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Assessing Health Risks Associated with Antibiotic Residues in Armenian Honey

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ABSTRACT

This study assessed potential risks associated with antibiotic residues in Armenian honey. Honey sample analysis revealed multiple antibiotics, in varying concentrations. Estimated daily intakes of antibiotics were calculated for different consumer clusters. The margin of exposure was determined based on acceptable daily intake values. The findings indicate that there are no risks to consumers regarding antibiotic residues in honey. However, it highlights the importance of controlling antibiotics in beekeeping practices to ensure honey safety.

Introduction

Natural honey has long been known for its nutritional value and health benefits. Honey possesses antimicrobial properties and antioxidant activity due to its rich content of polyphenols, enzymes, and a combination of high osmolarity and low pH (Almasaudi, 2021; Nolan, et al., 2019). Additionally, honey has an attractive taste and high energy value due to the rich mixture of sugars (Ashagrie Tafere, 2021).

Honey composition varies depending on factors such as geographical and botanical origin, honey bee health and species, as well as honey processing and storage methods (Almasaudi, 2021; Mama et al., 2019; Nolan et al., 2019). Armenian honey, in particular, is unique due to the mountainous terrain, and special plant composition, including endemic plants. Different authors have mentioned the high quality and beneficial properties of Armenian multi-floral honey (Belyaeva, et al., 2020; Pipoyan, et al., 2019), leading to its growing popularity in the international market. Notably, Armenia's natural honey export has experienced substantial growth, with exports reaching $126 thousand in 2020 and $3.28 million in 2021 (Trend Economy, 2022).

The mentioned characteristics and increasing demand for Armenian honey emphasize the importance of its safety. While honey is generally considered a nourishing and beneficial product, there are growing concerns regarding antibiotic residues (Lima, et al., 2020). Antibiotics in various fields of agriculture, including beekeeping practices for disease prevention and treatment, raise issues related to the potential impact on product safety. Excessive or improper use of antibiotics can result in residues potentially posing risks to the population consuming contaminated food (Kumar, et al., 2020; Kim, et al., 2021; Ghimpețeanu, et al., 2022; Arsène, et al., 2020).

The global community is actively seeking optimal solutions to address the issue of substance residues in honey. It is also seeking to minimize the use of antibiotics along with the associated risks. There is a growing emphasis on regulatory measures to monitor and control antibiotic use in beekeeping. Governments and organizations are implementing guidelines and standards to prevent the occurrence of antibiotic residues in honey. Regular monitoring and testing of honey samples for residue detection play a crucial role in maintaining honey purity and consumer safety (Bonerba, et al., 2021; Lima, et al., 2020). The results of the annual residue analysis can provide comprehensive insights, enabling the identification and characterization of potential risks induced by antibiotic residues. Therefore, this study aims to assess the potential risks associated with antibiotic residues identified in Armenian honey within the framework of the national monitoring program.

Materials and methods

Consumption data collection and processing

The honey consumption data was collected via population survey conducted by the Informational-Analytical Center for Risk Assessment of the Food Chain, CENS in 2018. A Food Frequency Questionnaire (FFQ) was used, which included inquiries about portion size, consumption frequency, and demographic information. A total of 1040 residents from different districts of Yerevan, aged between 18 and 65 years, participated in the survey (Stepanyan, et al., 2022). The *FFQ* methodology ensured accurate data collection (Pipoyan, et al., 2020). The collected data were statistically analyzed using *SPSS* software (version 22.0). The cluster analysis method was employed to classify consumers into homogeneous clusters.

Analysis of samples

As part of the Armenian monitoring program on residues in animal-origin products, 32 multi-floral honey samples were collected in November 2019. These samples were obtained from various producers of natural multi-floral honey in Armenia, with each sample weighting between 0.5 and 1 kg. The samples were tested at the Republican Veterinary-Sanitary and Phytosanitary Laboratory Services Center. Honey samples were analyzed for the presence of residues of 10 antibiotics, namely dihydrostreptomycin (DHSTM), oxytetracycline (OTC), tetracycline (TC), sulfadiazine (SDZ), penicillin G (PenG), enrofloxacin (ENR), terramycin (TM), streptomycin (STM), sulfadimethoxine (SDMO), salinomycin (SM). The initial analyses were conducted using the ELISA method with MaxSignal ELISA Kits and a BioTek ELx800 analyzer. LC-MS/MS was used to analyze the primary screening results.

