
Determination of Tetracycline Residues in Honey from Tabora and Singida Regions Tanzania Produced Using Modern Beehives

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Abstract: A liquid chromatographic tandem mass spectrometric (HPLC MS/MS) method is proposed for identification and quantification of antibiotics residues and metabolites in honey and other matrices such as water, manure, soil, meat and sediments. This method was used to detect tetracycline derived residues in honey samples from Tabora and Singida regions, Tanzania. This honey was obtained from modern beehives. Three hundred honey samples were tested for the presence of tetracycline derivative residues. Of the examined samples 15% had these drug residues. Majority of samples contained residues to a proportion of 0.002–0.008 mg/kg. The findings show that tetracycline antibiotic residues are present in honey from Tabora and Singida regions, although in small concentrations, however, this can possibly influence the naturally occurring protective properties of this medicinal food. Also can lead to drug resistance to consumers. Prospective studies that simultaneously track both resistance genes and antibiotic residues will go far in resolving some of the gagging questions that cloud our understanding of antibiotic resistance dissemination.

Keywords: Honey, Tetracycline Residues, Mass Spectrometry, Tabora, Singida, Tanzania

1. Introduction

The association of human being and honey bees has existed since time immemorial [8], and honey remains an important product for human wellbeing. It is widely known and consumed as a food and medicine in various places all over the world [1]. It has also non consumptive uses. Also honey is historically an essential ingredient of diet and has been promoted currently around the world as a naturally protective product against various infections [11], or burns and wounds [7]. The antimicrobial effects of honey, attributed to the presence of hydrogen peroxide and the level of antioxidant power [11], appear to be influenced by type of honey. The dark colored varieties of honey are thought to possess more inhibitory properties on selected pathogens than light colored ones. Other properties of honey, among which is its antiinflammatory activity, contributes to rapid pain, edema

and exudates reduction.

Moisturing of wounds prevents tissue maceration and adherence of dressing to the wound bed [7]. However, the purity of the honey would determine the strength of these properties, including antimicrobial action. Drug residues reduce these natural 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 inhibine [11, 16], that have intrinsic antibacterial actions, including activities against bacterial strains that are generally resistant to commonly used drugs [2, 3, 12]. This honey protective property is making it a preferred natural product for studying less intrusive alternative for protection of patients.

The rediscovered medicinal properties have also increased

in 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 [14]. Improved detection methods would naturally give rise to more classes of drugs and other chemical residues being detected [6, 15]. With the contaminant increase in proportional of honey samples reported as positive [5, 13, 14].

However, bees like other living organisms, suffer the consequence of disease, and beekeepers often use either preventive or therapeutic antimicrobials to protect bees, health and levels of honey production. The tetracyclines, among other agents, have been used consistently for treating foulbrood caused by *Paenibacillus larvae*. Thus honey could have drug residues in detectable amounts, possibly having considerable consequences to consumer. Various methods used in detecting residual antimicrobials in food, may not be suitable for honey, a product having naturally occurring bacteriostatic substances [2, 16], but technological improvements in available methods are giving promising results [14]. The modified [10] Oka *et al.*, 1987 method was used for detecting minute amounts of tetracycline derivatives.

2. Materials and Methods

2.1. Standard Solutions

Tetracyclines, Oxytetracycline, Chlortetracycline and Doxycycline standards were purchased from Sigma Aldrich, Germany. Standard stock solutions at 100 mg/l were prepared by dissolving 10 mg of standard powdered in 10 ml ethanol. Working standard solutions at 1mg/l were prepared by dilutions from the standard stock solution in ethanol. Roxithromycin, used as internal standard was purchased from Sigma. Standard stock solutions at 1000 mg/l were prepared in ethanol. Working standard solutions at 10mg/l were prepared by dilution from standard stock solutions in ethanol. Standard stock solutions were stored in dark bottles at 4°C and were stable for two months. Working standard solutions were stored in same conditions for one month.

2.2. Fortified Samples

Tetracycline, Oxytetracycline, Chlortetracycline and Doxycycline concentrations in honey samples of 10, 15, 20, 25 and 30 µg/kg were obtained by spiking 50g of negative control honey with 55, 120, 210, 350 and 600 µl of working solutions respectively. Spiked and blank honey samples were mixed with a small volume of 100 µl of roxithromycin working standard solutions.

2.3. Reagents and Equipment

Ammonium acetate, Tris (Hydroxymethyl amonmethane) and 30% aqueous ammonia solution were purchased from

Sigma Aldrich, were of the highest purity grade. Solvents such as acetonitrile, ethanol, methanol were purchased from Sigma Aldrich were all high performance liquid chromatography grade. Distilled water was deionised by MilliQ apparatus from Millipore.

