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ANTIBIOTIC RESIDUES IN HONEY SAMPLES COLLECTED WITHIN ONE YEAR PERIOD IN AP VOJVODINA, SERBIA

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Summary: The illegal use of antibiotics in honey production has recently been focused and the presence of antibiotic residues in honey is a critical parameter for its quality as it affects the safety of honey for human population. The need for simple test methods for determination of the presence of antibiotic residues in honey certainly exists. A modified method 4 plates that was primarily developed for detection of antibiotic residues in food was qualitatively applied for the screening of the residues of antibiotics in honey. The test is rapid, simple and cheap. In the present study, the investigations of the presence of antibiotic residues in honey result in 2.59% positive findings, i.e. in 5 of 193 examined honey samples collected from the territory of Vojvodina. Rapid analysis of the presence of antibiotic residues in honey certainly plays an important role in determining the overall safety of honey and final assessment of its usability.

Keywords: honey, quality, safety, antibiotic residues, modified method 4 plates

INTRODUCTION

Growing knowledge and consequent concern about the relationship between nutrition and human health are fast changing consumer habits, and there has been increasing demand for safety foods with health improved properties, such as honey and other bee products without residues of antibiotics. The presence of such residues in honey can lead to allergic reaction following the ingestion of contaminated honey (van Asselt et al., 2013). There is also enlarged concern about increasing bacterial resistance to antibiotics, which has been observed in various domestic animals (Kemper, 2008). Moreover, the low dosages of antibiotics used for growth promotion or in 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, van Asselt et al. 2013). The transmission of these resistance factors to human population could be harmful and furthermore could drastically reduce the effectiveness of antibiotics against various human diseases (Pyun et al. 2008). However, huge pools of information available to

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modern consumers result in their increased expectations and demands in view of the safety and quality of food (Prca et al., 2009), including honey (Prca et al., 2014).

Sulfonamides, tetracyclines (TCs), aminoglycosides, nitrofurans and macrolides are used by beekeepers to prevent and to combat diseases in honeybees (Bargańska et al. 2011, Apić et al. 2014). Generally, the use of antibiotics is prohibited in the European Union (EU) and European Commission (EC) Directive 2377/90 with annexes states that honeys should be free of antibiotic residues (EEC Regulation 2377/90 and amendments), so honey containing antibiotic residues cannot be sold in most EU countries and no maximum residue levels (MRLs) of antibiotic residues have been established. However, the application of the law in relation to the presence of antibiotic residues in honey is not harmonised across all member states of the EU and some countries, such as Switzerland and Belgium, have set MRLs for the for each class of antibiotics in honey at 10-50 μgkg^{-1} (Hammel et al., 2008, Carrasco-Pancorbo et al., 2008). In Republic of Serbia, the use of antibiotics by beekeepers to prevent and to combat bacterial diseases is prohibited and accordingly no MRL of antibiotic residues have been established (Sl.list SRJ, 5/92, 11/92, 32/2002). It is very important to establish a good approach for assessment and graduation of the quality and safety of honey. One of the possible approaches to evaluate the quality of honey includes implementation of relevant methods for determination of the presence of antibiotic residues in this foodstuff. Microbial inhibitions assays, including the tube test and the (multi-) platetest, were the first methods used for the detection of antibiotic residues and they are still widely used, due to their cost-effectiveness and ability to cover the entire antibiotic spectrum (Pikkemaat, 2009). A (multi) plate test consists of a layer of inoculated nutrient agar, with samples applied in wells in the agar or on top of the layer and bacterial growth will turn the agar into an opaque layer, which yields a clear growth-inhibited area around the sample if it contains antibiotic residues substances (Pikkemaat, 2009). The first results of our investigations on the antibiotic residues presence in honey samples in the territory of Vojvodina were published previously (Apić et al., 2014).

The objective of this study was to present the results of complete investigation on the presence of antibiotic residues in the various honey types, collected from April 2013 to April 2014 in the territory of Vojvodina.

MATERIAL AND METHODS

Honey samples obtaining. For the purpose of determining the presence of antibiotic residues in honey, 193 samples of different honeys originating from Vojvodina region were collected, within the period from April 2013 to April 2014. Honey samples were collected from two sources: honey samples that were brought by the owners at the Scientific Veterinary Institute "Novi Sad" and honey samples that were collected by experts from the Scientific Veterinary Institute "Novi Sad" on-site production. All samples were in their original packages and were transferred to the laboratory, properly labeled and stored in a cold and dark place. The investigated samples included 68 samples of meadow honey, 46 samples of acacia honey, 35 samples of linden honey, 12 samples of multiflower honey, 19 samples of sunflower honey and 13 samples of forest honey.

