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ELISA Evaluation of Erythromycin Residues in Honey Samples Collected from Different Areas of Qazvin, Iran

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KEYWORDS

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ABSTRACT: Nowadays, antibiotic residues as a global concern pose a threat not only to public health, but also to the food industry all around the world. There have been many studies on contamination sources of bee products, which the most important of them is evaluation of honey contamination extent with various antibiotics used in colonies. Antibiotic residues have a relatively long half life and may have direct toxic effects on consumers. Considering the adverse effects of this residues, we decided to measure the residual erythromycin in different samples of honey consumes in Qazvin, Iran. The present study is a cross-sectional study which conducted in different regions of Qazvin, Iran, in 2019. 80 samples of honey were collected from different regions in Qazvin. Using semi-quantitative ELISA, honey samples were evaluated for the presence of residual erythromycin. Based on the results obtained by ELISA, residual erythromycin was detected only in 8 samples (10.66%), of which, 5 samples (6.66%) and 3 samples (4%) had between 10-120 ng g^{-1} erythromycin and higher than 120 ng g^{-1} erythromycin (higher than detection limit of kit), respectively. According to the results, more than 96 percent of our honey samples lacked any erythromycin or had erythromycin concentration lower than allowable limit. The present study demonstrated that most of the honey samples collected from different regions of Qazvin lacked erythromycin residues, but due to the importance of drug residues on community health, annual screening of produced honey for the presence of drug residues by competent authorities.

INTRODUCTION

Honey is produced by bees collecting the nectar and honeydew and living parts of plants, and is one of the most widely used natural foods worldwide [1]. Due to the high percentage of easily absorbed carbohydrates, honey is a nutritious food and is used extensively in food industry [2]. Honey is a mixture of different sugars (mainly fructose and glucose), water, and other minor compound's such as proteins, amino acids, enzymes,

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organic acids, vitamins, minerals, polyphenols, and volatile compounds [3, 4]. Honey contains antioxidant and antibacterial compounds such as phenolics and carotenoids. Flavonoids and polyphenols are the two main reactive molecules of honey which act as antioxidant. The presence of these two and other valuable compounds has given it medical and therapeutic, antioxidant, antimicrobial, anti-inflammatory, anti-viral, and anti-ulcer properties [5]. The main antibacterial agent of honey is hydrogen peroxide, which is produced by glucose-oxidase activity. Researchers have reported the bacteriostatic and bactericidal properties of honey especially in pathogenic strains such as klebsiella pneumoniae, Staphylococcus aureus, *Salmonella typhimurium*, etc. [6]. Annual honey production is estimated 4.1 million tons, worldwide. Asia is the largest producer of honey, accounting for approximately 40% of world production [7]. The global honey market is anticipated to reach 2.4 million tons by 2020, and most of this growth is thought to be the result of this fact that honey is a natural food and its sugar content don't contribute in obesity [8]. Antibiotic residues have a relatively long half life and may have direct toxic effects on consumers. Monitoring pesticide residues in honey, wax, and bees helps to assess the potential risk of these products to human health and provides data on the extent of pesticide treatments that have been used on the field crops surrounding the hives [9]. Antibiotic residues originating from agricultural use should be carefully monitored as they can adversely impact public health due to allergenic and carcinogenic factors, and may contribute to bacterial resistance [10]. The global antibiotic crisis poses a threat not only to public health but also to the food industry [11]. Many studies have investigated the contamination sources of bee products. Many studies investigated the contamination of honey with various antibiotics used in colonies. Application of various antibiotics against American foul brood, European foulbrood, etc. and antibiotic prophylaxis results in considerable antibiotic residues in bee products [12]. Erythromycin is one of the broad-spectrum polyketide antibiotics produced by industrial fermentation using *Sacaropolyspora erythrae* (Enzyme-Linked Immuno Sorbent Assay) [13]. Erythromycin is an important industrial compound used in synthesis of other derivatives such as clarithromycin, azithromycin, telithromycin, and roxithromycin [14]. Antibiotic microbial resistance is considered a public health threat. Improper administration of antibiotics has distributed the ecological balance between the host and microorganisms. Also, antibiotic resistance in pathogens will also increase and lead to sever infections [15]. Today, honey is produced in contaminant environments. Environmental contaminants include pesticides, heavy metals, bacteria, and radioactive materials which found in air, water, soil, and plants and are transmitted from one bee to another. Antibiotics are found in large amounts in the honey. While, antibiotic residues have a relatively long half-time, they may directly affect the health of consumers, and cause severe reactions in susceptible people [7]. Nowadays, different methods are used to identify and determine drug residues in foods [16]. Among these methods, ELISA showed high ability in data analysis. In addition, many authors used ELISA to detect antibiotic residues [17]. In this study, we evaluated the residual erythromycin in honey samples collected from different regions of Qazvin, Iran, by ELISA.

