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Original Contribution

DETERMINATION OF TETRACYCLINE RESIDUES IN GREEK HONEY

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ABSTRACT

Liquid chromatography was used to detect tetracycline-derived residues in Greek honey. Of the examined samples 29% had these drug residues. 20.3% of these contained more than one of the observed tetracycline derivatives. Majority of samples contained residues to a proportion of 0.018-0.055 mg/kg of honey while some others had residues in excess of 0.100mg/kg. The findings show that tetracycline drug residues in Greek honey are present and possibly influence the naturally occurring protective properties of this medicinal food.

Key Words: honey, tetracycline, liquid chromatography

INTRODUCTION

Honey is historically an essential ingredient of Mediterranean diet (Skidas and Lascaratos, 2001) and is also promoted currently around the world as a naturally protective product against various infections (Basson et al. 1994; Drouin, 1999; Taormina et al. 2001) or burns and wounds (Molan, 2002)$_1$ The antimicrobial effects of honey, attributed to the presence of hydrogen peroxide and the level of antioxidant power (Taormina et al. 2001), appear to be influenced by the type of honey. The dark coloured varieties of honey are thought to possess more inhibitory properties on selected pathogens than the light coloured ones. Other properties of honey, among which is its anti-inflammatory activity, contribute to rapid pain, oedema and exudate reductions. Moisturising of wounds prevents tissue maceration and adherence of dressings to the wound bed (Molan, 2002). However, the purity of the honey would determine the strength of these properties, including the antimicrobial action.

Drug residues reduce these natural protective properties in honey and may consequently affect adversely its antimicrobial activity. Specifically, these residues may contribute to microbial resistance thereby altering the beneficial effects of the honey. The antimicrobial properties of honey appear to result from its naturally occurring "inhibines" (Taormina et al. 2001; Zaghloul et al. 2001) that have intrinsic antibacterial actions, including activities against bacterial strains that are generally resistant to commonly used drugs (Tichy and Novak, 2000; Ceyhan and Ugur, 2001; Dixon, 2003). This honey protective property is making it a preferred natural product for studying less intrusive alternatives for protection of patients.

The rediscovered medicinal properties of honey have also increased interest in the quality of this food. Among the factors determining honey quality, purity is the most important. Various chemical contaminants or residues of chemicals, including drugs used for bee diseases, are currently being researched vigorously and detection methods are continuously improving (Vinas et al. 2004). Improved detection methods would naturally give rise to more classes of drug and other chemical residues being detected (Kaufmann et al., 2002; Evans, 2003;

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Verzegnassi et al., 2003; Wang, 2004), with the concomitant increase in proportion of honey samples reported as positive (Heering et al., 1998; Verzegnassi et al., 2003; Vinas et al. 2004).

However, bees, like other living organisms, suffer the consequence of disease, and beekeepers often use either preventive or therapeutic antimicrobials to protect bee health and levels of honey production. The tetracyclines, among other agents, have been used consistently for treating foulbrood caused by *Paenibacillus larvae* (Evans, 2003). Thus, honey could have drug residues in detectable amounts, possibly having considerable consequences to the consumer. Detecting drug residues in honey is one objective of state food inspecting agencies charged with the responsibility of ensuring the safety level of honey. Various methods used in detecting residual antimicrobials in foods (Mitchell et al. 1998) may not be suitable for honey, a product having naturally occurring bacteriostatic substances (Ceyhan and Ugur, 2001; Zaghloul et al. 2001), but technological improvements in available methods are giving promising results (Vinas et al. 2004). Greek honey, in order to be exported, must conform to EU directives, thus must be free of chemical contaminants and antimicrobial substances. To meet this requirement, the Veterinary Laboratory Services of the Greek Ministry of Agriculture in Chania, Crete, usually tests honey for tetracycline residues before exportation. This effort, which started officially in 2003, is revealing interesting results. The preliminary results reported here were produced using the slightly modified method of Oka et al. (1987) for detecting minute amounts of tetracycline derivatives.

