.24-9.78 |
| Imported | 24 | 11 (45.8) | 2.2-9.8 |
| **Total** | 155 | 45 (29.0) | 2.1-9.8 |

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

| Regions | Difference between regions | Mean Difference | SE of difference | T-Valeue | P- Valeue |
|---|---|---|---|---|---|
| $r_1$ (n=22) | r2-rt1 | 1.39 | 0.77 | 1.81 | 0.073 |
| | r3-r1 | 0.17 | 0.74 | 0.23 | 0.816 |
| | r4-r1 | 1.82 | 0.64 | 2.86 | 0.005 |
| | r5-r1 | 5.56 | 0.86 | 6.49 | 0.000 |
| | r6-r1 | 4.79 | 0.73 | 6.58 | 0.000 |
| | r7-r1 | 2.021 | 0.70 | 2.87 | 0.005 |
| $r_2$ (n=17) | r3-r2 | 1.22 | 0.79 | 1.55 | 0.124 |
| | r4-r2 | 0.43 | 0.69 | 0.62 | 0.537 |
| | r5-r2 | 4.17 | 0.90 | 4.63 | 0.000 |
| | r6-r2 | 3.40 | 0.78 | 4.37 | 0.000 |
| | r7-r1 | 0.63 | 0.76 | 0.83 | 0.408 |
| $r_3$ (n=20) | r4-r3 | 1.65 | 0.66 | 2.51 | 0.013 |
| | r5-r3 | 5.39 | 0.87 | 6.18 | 0.000 |
| | r6-r3 | 4.62 | 0.75 | 6.19 | 0.000 |
| | r7-r3 | 1.85 | 0.72 | 2.56 | 0.012 |
| $r_4$ (n=39) | r5-r4 | 3.74 | 3.79 | 4.74 | 0.000 |
| | r6-r4 | 2.97 | 0.65 | 4.60 | 0.000 |
| | r7-r4 | 0.20 | 0.62 | 0.32 | 0.749 |
| $r_5$ (n=12) | r6-r5 | 0.76 | 0.86 | 0.89 | 0.377 |
| | r7-r5 | 3.54 | 0.84 | 4.19 | 0.000 |
| $r_6$ (n=21) | r7-r6 | 2.77 | 0.14 | 3.89 | 0.000 |

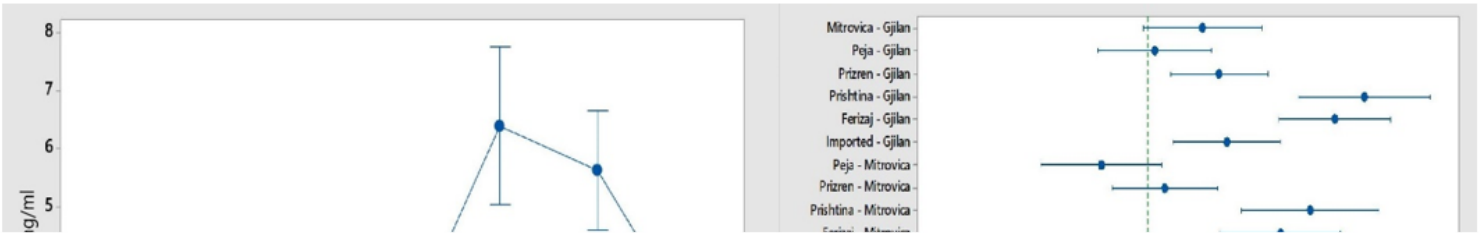
Note: Given characters denote: r-regions; $r_1$-Gjilan; $r_2$-Mitrovica; $r_3$-Peja; $r_4$- Prizren; $r_5$- Prishtina; $r_6$-Ferizaj; $r_7$-Imported. Significance of variations is denoted as follows: *P≤0.05; **P≤0.01; *** P≤0.001.

# Figures




## Figure 1

Sample collection sites in six regions of Kosovo: Gjilan, Mitrovica, Peja, Prizren, Prishtina, and Ferizaj.



## Figure 2

Interval plots of Streptomycin in local and imported bee honey