MATERIALS AND METHODS

Sampling

80 honey samples were collected from different regions of Qazvin (Eastern Alamut, Western Alamut, Takestan, and Abyek) during 2019 summer. The samples were transferred to the food safety laboratory of Qazvin University of medical sciences, under appropriate conditions.

Analysis of erythromycin residue

ELISA assay

We used ELISA to measure the erythromycin content of honey. First, the honey samples were completely homogenized. Then, 1g of homogenized samples added to 4ml 15% methanol and vortexed for >15min. The resulting solution was centrifuged at 2000rpm for 10 minutes. Then, 250µl of supernatant diluted with 750 µl PBS (Phosphate Buffer Solution). 50µl of the compound (erythromycin-HRP) was added to all wells except G1 and G2, microplate was placed in shaker for a few

minutes and incubated at 4˚C in a dark place for 60 minutes. Microplate contents were discarded and washed 3 times with a special buffer. In next step, 100 µl substrate was added to each well, and microplate was shaken for a few minutes again. Microplate was incubated in dark place and 20-25˚C for 30 minutes. 100µl stop solution was added to each well. The absorbance was read at 450 nm. Finally, 50µl of solution analyzed by ELISA reader according to manufacture instructions [18]. Europroxima kit (Made in The Netherlands) was used to determine erythromycin.

Statistical analysis

Data were analyzed by SPSS software version 23 using one-way analysis of variance (ANOVA) and P<0.05 was considered statistically significant.

RESULTS AND DISCUSSION

According to the results, erythromycin residues were only detected in 8 out 80 honey samples (10.66%), of which 5 samples (6.66%) had 10-120 ng/g erythromycin and 3 samples (4%) had more than 120 ng g^{-1} erythromycin (limit of detection). According to the results, more than 96% of our honey samples either did not have erythromycin or contained lower than allowable limit. The minimum and maximum values were 7.50ppb and 120ppb, respectively. Also, the mean residual amount of erythromycin was 20.32 ppb. According to the EU and veterinary organization standards, most of our samples contained lower antibiotic residues than standards. The comparison results of the samples collected from different regions of Qazvin demonstrated that most contaminated samples were collected from Eastern Alamut, Western Alamut, Abyek, and Takestan, respectively; and the differences were not significant (Table 1).

Table 1. Results obtained from analysis of different honey samples (collected from different regions of Qazvin) by ELISA.

Location	Number	Mean+/- SD	Min (ppb)	Max (ppb)	MRL(Maximum residue level) (ppb)	Samples containing erythromycin with values above the kit's LOD (%)	P- value of ANOVA
abyek	22	17.0764±11.43	7.50	34.33	100	9.09	
Western Alamut	22	19.1373±24.37	7.50	120	100	9.09	0.898
Eastern Alamut	32	19.7806±24.10	7.50	120	100	12.5	
Takestan	4	12.3300±3.61	7.50	16.04	100	0	
Total	80	18.4875±20.60				30.68	

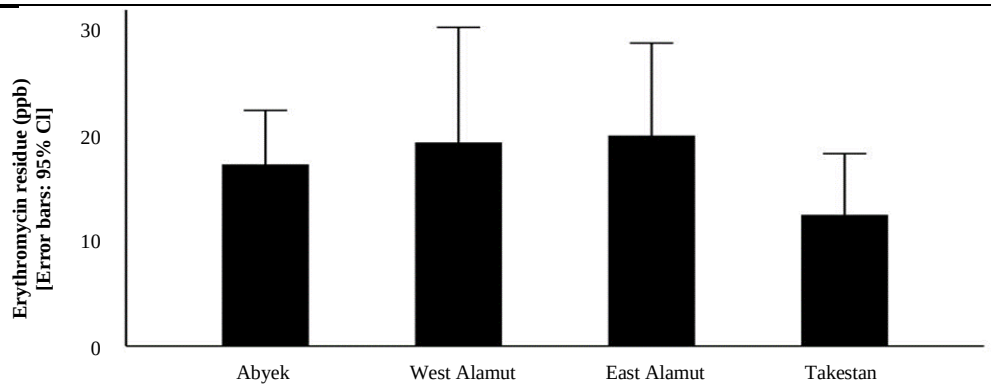

Figure 1. Residual erythromycin in different studies regions diagram.