Risk assessment

The risks associated with antibiotic residues were assessed based on the Margin of exposure (*MOE*) approach using the following equation:

$$MOE = \frac{HBGV}{EDA}, \quad (1)$$

where *HBGV* is the toxicologically established health-based guidance value (mg/kg/day); and the *EDA* is the estimated daily intake of antibiotics through honey consumption (mg/kg/day).

The *EDI* of antibiotics was calculated for each cluster of honey consumers, using the following equation:

$$EDI = \frac{C_{honey} \times C_{antibiotic}}{BW}, \quad (2)$$

where C_{honey} is the mean daily intake (consumption) of honey (kg/day); $C_{antibiotic}$ is the mean content of antibiotic residue in food (mg/kg). In the case of content data on antibiotic residues below the Limit of Detection (*LOD*), commonly called “left-censored”, the value of *LOD/2* was used. *BW* is the body weight of consumers, averaged at 65 kg per population survey. For *PenG*, the *EDI* was calculated by multiplying the content with the honey consumption, without dividing by the body weight (*BW*), since the acceptable daily intake, presented as *HBGV*, is expressed in “mg/person/day”.

The worst-case scenario

Risk assessments commonly incorporate various scenarios, including the worst-case scenario, to ensure a comprehensive evaluation and avoid underestimating risks. In this study, the worst-case scenario refers to a hypothetical situation where all variables, such as residue and consumption data, are the highest when calculating the potential risk associated with antibiotic exposure. By considering this scenario, the study aims to evaluate the maximum possible risk. This provides valuable insights into the upper limit of risks associated with antibiotic exposure.

Results and discussions

Honey consumption

Through analysis of the survey data obtained from the Food Frequency Questionnaire (*FFQ*), three consumer clusters were identified based on their daily honey consumption. The first cluster, comprising 80 % of respondents, has an average daily consumption of 0.006 kg of honey. In contrast, the second cluster, representing 15 % of respondents, showed higher daily honey consumption at 0.028 kg/day. The third cluster, consisting of 5 % of respondents, exhibited the highest honey consumption daily, amounting to 0.059 kg/day.

Antibiotic residues in honey

Analyses showed that the studied honey samples did not contain residues of *DHSTM, OTC, ENR, TC*, and *SDMO* antibiotics. Therefore, exposure to these antibiotics and associated risks were not addressed in this research paper.

The study results revealed that out of the 32 honey samples analyzed, 8 tested positive for *TC*, 28 for *SDZ*, 31 for PenG, 32 for *STM*, and 6 for *SM*. Table 1 shows the minimum and maximum antibiotic concentrations as well as the calculated means and standard deviations (*SD*).

Table 1. Antibiotic residues in the studied honey samples*

Antibiotics	Antibiotic residues (µg/kg)			
	Min	Max	Mean	SD
TC	3.3	195.9	14.7	45.3
SDZ	4.7	40.9	10.5	8.1
PenG	1.9	4.5	3.2	0.7
STM	11.9	554.5	50.1	106.3
SM	1.2	1.4	0.8	0.3

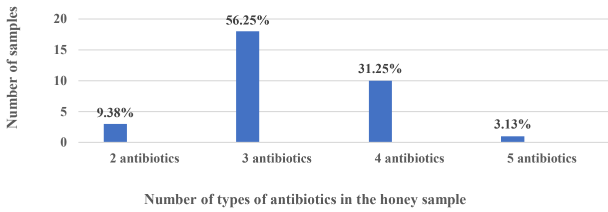

Figure. Number of types of antibiotics and percentage of honey samples (*composed by the authors*).

The honey samples analyzed contained varying numbers of antibiotic types (Fig.). Out of the 32 honey samples analyzed, none were "antibiotic-free". Each sample simultaneously contained residues of varying numbers of different antibiotics.

Approximately 9.38 % of the samples contained 2 different antibiotics *PenG* and *STM*. The residues of three different antibiotics were found in 56.25 % of the analyzed honey samples (18 samples), with 17 samples containing *PenG*, *STM*, and *SDZ*, and one sample containing *PenG, STM*, and *TC*. In 31.25 % of the samples (10 samples), four types of antibiotics were detected. The antibiotic composition varied among these samples, with five samples containing *TC, SDZ, PenG*, and *STM*, four samples containing *SDZ, PenG, STM,* and *SM,* and one sample containing *TC, PenG, STM,* and *SM*. Finally, all five detected antibiotics (*PenG, STM, SDZ, SM, and TC*) were present in only one sample.

