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Detection of Antibiotic Residues in Honeys from Different Regions in Türkiye by Liquid Chromatography-Tandem Mass Spectrometry Method

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Abstract: This study aimed to investigate 29 antibiotics and their metabolites in 27 honey samples obtained from different provinces of Turkey by Liquid Chromatography-Tandem Mass Spectrometry Method (LS-MS/MS). This study showed that the correlation coefficients of the calibration graphs were 0.999, the limit of detection (LOD) was 0.94–3.40 ng/g, and the limit of quantification (LOQ) was 3.11–11.22 ng/g. To express the accuracy of the method, intra- and inter-day recoveries were tested using three different concentrations from 0.25 to 1 µg/kg. Intra-day recoveries for antibiotics and metabolites were found to be 95.56–115.56% with relative standard deviation values between 0.43 and 6.58; inter-day recoveries were found to be 90.00–108.89% with relative standard deviation values between 0.54 and 5.31. The analysis results showed that no antibiotic residues were found in any of the honey samples. The honey did not pose any danger to food safety or public health.

Keywords: Antibiotic residue, honey, LC-MS/MS.

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1. INTRODUCTION

Two types of honey are produced worldwide: honey produced by traditional honey bees (*Apis mellifera*) and stingless bee honey (Ranneh et al., 2021). Honey is a plant-based natural food containing about 200 substances, including carbohydrates (especially fructose and glucose), enzymes, minerals, vitamins, phenolic acids, amino acids, volatile compounds, flavonoids, carotenoids, and organic acids (gluconic acid and acetic acid) (Ranneh et al., 2021; Valverde et al., 2022; Brar et al., 2023). Besides its rich nutritional content and organoleptic properties, honey provides numerous health benefits such as antifungal, antibacterial, hepatoprotective, antioxidant, hypoglycaemic, antimutagenic, antihypertensive, and anti-inflammatory effects (Ranneh et al., 2021; Brar et al., 2023).

Turkey has approximately 500 nectar plant species for bees. Due to this rich ecosystem, Turkey enjoys favourable conditions for beekeeping activities and thus for the production of various bee products such as honey, propolis, bee bread, bee pollen, and royal jelly (Bayram, 2023). China is the world's leading producer of natural honey with over 472.7 metric tonnes, followed by Turkey, the second largest producer with 96.34 metric tonnes (Anon, 2023).

Food safety is one of the world's major concerns due to rapid urbanisation and population growth. Overpopulation leads to high demand for food production and commercialisation, which in turn calls for attention to maintain food safety and quality control to meet consumer expectations and mitigate the critical problem of foodborne diseases. The main causes of foodborne diseases are attributed to food hazards such as pathogenic microorganisms, heavy metals, toxic substances, pesticides, and veterinary drugs. Therefore, it is very important to detect and identify hazardous substances contained in foods in inspection procedures and food control systems (Hitabatuma et al., 2022).

Organic pollutants that may be contained in foods of natural or anthropogenic origin are divided into four main categories:

pesticides, persistent environmental chemicals, naturally occurring toxins, and veterinary drugs. Honey has been used in the treatment of diseases in recent years due to its natural raw materials and provides numerous health benefits, but it also poses a danger and raises concern due to various contaminants, including antibiotics (Marazuela and Bogialli, 2009; Shoaei et al., 2023; Zergui et al., 2023). Antibiotics are natural, semi-synthetic, and synthetic drugs used to treat diseases caused by bacteria in human and veterinary medicine (Gürel Yücel et al., 2023). Honey may be contaminated with antibiotics due to intensive agricultural and industrial activities or due to their use in beekeeping for the treatment of bacterial diseases (Bonerba et al., 2021; Er Demirhan and Demirhan, 2022). Bees can fly to regions approximately 3-6 km away, and the honey they produce from waters, nectars, and flowers in these regions can be contaminated with environmental pollutants such as heavy metals, radioactivity, and pesticides (Tutun et al., 2019; Savarino et al., 2020). Honey bees are unable to metabolise most of the antimicrobial substances, and drug residues have been reported to be found even after a considerable period in honey harvested after drug administration (Er Demirhan and Demirhan, 2022). Since honey is a natural product widely consumed by all population groups for both nutritional and medicinal purposes, monitoring antibiotic residues and other contaminants in honey is becoming more important to help protect food safety and human health (Bonerba et al., 2021; Cunningham et al., 2022). As these drug residues or metabolites are harmful to humans, animals, and the environment, the most important task is to identify, monitor, and evaluate trace amounts of these residues (Hitabatuma et al., 2022). Liquid chromatography-mass spectrometry (LC-MS) methods, high-performance liquid chromatography (HPLC) methods, and enzyme-linked immunosorbent assays (ELISA) are mostly employed to identify antibiotic residues in different food samples (Shoaei et al., 2023).