The results showed that 8 of 80 samples (10.66%) contained erythromycin residue. The minimum and maximum detected values were 7.50ppb and 120 ppb, respectively. The results of analysis of different samples collected from different regions demonstrated that Eastern Alamut's samples have the highest erythromycin

residue. Also, statistical analysis by one-way analysis of variance (ANOVA) test showed that there was no significant difference between the different regions of Qazvin (Eastern Alamut, Western Alamut, Abyek, and Takestan) in terms of antibiotic contamination of honey samples. Antibiotic residues in foods have industrial, economic and health consequences. Health effects include a variety of allergic reactions in susceptible people, increasing the antibiotic-resistant bacteria, and the occurrence of variety of disease in consumers. One of the industrial consequences of residual antibiotics in foods is the elimination of beneficial bacteria used to produce fermented foods. Economically, unhealthy foods lead to consumer pessimism, non-use of the product, and ultimately economic losses. However, continuous field study of possible and widely used antibiotics are needed to monitor the current situation and take preventive measures. Considering the importance of antibiotic residues in different foods, different studies were conducted in this area and attracted special attention. In Iran, Standard No. 7087 on the method of honey sampling for residual drugs analysis was approved by Iranian Institute of Standards and Industrial Research, in 2003 [19]. However, there is no allowable limit (3MRLS) for veterinary antibiotics use in bees. In the United States, Canada, and Argentina, the use of antibiotics to treating foulbrood are banned. In Australia, 300ppb oxytetracycline is allowed and other antibiotics are banned. In Canada, allowable limits for oxytetracycline and erythromycin in honey are 300ppb and 30ppb, respectively [20]. Worldwide, various studies have been conducted on the different antibiotic residues of honey. In 2005, in the United Kingdom, different honey samples were tested by HPLC during the 12 weeks after the treatment (liquid and powdered drugs). Residual tetracycline was higher in the liquid treatment method and reached 3.7 mg kg^{-1} in 8 weeks after treatment [21]. A study of 576 honey samples in northern Spain showed that 24 samples (4%) contained tetracycline residues in the range between 15 and 920µg kg^{-1} [22]. In Greece, 251 honey samples were evaluated for tetracycline using HPLC method, of which 29% were positive. Most of the samples contained 0.018 to 0.055 mg kg^{-1} and some of them contained more than 0.100 mg kg^{-1} [23]. In Spain, the presence of 17 antibiotics (macrolides, tetracyclines, quinolones, and sulfonamides) were tested in 16 and 5 honey samples collected from supermarkets and beekeepers, respectively. One sample (from supermarket) contained 8.6 µg kg^{-1} sarafloxacin, and one of the samples (beekeeper) contained trace amounts of tylosin, sulfadimidine, and sulfachlorpyridazine [24]. In Germany, 30 and 47 positive imported honey samples were detected and 22 samples (approximately 50%) of imported honey sampled (from Argentina, China, and Canada) contained mainly sulfamethoxazole, while only one domestic sample contained antibiotic residues [25]. In Turkey, 50 honey samples were free of sulfonamides and oxytetracycline [26]. In Italian honey samples, A total of 27 types of veterinary drugs in sulfanamide, nitromidazole and quinolone groups were determined by LC-MS / MS. According to the results obtained from 74 honey samples, the level of sulfonamides was confirmed in 9 samples (12%) [27]. In Italy, honey samples were collected from different provinces of Italy and experiments were performed to evaluate 6 types of antibiotics. According to the study in 66 honey samples, 40 samples had antibiotic. 38 honey samples contained antibiotic tylosin, 36 samples had tetracycline, 27 samples contained quinolone, 21 samples contained thiamphenicol, 19 samples contained cephalosporin, and in one of the honey samples, streptomycin was found [28].

CONCLUSIONS

According to studies that have been conducted on the honey properties, honey has been introduced as a useful and healthy food, and health monitoring of it is of particular importance. Antibiotic residues in honey and its possible harms are a special concern, worldwide. Therefore, monitoring and control of antibiotic residues in hone is essential. Among the methods used to control drug residues in honey, are to increase the knowledge and awareness of beekeepers about the amount and how to use antibiotics and drugs, using drugs according to manufacture instructions, and under the supervision of a veterinarian. According to the results, it is necessary that responsible organizations determine the standards in accordance with the condition of each country. Improving the knowledge of farmers about the correct

use of drugs and the consequences of their excessive use, should be considered, too. Responsible organizations should set up and implement programs in order to control the drug use in breeding unites, and antibiotic should prescribe only after examinations, clinical tests, and antibiogram test. Drug prescription should perform under the supervision of a veterinarian and according to the manufacture instructions. A complete and appropriate explanation about the residual drug, tolerance, dose and administration rout, and when to dispose products due to contamination, should be considered [29]. Today, the presence of antibiotic residues in honey has resulted a serious problem for the trade of honey between countries. There are few articles about erythromycin residues in honey, so future studies are needed on this antibiotic. It should be noted that in the cases there the antibiotic levels were higher than the detection limit of kit; the exact levels should be determined by chromatographic techniques such and HPLC, LC-MS, etc. Our results demonstrated that most of the our honey samples are free of erythromycin residues, but considering the importance of residual drugs in public health, annual screening of honey produced in Qazvin, Iran, in terms of presence of medicinal residues is recommended by competent centers such ad veterinary administration and food and drug administration of ministry health.

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Conflict of interest

The authors declared no conflict of interest.

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