MATERIALS AND METHODS

Two hundred and fifty one samples of honey produced across Greece and waiting certification for exportation were mailed between 2003 and 2004 to this laboratory. Producers wanting state permission to export their product submitted these samples for evaluation following government controls on the use of antibiotics for treating bee diseases. Most samples were either pine tree or spring wild flower honeys. Tetracycline (TC), oxytetracycline (OTC), chlortetracycline (CTC) and doxycycline (DC) were detected according to Oka et al (1987) by a Hewlett Packard 1100 liquid chromatographer (Hewlett Packard Inc) with a Diode Array Detector and a Discovery C8 separation column (25 cm x 4.6 mm, 5μm) at a light wavelength of 360 nm. Specifically, purification of samples was achieved by diluting 15 g of honey in 30 ml of EDTA (0.1M and pH of 4.0). The solution was passed through glass wool (SUPELCO) filters before it was further purified through a Baker column $10C_{18}$ (SUPELCO) suitable for separating organic compounds. The column was washed with 10 ml distilled water and further cleaned by the passing of high speed N_2 gas. Any remaining organic compounds were removed by passing through the column of 5 ml of ethyl acetate having 10% of MeOH. The collected solutions were passed again through a 10 COOH Baker column, which was washed with 3 ml of MeOH. Final purification was achieved with the through passing of 0.01M and pH of 3.0 Oxalic acid solution (Fluka), Methanol (LiChrosolv, Merck) and Acetonitrile (LiChrosolv, Merck) in a ratio of 5:4:1 respectively. The final solutions were tested by the liquid chromatographer using as positive controls samples having 0.30, 0.20, 0.05 and 0.100 mg/Kg of TC, OTC, DC and CTC respectively.

RESULTS

Twenty nine percent of the examined samples had drug residues of at least one of the tetracyclines. Of them 17 (20.3%) samples had residues of more than one derivative. The range of the detected amounts of each observed drug residue in the examined samples was 0.018-0.057, 0.023-0.335, 0.018-0.190 and 0.013-0.393 for TC, OTC, DC and CTC respectively. With an intra-laboratory reproducibility of 97%, 94%, 90% and 96% for TC, OTC, CTC DC, the positive samples detected and the areas they have originated from are detailed in **Table 1**. The highest proportion of positive samples had originated from Thrace (53%), with the islands second (37.5%) and Macedonia third (30.9%). Most of the positive samples were positive for OTC (34 samples) with DC second (27 samples). One sample was found to have 0.335 and 0.393 mg/kg of OTC and CTC respectively. Honey from the islands appears to have the lowest concentration of drugs and that of Macedonia the highest. Among the drug classes used OTC residue was found in 14% of the examined samples and DC in 10%.

DISCUSSION

Very little to no information is available in the literature regarding the presence of tetracycline residues in honey, and this is the

first ever for Greece. The findings are evidence of a rising problem with possible health consequences to honey consumers.

Thus, one understands that, in the absence of internationally set acceptable amounts of tetracycline residues or standard methods for detecting them, much depends on the sensitivity of the method used in the detection of antimicrobials.

Table 1: Number of positive samples to each antimicrobial agent, area of origin and range of drug residues

Area	*No*	*Pos (+) Samples*	*Neg (-) Samples*	*Antimicrobial (+)*			
				TC	*OTC*	*DC*	*CTC*
Attika	16	1	15				1
Islands	32	12	20	1 0.018	6 0.022-0.054	5 0.015-0.035	4 0.022-0.032
Macedonia	123	38	85	10 0.018-0.037	19 0.023-0.135	13 0.020-0.190	12 0.013-0.90
Peloponissos	48	11	37		5 0.047-0.085	6 0.020-0.111	3 0.026-0.096
Sterea Hellas	7	1	6		1 0.089		
Thessaly	10	2	8		2 0.047-0.049		
Thrace	15	8	7	1 0.018	3 0.043-0.335	1 0.020	1 0.393
Total	***251***	***73***	***178***	***12***	***36***	***25***	***21***

The present findings compare well with those of others (Vinas et al. 2004) using a similar methodology. The method used here detects minute amounts of drug; thus it has high reproducibility and sensitivity with the attendant increase in the proportion of true positive samples. A relatively high proportion of examined samples are, thus, positive to one or more of the tetracycline derivatives. The maximum amounts detected indicate an excessive use of the observed substances, of which OTC and DC are more often detected. The reason for this frequent use could have arisen from easier access connected with pricing, flexibility of use or the need to go above the normal dose in response to dwindling efficacy. These scenarios inexorably give rise to residue accumulation in honey or a concomitant increase in microbial resistance when the honey is used against microorganisms (Heering et al. 1998; Evans, 2003; Verzegnassi et al. 2003).