Turkey occupies a strong position in the extraction of bee products, with honey being the most popular. Due to uncontrolled and unconscious antibiotic abuse and indirect contamination from the environment, antibiotic residues in the honey lead to significant problems in food safety, public health, and exports. Therefore, this study aimed to determine the presence and levels of various antibiotics in honey by Liquid Chromatography-Tandem Mass Spectrometry Method (LC-MS/MS), to evaluate the results thereof according to national and international legislation, and to compare the data reported in published studies.

2. MATERIAL AND METHOD

2.1. Sampling

A total of 27 commercial honey samples were collected from Marmaris (n = 12), Datça (n = 4), Adana (n = 4), Çanakkale (n = 2), Erzincan (n = 1), Ankara (n = 2), Istanbul (n = 1), and Izmir (n = 1) provinces in Turkey. Honey samples were collected in their original packaging and stored at room temperature and in the dark until analysed.

2.2. Chemicals and reagents

The standards for tetracycline, 4-epi tetracycline, chlortetracycline, 4-epichlorotetracycline, oxytetracycline, 4-epi oxytetracycline, doxycycline, demeclocycline, sulphonamide, sulfamethoxazole, sulfamethazine, penicillin, ampicillin, amoxicillin, streptomycin, neomycin, kanamycin, gentamicin, chloramphenicol, lincomycin, bacitracin A, enrofloxacin, ciprofloxacin, cephalexin, ceftiofur, novobiocin, potassium clavulanate, tylosin, tilmicosin were obtained from Sigma-Aldrich (St. Louis, MO, USA). Methanol, EDTA, acetonitrile and formic acid were obtained from Merck (Darmstadt, Germany). Ultra-purified water was obtained using an H2OPRO-VF-T/Arrium Ultrapure device (Sartorius, Germany).

2.3. Standard solutions

Standard antibiotic solutions (10 µg/mL) were prepared in acetonitrile and stored in amber glass at 2-4ºC. Different working standard solutions were prepared by diluting the stock solutions in the same solvent.

2.4. Sample extraction

For the extraction of honey samples, the method used by Yang et al. (2022) was modified and followed. Each honey sample was weighed at 2 g in polypropylene tubes, and 10 mL of 70% methanol and 200 µL of 0.1 M EDTA were added. The mixture was homogenised by vortexing for 1 minute and then centrifuged at 9000 rpm for 5 minutes. The supernatant (500 µL) was taken into polypropylene tubes, and 2 mL of distilled water was added and mixed. Then, it was filtered through a 0.45 µm filter, and 1.5 mL was taken into vials and made ready to be injected into the LC-MS/MS system.

2.5. Device used and operating conditions

Antibiotics and their metabolites in honey samples were chromatographically separated according to the method used by Tasci et al. (2021). The extracts were injected 1 µL into the LC-MS/MS system.

2.6. Validation

The correlation equation, correlation coefficient (R^2), limit of detection (LOD), limit of quantification (LOQ), recovery rate, intra-day precision, and inter-day precision were determined as quality parameters (ICH, 2005).

2.7. Statistical analysis

Data were analysed by descriptive statistics using Minitab for Windows Version Release 16.1 (Minitab Inc., 2011).

3. RESULTS

The correlation equation, correlation coefficient values (R^2), the limit of detection (LOD), the limit of quantification (LOQ), recovery rate, and intra-day and inter-day precision of the graphs of antibiotics analysed in 27 honey samples are shown in Table 1 and Table 2 respectively. No antibiotic species were detected in the 27 honey samples analysed. .

Table 1. Validation parameters of antibiotic and metabolites analyses in honey samples.