Estimated daily intake (EDI) and Margin of exposure (MOE) of antibiotic residues

*EDI*s of antibiotics from honey for three consumer clusters are represented in Table 2.

Acceptable daily intake (*ADI*) values were taken as *HBGV* for the studied antibiotics. The estimated daily intake of all studied antibiotic residues was much lower than the established *ADI*s.

Table 2. The *EDI* of antibiotic residues through honey consumption, mg/kg/day*

Consumers	*TC*	*SDZ*	*PenG***	*STM*	*SM*
Cluster 1	1.36E-06	9.68E-07	1.90E-05	4.63E-06	7.70E-08
Cluster 2	6.33E-06	4.52E-06	8.86E-05	2.16E-05	3.59E-07
Cluster 3	1.33E-05	9.52E-06	1.87E-04	4.55E-05	7.57E-07

***is expressed in "mg/person/day"*

*Composed by the authors.

The *ADI* for TC is 0.03 mg/kg/day (JECFA, 1998). Due to its poor absorption from the intestinal tract, the toxic effects of *TC* on the body are relatively low. *ADI* is primarily associated with the risk of resistant bacterial strains (JECFA, 1998). The *ADI* for *SDZ* is 0.02 mg/kg bw/d (NRA, 2000). This *ADI* was derived by applying a safety factor of 2000 to the No-Observed-Adverse-Effect Level (NOAEL) of 37.5 mg/kg bw, which indicated fetotoxic effects in rats (NRA, 2000). The *ADI* for *PenG* is 0.03 mg/person/day (JECFA, 1990). The most common adverse effect of *PenG* is hypersensitivity reactions, but such cases are not associated with residual drug levels in food (WHO/JECFA). The *ADI* for *STM* is 0.05 mg/kg/day (JECFA, 2002). The *ADI* is obtained by applying a safety factor of 100 to NOAEL (5 mg/kg), which is based on the decreased body weight gain in rats in a two-year dietary study. And although these studies were performed with dihydrostreptomycin, the results apply to streptomycin due to their close relationship (NCBI-a). The *ADI* for *SM* is 0.01 mg/kg/day (APVMA, 2023). A study in human cell cultures showed the cytotoxic effect of *SM*. Scientific research and investigation of domestic cases with various animals revealed that high doses and long-term use of *SM* in animals have a neuropathic effect, can reduce the growth rate and reproductive functions of animals, and damage the skeletal and cardiac muscles. The toxicity of *SM* has not been extensively studied, but due to its identified antitumor activity, it is the subject of research (NCBI-b). Since the studied substances are medicines, they have undergone numerous studies and tests, as a result of which hazard categories have been identified for each drug. We referred to data from the European Chemicals Agency (ECHA) and the National Center for Biotechnology Information (NCBI-c) to compare antibiotic hazard statements (Table 3).

The statements listed in Table (3), provide information about the potential hazards associated with each antibiotic. SDZ appears to have the highest number of hazard statements among the studied antibiotics. The hazard statements for antibiotics include two types: health hazard (H3--) and environmental hazard (H4--). While *TC* and *SDZ* both have these hazard classifications, *SDZ* has a more significant number of them. In general, the most common hazard classes are related to ingestion, dermal effects, and reproductive toxicity. It is important to note that antibiotic *ADI*s may not always be directly associated with reported hazards, as these hazards are often linked to long-term therapeutic doses or overdoses. However, it should be recognized that these substances can have negative effects on the body. Furthermore, individual consumers may vary in terms of their metabolism and sensitivity to specific substances, potentially experiencing adverse effects at lower doses.