2.4. Sample Preparation

Three hundred samples of honey produced across Tabora and Singida regions using modern beehives were evaluated for tetracycline residues. 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 C18 separation column (25 cm x 4.6mm, 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). The solution was passed through glass wool (SUPELCO), filters before it was further purified through a Baker column 10C18 (SUPELCO) suitable for separating organic compounds. The column was washed with 10ml distilled water and further cleaned by passing of high speed N₂ gas. Any remaining organic compounds were removed by passing through the column of 5ml of ethyl acetate having 10% of MeOH. The collected solutions were passed again through a 10COOH Baker column, which was washed with 3ml of MeOH. Final purification was achieved with the passing of 0.01m and pH 3 oxalic acid solutions, methanol and acetonitrile in a ratio of 5.4:1 respectively. The final solutions were tested by liquid chromatographer using positive control samples having 0.30, 0.20, 0.04 and 0.001 mg/kg of OTC (Oxytetracycline), TC (Tetracycline), CTC (Chlorotetracycline) and DC (Doxytetracycline) respectively.

3. Results

Fifteen percent of the examined samples had drug residues. The range of detected amount of each observed drug residues in examined samples was 0.002–0.005, 0.006–0.008, 0.001–0.031 and 0.004–0.0072 mg/kg, doxycycline, chlorotetracycline, oxytetracycline and tetracycline respectively. With reproducibility of 90%, 93%, 91% and 97% for doxycycline, chlortetracycline, oxytetracycline and tetracycline respectively. Positive samples detected and regions they have originated are detailed in Table 1. The highest proportion of positive samples originated from Tabora which was 23% and only 3.8% from Singida region. Most of the positive samples were positive for tetracycline 29 samples, oxytetracycline 14 samples. Honey samples from Singida region appear to have the lowest concentrations of drugs and that of Tabora the highest.

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

Area	No	positive samples	Negative samples	Antimicrobial (+)			
				TC	DC	OTC	CTC
Sikonge	90	23	67	10	13		
	0.005 - 0.0054 0.002-0.007						
Uyui	40	10	30	9			
	0.001 - 0.0032 0.003 - 0.005	1					
Urambo	41	7	34	7			
	0.004 - 0.006						
Iramba	52	1	51	1			
	0.0011 - 0.0032						
Manyoni	30	4	26	3			
	0.002 - 0.008 0.002 - 0.007	1					
Mkalama	47	0	47				
Total	300	45	255	29	1	14	1

Key. TC (Tetracycline), DC (Doxycycline), OTC (Oxyteracycline), CTC (Chlortetracycline).

4. Discussion

Beekeeping in Tanzania plays a major role in socio economic development and environmental conservation. It is a source of food (e.g. honey, pollen and brood), raw materials for various industries (e.g. beeswax candles, lubricants), medicine (honey, propolis, beeswax bee venom) and source of income to beekeepers. Also beekeeping plays a major role in improving biodiversity and increasing crop production through pollination. In Tanzania honey is highly produced in Tabora region (Sikonge, Nzega, Igunga, Urambo, Uyui and Tabora districts), Singida region (Manyoni, Iramba and Mkalama districts), Shinyanga (Kahama and Bukombe districts), Rukwa (Mpanda district), Songwe (Chunya district) [9]. Scarce information is available in literature in Tanzania concerning the presence of antibiotic residues in honey. The findings are evidence of rising problem with possible health consequences to honey consumers. Thus one understand that, in the absence of international set acceptable amounts of antibiotic residues including tetracycline residues or standard methods for detecting them, population is involuntarily exposed to antibiotics residues unknowingly through honey consumption.

The present findings compare well with those others [14], using similar methodology. The method used here detects minute amounts of drugs residues, thus it has high reproducibility and sensitivity with tendency increase in proportion of true positive samples. A relatively high proportion of examined samples are thus positive to one or more of the tetracycline derivatives. The findings indicate a frequent use of tetracyclines derivatives. 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 residues accumulation in honey or a concomitant increase in microbial resistance when the honey is used against microorganisms [5, 4, and 13]. 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 will help food agencies and government to further regulate the use of antibiotics and antiparasitic drugs in apiculture. Currently, world restrictions of common antibiotics in apiculture call for more research on the suitable drugs used in combating bee infections or parasitic diseases.

5. Conclusion

Beekeeping in Tanzania has a great potential of contributing to poverty reduction through income generation to beekeepers and government, creating employment to community and improving biodiversity. However, application of antibiotics for therapeutic and prophylaxis should be monitored to prevent antibiotic residues accumulation in honey, which may have public health concern to consumers.

Conflict of Interest

We declare that there is no conflict of interests.

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