Antibiotics	Calibration equation	R^2	LOD (ng/g)	LOQ (ng/g)
Tetracycline	$Y=2.12*10^{-6}x+1.29*10^{-4}$	0.999	3.40	11.22
4-epitetracycline	$Y=3.13*10^{-6}x+2.24*10^{-5}$	0.999	1.54	5.10
Chlortetracycline	$Y=7.25*10^{-6}x+6.21*10^{-5}$	0.999	1.03	3.41
4-epichlorotetracycline	$Y=2.89*10^{-6}x+6.08*10^{-5}$	0.999	1.02	3.35
Oxytetracycline	$Y=1.24*10^{-6}x+2.29*10^{-5}$	0.999	0.94	3.11
4-epioxytetracycline	$Y=4.13*10^{-6}x+5.01*10^{-5}$	0.999	1.37	4.50
Doxycyline	$Y=2.00*10^{-6}x+5.31*10^{-5}$	0.999	3.06	10.11
Demecloycline	$Y=2.00*10^{-6}x+5.24*10^{-4}$	0.999	2.73	9.01
Sulfonamide	$Y=2.13*10^{-6}+3,18*10^{-5}$	0.999	3.18	10.51
Sulfamethoxazole	$Y=2.04*10^{-6}x+4.43*10^{-4}$	0.999	0.94	3.11
Sulfamethazine	$Y=1.95*10^{-6}x+2.25*10^{-5}$	0.999	2.06	6.79
Penicilin	$Y=4.04*10^{-6}x+8.89*10^{-4}$	0.999	1.84	6.09
Ampicilin	$Y=1.00*10^{-6}x+5.52*10^{-4}$	0.999	1.76	5.80
Amoxicilin	$Y=4.21*10^{-6}x+1.53*10^{-5}$	0.999	1.42	4.70
Streptomycin	$Y=1.32*10^{-6}x+8.35*10^{-5}$	0.999	1.02	3.35
Neomycin	$Y=2.15*10^{-6}x+3.05*10^{-5}$	0.999	1.11	3.65
Kanamycin	$Y=6.21*10^{-6}x+7.04*10^{-5}$	0.999	1.96	6.47
Gentamicin	$Y=2.03*10^{-6}x+2.46*10^{-5}$	0.999	0.96	3.16
Chloramphenicol	$Y=4.33*10^{-6}x+3.12*10^{-5}$	0.999	2.75	9.09
Lincomycin	$Y=7.23*10^{-6}x+4.08*10^{-5}$	0.999	1.18	3.89
Baciratcin A	$Y=4.79*10^{-6}x+3.07*10^{-4}$	0.999	2.36	7.80
Enrofloxcain	$Y=9.55*10^{-6}x+2.25*10^{-5}$	0.999	1.59	5.23
Ciprofloxacin	$Y=8.12*10^{-6}x+3.12*10^{-4}$	0.999	1.11	3.68
Cephalexin	$Y=1.78*10^{-6}x+2.12*10^{-5}$	0.999	2.23	7.35
Ceftiofur	$Y=5.21*10^{-6}x+3.29*10^{-4}$	0.999	1.01	3.33
Novobiocin	$Y=1.01*10^{-6}x+1.21*10^{-5}$	0.999	1.98	6.52
Potasyum clavulanate	$Y=1.23*10^{-6}x+8.29*10^{-4}$	0.999	2.53	8.34
Tylosin	$Y=1.21*10^{-6}x+8.22*10^{-5}$	0.999	1.31	4.34
Tilmicosin	$Y=4.12*10^{-6}x+1.25*10^{-5}$	0.999	1.40	4.61

Explanations; R^2: Correlation Coefficient; LOD: Limit of Detection; LOQ: Limit of Quantification

Table 2. Precision and repeatability results for antibiotics and their metabolites in honey samples.