Antibiotic residues determination. The presence of antibiotic residues in honey was determined by microbiological method "Modified method 4 plates" (Heitzman, 1994). This is the most commonly used method in routine testing of food. The sensitivity and speed are the main characteristics of the test. The detection limit of the test depends on the inherent sensitivity of the test bacteria, the pH and the thickness of growth medium. This method is sensitive enough to regulate MRL. The sensitivity of the method is the best for beta-lactams, but is also very good for sulfonamides, aminoglycosides, tetracyclines and quinolones, while the sensitivity for chloramphenicol is insufficient and above the MRL. A test consists of a layer of inoculated nutrient agar. Principle of the method is based on detection of test microorganism growth inhibition in the presence of antibiotics, and this is manifested in the illumination zone of the substrate around the positive sample. Standard test microorganisms are: *B. subtilis*, *B. cereus*, *K. rizophila*, *B. stearothermophilus*, *B. megatherium*. The test microorganism was inoculated onto or into an agar medium in a Petri dish, then the honey sample was placed on the surface of the inoculated medium, which was incubated at the optimal temperature and time (24 h) for growth of the test-organism. There will not be observed growth of the test microorganism in the diffusion zone and a clear growth-inhibited area will be around the sample if it contains antimicrobial substances. The detection of an antibiotic in honey samples was determined positive when at least one of the four agar diffusion plates was considered to contain residues of antibacterial substances as shown by zones of inhibition of at least 2 mm in width. Width of the growth-inhibited area was measured from the edge of the hole in the agar to the limits of growth of test microorganisms. The simplicity and low cost of inhibition tests make it suitable for analysis of the large number of samples in monitoring programs.

RESULTS

The obtained results on the presence of antibiotics residues in the honey samples are shown in Table 1.

Table 1. The presence of antibiotic residues in the samples of various honey types

Type of honey	No. of samples	Number of positive samples	Positive samples (%)	Inhibition zone (mm)
Meadow honey	68	0	0	0
Acacia honey	46	1	2.17	>4
Linden honey	35	1	2.86	>4
Multiflower honey	12	1	8.33	>4
Sunflower honey	19	1	5.26	>4
Forest honey	13	1	7.69	>4
Total	193	5	2.59	?

Our results showed that 5 honey samples (2.59%) contained the residues of antibiotics. Further analysis of the results obtained for the investigated parameter in honey samples revealed the lowest presence of antibiotics residues in samples of meadow honey where all tested samples were negative on the presence of residues of antibiotics (Table 1). The highest percentage of antibiotic residues among examined honey types were established in multiflower honey samples (8.33%).

DISCUSSION

There are several international reports of antibiotic residues in honey samples (Reybroeck, 2003; Ortelli et al., 2004; Saridaki-Papakonstadinou et al., 2006; Gunes et al., 2008; Vidal et al., 2009). However, there are very few reports of antibiotics in Serbian honey (Dugalić-Vrndić et al., 2005; Dugalić-Vrndić et al., 2011; Petrović et al., 2009). Dugalić-Vrndić et al. (2005) determine the antibiotics and sulphonamides residues the antibiotics and sulphonamides residues in hundred honey samples from Belgrade market and supermarket by using the EEZa (four plate) modified microbiology method and the presence of the investigated residues was found in 18 (18%) of the honey samples whose inhibition zone was 0.5 to 4 mm. The residues of antibiotics and sulphonamides in samples from market were determined. Samples obtained from market places had a greater number of positive samples (13 samples, or 7.8%) than samples from supermarkets (5 samples, or 2%). Petrović et al. (2009) showed that 23.08% of the honey samples originating from the Vojvodina Province (Serbia) retail, contained antibiotics residues. Dugalić-Vrndić et al. (2011) tested 65 honey samples and found the presence of residues of antibiotics and sulfonamide in 8 samples by using two microbiological methods - Premi® test and Four plate methods. Mujić et al. (2011) conducted research to evaluate the health safety of honey (meadow, chestnut, acacia, amphora, and honeydew) produced in Bosnia and Hercegovina and determined antibiotic residues by disc plate method of microbiological antibiotic assay in 46 samples whereby all honey samples were negative. Saridaki-Papakonstadinou et al. (2006) found tetracycline residues in Greek honey, and Ortelli et al. (2004) found residues of chloramphenicol in honey collected from Switzerland market. In Greece, 251 honey samples were analysed by liquid chromatography by Saridaki-Papakonstadinou et al. (2006) to detect tetracycline residues. They have found that 29% of the samples contained tetracycline residues. Ortelli et al. (2004) reported that of the 75 samples of honey obtained in Switzerland, 34 which originated from Asian countries, 13 samples (17%) contained chloramphenicol residues. Gunes et al. (2008) have analysed 50 honey samples comprising chestnut, pine, linden and multiflower honeys collected from the hives in Turkey. The samples were analysed for erythromycin residues by liquid chromatography-mass spectrometry using electrospray ionization in the positive ion mode (LC-ESI-MS) and four of the honey samples (8% of the honey samples) were contaminated with erythromycin residues. Vidal et al. (2009) reported presence of erythromycin residues in three out of 16 samples of honey in Spain by using ultra-performance liquid chromatography tandem mass spectrometry. Reybroeck (2003) monitored 248 samples of locally produced and imported honey on the Belgian market for the presence of residues of antibiotics in the period 2000-2001. According to them residues of antibiotics were found in a very limited number of honey samples produced in Belgium and streptomycin was detected in 4 out of 248, tetracycline in 2 out of 72, sulfonamides in 3 out of 72 samples, while no residues of β -lactam antibiotics and chloramphenicol were found. In imported honey samples streptomycin was detected in 51 out of 102