However, lack of information on findings and methods used for drug detection in honey point to the need for more work. This work should show the spread and levels of honey contamination by drugs used in apiculture, with or without official permission, and help food agencies and governments to further regulate the use of antibiotic and antiparasitic drugs in apiculture. Current EU restrictions of common antibiotics in apiculture call for more research on the suitable drugs or dose of various drugs used in combating bee infections or parasitic diseases.

REFERENCES

Basson, N. J., du Toit, I. J. & Grobler, S. R., 1994. Antibacterial action of honey on oral *Streptococci*. Journal of Dental Association of South Africa 49**,** 339-341.

Ceyhan, N. & Ugur, A., 2001. Investigation of in vitro antimicrobial activity of honey. Reviews in Biology 94, 363-371.

Dixon, B., 2003. Bacteria can't resist honey. Lancet Infectious Diseases 2, p116.

Drouin, E., 1999. Helicobacter pylori: novel therapies. Canadian Journal of Gastroenterology 13, 581-583.

Evans, J. D., 2003. Diverse origins of tetracycline resistance in the honey bee bacterial pathogen *Paenibacillus larvae*. Journal of Invertebrate Pathology 83, 46-50.

Heering, W., Usleber, E., Dietrich, R. & Martlbauer, E., 1998. Immunochemical screening for antimicrobial drug residues

in commercial honey. Analyst 123, 2759-2762.

Kaufmann, A, Roth, S., Ryser, B., Widmer, M. & Guggisberg, D. 2002. Quantitative LC/MS-MS determination of sulfonamides and some other antibiotics in honey.

Journal of AOAC International 85, 853-860.

Mitchell, J. M., Griffiths, M. W., McEwen, S. A., McNab, W. B. & Yee, A.J., 1998. Antimicrobial drug residues in milk and meat: causes, concerns, prevalence, regulations, tests, and test performance. Journal of Food Protection 61, 742-756.

Molan, P. C., 2002. Re-introducing honey in the management of wounds and ulcers - theory and practice. Ostomy/wound management 48, 28-40**.**

Oka, H., Ikai, Y., Kawamura, N., Uno, K., Yamada, M., Harada, K. & Suzuki, M., 1987. Improvement of chemical analysis of antibiotics. XII. Simultaneous analysis of seven tetracyclines in honey. Journal of Chromatography 400, 253-261.

Skiadas, P. K. & Lascaratos, J. G., 2001. Dietetics in ancient Greek philosophy: Plato's concepts of healthy diet. European Journal of Clinical Nutrition 55, 532-7.

Taormina, P. J., Niemira, B. A. & Beuchat, L. R., 2001. Inhibitory activity of honey against food-borne pathogens as influenced by the presence of hydrogen peroxide and level of antioxidant power. International Journal of Food Microbiology 69, 217-225.

Tichy, J. & Novak, J., 2000. Detection of antimicrobials in bee products with activity against viridans streptococci. Journal of Alternative and Complementary Medicine 6, 383-389.

Verzegnassi, L., Royer, D., Mottier, P. & Stadler, R. H., 2003. Analysis of chloramphenicol in honeys of different geographical origin by liquid chromatography coupled to electrospray ionisation tandem mass spectrometry. Food additives and contaminants, 20, 335-342.

Vinas, P., Balsalobre, N., Lopez-Erroz, C., Hernandez-Cordoba, M., 2004.

Liquid chromatography with ultraviolet absorbance detection for the analysis of tetracycline residues in honey. Journal of Chromatographic Analysis 1022, 125-129.

Wang, J., 2004. Confirmatory determination of six penicillins in honey by liquid chromatography/electrospray ionisation-tandem mass spectrometry. Journal AOAC International 87, 45-55.

Zaghloul, A. A., el-Shattawy, H. H., Kassem, A. A., Ibrahim, E. A., Reddy, I. K. & Khan, M. A., 2001. Honey, a prospective antibiotic: extraction, formulation, and stability. Die Pharmazie 56, 643-647.