Antibiotics	Spiked level (μg/kg)	Intra-day Recovery (%,n:3)	Intra-day RSD (%)	Inter-day Recovery (%,n:3)	Inter-day RSD (%)
Tetracycline	0.45	102.22	1.26	97.78	2.27
		100.00	1.28	95.56	2.32
		102.22	1.24	100.00	2.13
	0.75	100.00	0.78	98.67	0.78
		98.67	0.70	98.67	0.70
		98.67	0.68	97.33	0.70
	1	99.00	1.54	99.00	0.59
		98.00	1.59	98.00	0.60
		101.00	1.43	98.00	0.54
4-epitetracycline	0.45	100.00	1.26	100.00	1.26
		102.22	1.29	102.22	1.29
		102.22	1.18	102.22	1.28
	0.75	98.67	1.33	98.67	1.33
		100.00	1.20	100.00	1.31
		101.33	1.19	101.33	1.26
	1	101.00	1.98	101.00	1.98
		103.00	2.02	103.00	2.02
		99.00	1.82	99.00	2.04
Chlortetracycline	0.45	100.00	1.26	97.78	2.27
		102.22	1.28	100.00	2.30
		102.22	1.16	95.56	2.21
	0.75	100.00	2.03	98.67	1.57
		102.67	1.88	98.67	1.59
		98.67	1.86	96.00	1.62
	1	110.00	5.55	101.00	1.54
		108.00	5.66	99.00	1.52
		99.00	5.60	98.00	1.51
4-epichlorotetracycline	0.45	97.78	2.59	97.78	2.22
		97.78	2.64	100.00	2.24
		102.22	2.42	102.22	2.22
	0.75	98.67	2.02	97.33	2.83
		101.33	1.82	96.00	2.74
		102.67	1.80	101.33	2.77
	1	106.00	4.90	99.00	2.65
		109.00	4.95	103.00	2.59
		99.00	4.46	98.00	2.62
Oxytetracycline	0.3	96.67	3.89	93.33	2.09
		96.67	3.93	93.33	2.15
		103.33	3.82	90.00	2.05
	0.5	108.89	2.32	108.89	2.39
		108.89	2.09	108.89	2.41
		113.33	2.03	104.44	2.44
	0.75	101.33	1.53	98.67	0.79
		101.33	1.58	97.33	0.80
		98.67	1.42	97.33	0.79
4-epioxytetracycline	0.45	100.00	1.26	100.00	1.26
		102.22	1.28	102.22	1.29
		102.22	1.24	102.22	1.18
	0.75	98.67	1.33	98.67	1.33
		100.00	1.20	100.00	1.20
		101.33	1.17	101.33	1.19
	1	101.00	1.98	101.00	1.98
		103.00	2.04	103.00	2.00
		99.00	1.84	99.00	1.80
Doxycyline	0.5	102.00	1.92	98.00	1.19
		104.00	1.96	96.00	1.21
		106.00	1.91	96.00	1.19
	0.75	98.67	0.79	98.67	0.78
		97.33	0.78	98.67	0.79
		97.33	0.79	97.33	0.80
	1	108.00	5.79	97.00	0.59
		109.00	5.85	98.00	0.61
		98.00	5.57	98.00	0.60
Demecloycline	0.45	100.00	1.30	97.78	2.66
		97.78	1.33	97.78	2.72
		97.78	1.29	93.33	2.61
	0.75	98.67	0.79	98.67	0.79
		97.33	0.78	97.33	0.72
		97.33	0.79	97.33	0.69
	1	105.00	3.13	99.00	1.02
		104.00	3.23	98.00	1.04

		99.00	3.20	97.00	0.94
Sulfonamide	0.3	96.67 100.00 96.67	1.97 2.01 1.88	96.67 90.00 90.00	4.17 4.22 3.83
	0.5	108.89 113.33 115.56	3.01 2.87 2.79	108.89 106.67 104.44	2.08 1.93 1.91
	0.75	101.33 101.33 98.67	1.53 1.55 1.53	98.67 98.67 97.33	0.78 0.80 0.79
Sulfamethoxazole	0.5	98.00 102.00 96.00	3.10 3.16 2.90	98.00 94.00 94.00	2.42 2.50 2.38
	0.75	98.67 98.67 101.33	1.55 1.39 1.38	97.33 100.00 97.33	1.57 1.58 1.60
	1	108.00 105.00 99.00	4.41 4.45 4.01	101.00 99.00 96.00	2.55 2.58 2.55
Sulfamethazine	0.5	98.00 96.00 104.00	4.19 4.23 4.11	96.00 94.00 98.00	2.08 2.13 1.95
	0.75	97.33 98.67 101.33	2.05 1.85 1.80	98.67 100.00 101.33	1.33 1.20 1.19
	1	101.00 108.00 98.00	5.01 5.17 4.66	103.00 98.00 97.00	3.24 3.27 2.94
Penicilin	0.45	100.00 102.22 95.56	3.42 3.45 3.35	102.22 100.00 100.00	1.27 1.30 1.24
	0.75	98.67 98.67 102.67	2.31 2.08 2.02	98.67 101.33 97.33	2.05 2.01 2.04
	1	109.00 112.00 99.00	6.38 6.58 5.93	99.00 97.00 97.00	1.18 1.19 1.14
Ampicilin	0.3	103.33 100.00 96.67	3.33 3.37 3.33	96.67 93.33 93.33	2.04 2.06 2.00
	0.5	106.67 106.67 104.44	1.21 1.18 1.13	108.89 106.67 104.44	2.08 1.88 1.82
	0.75	98.67 98.67 100.00	0.78 0.78 0.78	98.67 97.33 97.33	0.79 0.81 0.73
Amoxicilin	0.3	100.00 96.67 96.67	1.97 2.03 1.97	93.33 93.33 96.67	2.04 2.06 2.00
	0.45	100.00 97.78 102.22	2.22 2.12 2.05	97.78 97.78 100.00	1.30 1.17 1.14
	0.75	100.00 101.33 101.33	0.76 0.77 0.78	98.67 98.67 100.00	0.78 0.80 0.72
Streptomycin	0.5	102.00 100.00 98,00	2.00 2.02 2.00	100.00 96.00 94.00	3.16 3.19 3.10
	0.75	98.67 101.33 98.67	1.55 1.50 1.52	98.67 97.33 97.33	0.79 0.71 0.69
	1	107.00 101.00 101.00	3.36 3.30 3.33	99.00 98.00 97.00	1.02 1.05 0.95
Neomycin	0.3	96.67 96.67 103.33	3.89 3.97 3.64	93.33 96.67 93.33	2.04 2.06 1.87
	0.5	113.33 108.89 106.67	3.10 2.78 2.76	108.89 104.44 106.67	2.08 1.93 1.91
	0.75	100.00 98.67 98.67	0.78 0.79 0.71	97.33 98.67 98.67	0.78 0.80 0.79
Kanamycin	0.5	102.00 98.00 98.00	2.32 2.37 2.18	98.00 96.00 94.00	2.08 2.10 2.04