samples, tetracyclines in 29 out of 98 samples, sulfonamides in 31 out of 98 samples, chloramphenicol 40 out of 85 samples. Furthermore, Reybroeck (2003) and Orтели et al. (2004) showed that a great part of honey imported from China contains chloramphenicol in quantities greater than the EU regulatory standard. The presence of antibiotic residues in honey have recently become a great public health concern. Johnson et al. (2010) noted that residues of antibiotics in honey originate mostly from improper beekeeping practices and not from the environment. Paige et al. (1997) stated that some antibiotics have the potential to produce toxic reactions in consumers directly while some other have the potential to produce allergic or hypersensitivity reactions. β -lactam antibiotics can cause cutaneous eruptions, dermatitis, gastro-intestinal symptoms and anaphylaxis at very low doses (Gehrig and Warshaw, 2008). 3-nitrofurans and nitroimidazoles can cause cancer in human population (McEvoy, 2002). Muhammad et al. (2009) and Jeong et al. (2010) reported that indirect and long term consequence of the ingestion of low-dose of antibiotics by consumers include microbiological effects, carcinogenicity, reproductive effects and teratogenicity. Microbiological effects are one of the major health hazards in humans (Casewell et al., 2003). 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). The simplicity and low cost of inhibition tests make it suitable for analysis of the large number of samples in monitoring programs (Pyun et al., 2008). Confirmatory methods are not considered in the present study but it will be taken into consideration in our future work. As obvious from a brief review of control of the presence of antibiotic residues in honey in Serbia and worldwide, the mentioned analysis is of vital importance in quality and safety assessment. Although the aforementioned researches encompassed different types of honey, our research demonstrated that the safety of honey from Vojvodina corresponds to that of honeys available in international market.

CONCLUSION

Presented results clearly demonstrate that 5 (2.59%) out of the 193 samples of honey analyzed contain the residues of antibiotics. These are not compliant with EU standard for honey to be exported due to the presence of antibiotic residues. Furthermore, according to our results, the modified method 4 plates can be used as a first step in screening procedures, especially when fast results are necessary, such as regular monitoring of the presence of antibiotic residues in honey. The above mentioned test can also be used to identify the presence of antibiotics in honey samples, but cannot be used for quantification and the final identification and quantification of any antibiotic in honey requested the use of an appropriate confirmatory technique to prove that the honey samples are safe for human consumption. Nevertheless, potential harmful effects of the presence of antibiotic residues in honey on the human health strongly suggest the necessity of continuous monitoring of the aforementioned parameters throughout the year.

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PRISUSTVO REZIDUA ANTIBIOTIKA U UZORCIMA MEDA SAKUPLJANIH OD APRILA 2013. DO APRILA 2014. NA TERITORIJI AP VOJVODINE, SRBIJA

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Izvod: Upotreba antibiotika u proizvodnji meda je zakonom zabranjena. Od nedavno, velika se pažnja poklanja detekciji i determinaciji rezidua antibiotika u medu, koje predstavljaju jedan od najvažnijih parametara kvaliteta samog meda. Prisustvo rezidua antibiotika u medu dovodi u pitanje njegovu zdravstvenu bezbednost za u humanoj populaciji. S toga, svakako da postoji potreba za brzim, skrining, test metodama za detekciju antibiotskih rezidua. Modifikovana „metod 4 ploče“ primarno razvijena da detektuje rezidue antibiotika u hrani, upotrebljena je kao skrining metoda za otkrivanje rezidua antibiotika u medu. Ova metoda je relativno brza, jednostavna za izvođenje i jeftina. Na prisustvo antibiotskih rezidua, ovom metodom, je ispitano 193 uzorka meda, od kojih je 5 uzoraka bilo pozitivno. Uzorci meda sakupljeni su na teritoriji AP Vojvodine, Srbija. Brzina analiza na prisustvo antibiotskih rezidua u medu svakako igra važnu ulogu u određivanju zdravstvene bezbednosti meda i davanje konačne ocene njegove upotrebljivosti.

Ključne reči: med, kvalitet, bezbednost, rezidue Ab, modifikovana „metoda 4 ploče“.

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