Table 3. Hazard statements for antibiotics*

GHS** Code	Hazard Statements	Antibiotics				
		TC	***SDZ***	***PenG***	***STM***	***SM***
H300	Fatal if swallowed					+
H301	Toxic if swallowed	+				
H302	Harmful if swallowed	+	+	+		
H312	Harmful in contact with skin			+		
H315	Causes skin irritation	+	+	+		
H317	May cause an allergic skin reaction		+	+		
H319	Causes serious eye irritation		+	+		
H332	Harmful if inhaled			+		
H334	May cause allergy or asthma symptoms or breathing difficulties if inhaled		+	+		
H335	May cause respiratory irritation		+	+		
H341	Suspected of causing genetic defects		+			
H351	Suspected of causing cancer				+	
H361	Suspected of damaging fertility or the unborn child				+	
H361(d)	Suspected of damaging the unborn child	+	+			
H362	May cause harm to breast-fed children	+	+			
H400	Very toxic to aquatic life		+			
H410	Very toxic to aquatic life with long-lasting effects		+			
H411	Toxic to aquatic life with long-lasting effects	+	+			
H412	Harmful to aquatic life with long-lasting effects		+			

*Note: GHS** - Globally Harmonized System of Classification and Labeling of Chemicals*

*Composed by the authors.

Table 4. *MOE* of antibiotic residues*

Honey consumption	*TC*	*SDZ*	*PenG*	*STM*	*SM*
Cluster 1	$2.21*10^4$	$2.07*10^4$	$1.58*10^3$	$1.08*10^4$	$1.3*10^5$
Cluster 2	$4.74*10^3$	$4.43*10^3$	$3.39*10^2$	$2.32*10^3$	$2.78*10^4$
Cluster 3	$2.25*10^3$	$2.10*10^3$	$1.61*10^2$	$1.1*10^3$	$1.32*10^4$

*Composed by the authors.

We calculated the Margin of Exposure (*MOE*) to antibiotics (Table 4) in honey for the three consumer clusters using the above-mentioned *ADI*s. While Table 3 suggests the presence of suspected genotoxicity in *SDZ* and carcinogenicity in *STM*, it is important to note that there is currently no definitive or confirmed data available regarding these specific risks. Moreover, the *ADI*s of all studied antibiotics are not associated with genotoxic or carcinogenic risks. Therefore, the threshold for risk assessment is set at $MOE \geq 10^2$. If the *MOE* is less than 10^2, it indicates a potential risk to consumers (Chem Safety PRO, 2018; Scientific Committee, 2019).

The *MOE* values for the studied antibiotics in the different consumer clusters ranged from $1.61*10^2$ for *PenG* in the third consumer cluster to $1.3*10^5$ for *SM* in the first consumer cluster. The *MOE* of *PenG* in the third consumer cluster is the closest value to 10^2. It is worth noting that none of the *MOE* values is lower than 10^2. This indicates that there is no potential risk associated with the studied antibiotics for consumers.

The worst-case scenario

For the worst-case scenario, the highest value of each antibiotic residue and the consumption data from the third consumer cluster were used to calculate the Estimated Daily Intake (*EDI*) and Margin of Exposure (*MOE)* values (Table 5). The results from the worst-case scenario analysis indicated that all *MOE* values remained above 10^2, suggesting no significant risk associated with the studied antibiotics. However, it should be noted that the *MOE* value for *STM* is slightly below 10^2. Hence, it is plausible to assume that an increase in honey consumption and/or the presence of antibiotic residues could potentially raise concerns regarding consumer health.

Table 5. EDI and MOE in the worst-case scenario*

Antibiotic	Max residue (mg/kg)	*EDI* (mg/kg/day)	*MOE*
TC	0.1959	$1.78*10^{-4}$	$1.69*10^2$
SDZ	0.0409	$3.71*10^{-5}$	$5.39*10^2$
PenG	0.0045	$2.66*10^{-4}$•	$1.13*10^2$
STM	0.5545	$5.03*10^{-4}$	99.3
SM	0.0014	$1.27*10^{-6}$	$7.87*10^3$

Note: •– mg/person/day

*Composed by the authors.

Conclusion

The growing demand for Armenian honey emphasizes the critical importance of its safety, particularly regarding antibiotic residues. The absence of some studied antibiotics in the honey samples indicates a favorable outcome in terms of consumer safety. However, the presence of multiple antibiotic types in some samples raises concerns about their potential impact on consumer health. The study findings highlight the importance of antibiotic usage control in beekeeping practices to ensure the safety of honey products.

Overall, the MOE values, which were assessed for each antibiotic in different consumer clusters, exceeded the threshold of 10^2. This indicates a low likelihood of adverse effects. Nevertheless, it is crucial to consider the worst-case scenario, where increased honey consumption and/or higher levels of antibiotic residues could raise concerns regarding consumer health. Moreover, the potential long-term effects and uncertainties associated with certain antibiotics, such as suspected genotoxicity and carcinogenicity, should be acknowledged.

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