	0.75	101.33	1.55	98.67	0.78
		98.67	1.39	98.67	0.71
		98.67	1.38	97.33	0.69
	1	105.00	4.41	101.00	1.54
		108.00	4.50	99.00	1.59
		99.00	4.05	98.00	1.43
Gentamicin	0.45	97.78	1.30	97.78	1.32
		100.00	1.34	95.56	1.34
		97.78	1.30	97.78	1.30
	0.75	98.67	0.78	97.33	1.35
		100.00	0.74	100.00	1.22
		98.67	0.72	98.67	1.18
	1	105.00	3.70	99.00	3.76
		110.00	3.73	105.00	3.88
		113.00	3.77	98.00	3.49
Chloramphenicol	0.45	100.00	1.27	97.78	1.33
		102.22	1.29	95.56	1.35
		100.00	1.25	95.56	1.33
	0.75	101.33	2.31	98.67	0.79
		101.33	2.08	97.33	0.76
		97.33	2.02	97.33	0.77
	1	99.00	5.86	101.00	2.11
		111.00	6.04	98.00	2.07
		103.00	5.44	97.00	2.09
Lincomycin	0.3	96.67	3.89	96.67	2.01
		103.33	3.97	93.33	2.06
		96.67	3.64	96.67	1.89
	0.45	102.22	1.26	97.78	1.30
		102.22	1.14	97.78	1.17
		100.00	1.13	100.00	1.16
	0.75	98.67	1.55	98.67	0.78
		101.33	1.58	100.00	0.79
		98.67	1.42	98.67	0.71
Baciratcin A	0.5	98.00	1.19	102.00	3.53
		98.00	1.20	96.00	3.57
		96.00	1.09	96.00	3.47
	0.75	98.67	0.78	98.67	0.79
		100.00	0.72	97.33	0.71
		98.67	0.71	97.33	0.69
	1	105.00	2.23	99.00	1.02
		105.00	2.27	98.00	1.05
		101.00	2.25	97.00	0.95
Enrofloxcain	0.3	103.33	1.88	96.67	2.01
		100.00	1.92	96.67	2.06
		103.33	1.85	93.33	1.89
	0.45	97.78	1.30	102.22	3.45
		97.78	1.18	97.78	3.10
		100.00	1.15	95.56	3.07
	0.75	101.33	1.53	98.67	0.78
		101.33	1.56	98.67	0.79
		98.67	1.41	97.33	0.71
Ciprofloxacin	0.3	100.00	1.95	96.67	3.89
		100.00	1.99	96.67	3.97
		96.67	1.82	103.33	3.82
	0.45	100.00	1.29	97.78	1.32
		97.78	1.16	97.78	1.20
		100.00	1.15	95.56	1.17
	0.75	100.00	1.33	98.67	0.78
		101.33	1.35	98.67	0.80
		98.67	1.21	97.33	0.72
Cephalexin	0.3	103.33	1.84	96.67	5.21
		106.67	1.86	103.33	5.31
		103.33	1.81	93.33	4.87
	0.45	111.11	3.08	108.89	2.39
		113.33	2.77	108.89	2.15
		106.67	2.69	104.44	2.13
	0.75	101.33	1.53	97.33	1.35
		101.33	1.58	100.00	1.37
		98.67	1.42	98.67	1.23
Ceftiofur	0.25	100.00	2.28	96.00	2.44
		104.00	2.30	96.00	2.46
		100.00	2.23	92.00	2.26
	0.5	100.00	1.15	100.00	1.17
		102.00	1.03	98.00	1.04
		100.00	1.00	98.00	1.03
	0.75	100.00	0.78	98.67	0,79
		98.67	0.80	97.33	0.80

		98.67	0.72	97.33	0.72
Novobiocin	0.3	103.33 103.33 100.00	1.88 1.90 1.85	96.67 96.67 90.00	4.08 4.20 4.12
	0.45	102.22 97.78 100.00	2.22 2.01 1.91	97.78 95.56 100.00	2.27 2.05 1.99
	0.75	100.00 98.67 98.67	0.78 0.78 0.71	98.67 97.33 97.33	0.79 0.81 0.73
Potasyum clavulanate	0.75	98.67 100.00 98.67	0.78 0.78 0.76	98.67 97.33 97.33	0.79 0.80 0.74
	1	105.00 104.00 109.00	2.50 2.25 2.18	101.00 99.00 98.00	1.54 1.39 1.37
	1.25	98.40 99.20 99.20	0.47 0.48 0.43	99.20 98.40 97.60	0.81 0.82 0.74
Tylosin	0.5	98.00 98.00 104.00	3.46 3.57 3.40	98.00 98.00 94.00	2.39 2.44 2.37
	0.75	101.33 102.67 100.00	1.32 1.33 1.34	98.67 97.33 97.33	0.79 0.80 0.80
	1	112.00 105.00 101.00	5.25 5.31 5.25	103.00 97.00 97.00	3.50 3.46 3.50
Tilmicosin	0.5	102.00 104.00 98.00	3.01 3.05 2.96	94.00 96.00 94.00	1.22 1.23 1.20
	0.75	98.67 98.67 100.00	0.78 0.70 0.68	98.67 98.67 97.33	0.78 0.71 0.69
	1	103.00 101.00 98.00	2.50 2.58 2.32	102.00 98.00 97.00	2.67 2.76 2.48

RSD: Relative Standard Deviation

4. DISCUSSION

The calibration equation was established by providing reference standards at various concentrations (0.10 to 10 μg/mL), and the corresponding peak fields were obtained. The calibration curves showed good linearity, characterised by a high correlation coefficient ($R^2 > 0.999$). The LOD value for antibiotic residues was calculated as 0.94–3.40 ng/g and the LOQ value as 3.11–11.22 ng/g. To express the accuracy of the method, intra- and inter-day recoveries were tested using three different concentrations from 0.25 to 1 μg/kg. Intra-day recoveries for antibiotics and metabolites were found to be 95.56–115.56% with relative standard deviation values between 0.43-6.58; inter-day recoveries were found to be 90.00–108.89% with relative standard deviation values between 0.54–5.31. Recoveries and relative standard deviation values show the accuracy of the method. The validation parameters herein were compared with those in other studies. Günes et al. (2008) found a correlation coefficient of 0.996, a LOD of 6 ng/g, a LOQ of 20 ng/g, and a recovery rate of 85-89% for erythromycin in honey samples. Güneş et al. (2009) found that the LOD for oxytetracycline in honey was 10 ng/g, recovery 89–92%, and the LOD for sulphonamides was 6–12 ng/g, recovery 80–87%. Erdoğdu et al. (2011) determined correlation coefficient values of 0.9999, recovery rates of 50.7–62.2%, LOD values of 1.56-2.2 μg/kg, and LOQ values of 5.20–6.83 μg/kg for sulphonamide-derived antibiotics in honey samples. Ahmed et al. (2023) found that the accuracy rates for sulphonamides and tetracyclines in honey were between 83.07–86.93% and 86.90–91.19%, respectively, and the precision rates were below 9.54% with R^2 values between 0.978 and 1.00. The validation parameters determined herein were found to be compatible with those in other studies.

None of the antibiotics were found in the 27 honey samples analysed. Several studies have been conducted in Turkey and worldwide using different techniques to determine the presence and level of antibiotics in honey, and Table 3 and Table 4 summarise the results of these studies. A meta-analysis study conducted by Shoaei et al. (2023) to investigate antibiotic residues in honey showed that the antibiotic concentrations were fluoroquinolone 8.59 μg/kg, tetracycline 5.68 μg/kg, sulphonamides 5.54 μg/kg, and macrolides 4.19 μg/kg. There are differences between the results obtained herein and of other studies. These differences are correlated with factors such as difference in legislation and methods for determining antibiotic residues in honey, the use of various antibiotics for preventive and therapeutic purposes in beekeeping, indirect contamination from the environment, incorrect beekeeping practises, insufficient training of beekeepers on the dangerous effects of antibiotics, and inadequate monitoring system (Derebaşı et al., 2014; Savarino et al., 2020; Shoaei et al., 2023). Environmental conditions such as potable water around the apiary, surface, depth, and type of soil, as well as the type of plants grown therein, play an active role in antibiotic residues in honey samples (Ahmed et al., 2015). Chiesa et al. (2018) reported that close proximity of hives to agricultural activities due to different farming practices had a significant effect on antibiotic residues and honey quality. The level of antibiotic residues in honey samples herein was determined to be similar to the results of Güneş et al. (2009) and Kutlu et al. (2017) and much lower than the results of other researchers (Table 3 and Table 4). This may be due to the absence of residues in honey samples, the breakdown of antibiotics in honey over time, or the lower concentration than the detected concentrations of the method employed.

Table 3. The summary results of the studies related to the antibiotic residues in honey analysed by different methods in Türkiye.

Location	**Types of antibiotics**	**Analyses Methods**	**No. of honey sample**	**Incidence rate n (%)**	**Range (mean)**	**Reference**
Erzurum	Tetracyclin, Neomicin	ELISA	79	37 (46.8)	Tetrasiklin: 2.1-47.08 (9.33) ppb Neomisin: 0	Aydemir Atasever and Yüksel, 2022
Sivas	Tetracyclin group Streptomycin group	ELISA	60	Tetracycline: 22 (73.3) in packaged honey, 18 (60) in open sold honey; streptomycin: 30 (100) in packaged honey, 28 (93.3) in open sold honey	Tetracycline: 0.12-371.44 (13.91) ppb in packaged honey; 0.02-13.32 (1.75) ppb in open honeys. Streptomycin: 1.30-250.2 (25.8) ppb in packaged honey; 0.19-22.71 (8.2) ppb in open honeys.	Ağaoğlu et al., 2020
Bingöl	Sulfonamide group	LC-HRMS	13	n.r. (n.r.)	Sulfamethoxazole: 0.96-5.1 (n.r.) µg/kg	Kırkan et al., 2020
Kars	Multiple aminoglycoside and macrolide groups of antibiotics	Biochip array biosensors	45	Neomycin: 11 (24.4) Tylosin B: 2 (4.4) Amikacin: 8 (17.8) Lincosamides: 6 (13.3) Erythromycin: 41 (91.1 Streptomycin: 10 (22.2)	Neomycin: < 1.0-8.4 (0.81) ppb Tylosin B: < 1.0-55.2 (1.45) Amikacin: < 1.0-33.7 (4.11) Lincosamides: < 1.0-10.7 (1.23) Erythromycin: < 1.0-38.1 (6.71) Streptomycin: < 1.0-1000 (59.9)	Aksoy, 2019
Bitlis	Tetracylines, Sulfonamides	LC/MS/MS	20	0 (0)	0 (0)	Kutlu et al., 2017
Muğla	Amphenicols, Sulfonamides, Tetracyclines	UPLC-ESI-MS/MS	50	n.r. (n.r.)	Sulfamethazine: 647 µg/kg tetracycline: 968 µg/kg epitetracycline: 197 µg/kg oxytetracycline: 743 µg/kg epioxytetracycline: 158 µg/kg	Kıvrak et al., 2017
Black Sea Region	Streptomycin, Tetracyclines, Sulphonamides	Charm II tests	209	68 (32.5)	n.r. (n.r.)	Derebaşı et al., 2014
İzmir	Sulfonamide group	LC-MS/MS	536	Sulfanilamid: 2 (0.37) Sulfadiazin: 1 (0.19) Sulfametazine: 108 (20.15) Sulfamethoxazole: 9 (1.68) Sulfadimethoxie: 6 (1.12)	Sulfanilamid: 6.9-198 (102.45) µg/kg Sulfadiazin: 24.86 µg/kg Sulfametazine: 6.2-13356.7 (597.34) µg/kg Sulfamethoxazole: 10.66-70.1 (25.01) µg/kg Sulfadimethoxine: 25.4-542.5 (157.25) µg/kg	Erdoğdu et al., 2011
Southern Marmara region	Oxytetracyclin, Sulphonamides	LC-MS/MS	50	0 (0)	0 (0)	Gunes et al., 2009
Southern Marmara region	Erythromycin	LC-ESI-MS	50	4 (8)	50-1776 (n.r.) ng/g.	Gunes et al., 2008

n.r.: results not reported by author.

Table 4. The summary results of the studies related to the antibiotic residues in honey analysed by different methods in worldwide.

Location	Types of antibiotics	Analyses Methods	No. of honey sample	Incidence rate n (%)	Range (mean)	Reference
East Tennessee (USA)	Tetracycline, Erythromycin	cELISA	Tetracycline:9 Erythromyci:9	Tetracycline:9/9 (n.r.) Erythromycin:9/9 (n.r.)	Tetracycline: n.r. (77.86) µg/kg Erythromycin: n.r. (0.68) µg/kg	Sarkar et al., 2023
Egypt, Libya, Saudi Arabia	Sulfonamides, Tetracyclines	HPLC–DAD, and HPLC–MS/MS	Egyptian 33 Saudi Arabian 18 Libyan 24	Egyptian 19(57.6), Saudi Arabian 14(75), Libyan 18 (77.77)	Egyptian < d.l -275.080 (< d.l -96.825) µg/kg Saudi Arabian < d.l-151.066 ((< d.l-100.313) µg/kg Libyan < d.l -462.476 (< d.l-157.323)µg/kg	Ahmed et al., 2023
Iran	Erythromycin	ELISA	80	8 (10.66)	7.50-120 (20.32) ppb	Mehrabi et al., 2022
China	Quinolones, Sulfonamides, Tetracyclines, Phenicols	TQMS	94	All antibiotics: 79 (84.0)	All antibiotics: 0.04-7.84 (n.r.) ng/g	Wang et al., 2022
Italy	Amphenicols, Lincosamides, Macrolides, Nitroimidazoles, Pleuromutilins, Quinolones, Sulfonamides, Tetracyclines	LC-QTOF	55	3 (n.r.)	Sulfathiazole 0.5 µg/kg, Sulfamethazine 1.3 µg/kg, Tetracycline 0.5 µg/ kg; Oxytetracycline 1.1 µg/kg Tetracycline 0.5 µg/kg.	Paoletti et al., 2022
Indian	Oxytetracycline, Erythromycin, Chloramphenicol	HPLC	100	Oxytetracycline: 24/100 (24) Erythromycin: 2/100 (2) Chloramphenicol: 0/0 (0)	Oxytetracycline: 4.8-204 (69.3) ng/g Erythromycin: 51.0-55.0 (53.0) ng/g Chloramphenicol: 0(0)	Kumar et al., 2020a
Northwestern Himalayan Region of India	Oxytetracycline, Erythromycin, Chloramphenicol	HPLC	150	Oxytetracycline: 23/150 (15.3) Erythromycin: 8/150 (5.3) Chloramphenicol: 0/0 (0)	Oxytetracycline: 9-69 (28.9) ng/g Erythromycin: 50-112.0 (78.8) ng/g Chloramphenicol: 0(0) ng/g	Kumar et al., 2020b

n.r.: results not reported by author; < d.l.: below the detection limit

The European Union (EU) legislation has not set maximum residue limits (MRLs) for antimicrobial substances in honey, and therefore the European community does not allow the use of antibiotics in beekeeping. Therefore, the absence of MRLs means "zero tolerance" for antibiotic residues in honey, which is the same as the analytical method's limit of detection (EU, 2010). The legislation on veterinary drug residues for Turkey, prepared in compliance with EU legislation, has not set MRLs for antimicrobial substances in honey (TFC, 2017). However, some countries allow the use of a limited number of antibiotics in beekeeping and have set MRLs for these antibiotics. Although the Food and Drug Administration (FDA) in the United States authorises lincomycin 750 ppb, oxytetracycline (sum of tetracycline residues) 750 ppb, and tylosis 500 ppb for antibiotic residues in honey, it has not approved the use of chloramphenicol, nitrofurans, and/or fluoroquinolones to treat honey bees (FDA, 2023). Canada allows fumagillin at 0.025 ppm, oxytetracycline at 0.3 ppm, and tylosin at 0.2 ppm (Health Canada, 2022), while Australia and New Zealand allow oxytetracycline in honey at 0.3 ppm (FSANZ, 2016). As this study detected no antibiotic residues in honey samples, it was found to conform to national and international legislation and concluded that honey produced in Türkiye is safe for human consumption globally.

5. CONCLUSIONS

The absence of antibiotic residues in all the honey analysed in this study is satisfactory for food safety and public health, and the honey were found to be safe for human consumption. Implementation of antibiotic residue monitoring programmes to meet food safety requirements and scientific beekeeping practices is vital for the assessment of potential risks to human health. Therefore, appropriate antibiotic use and sale should be controlled, conscious beekeeping should be promoted, certified production should be compulsory, and legal inspections should be is recurrent. Hives should be placed at an appropriate distance from agricultural environment, and antibiotic residues in honey should be checked regularly.

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N/A

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