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# Research Article
# Residues of Tetracycline, Chloramphenicol and Tylosin Antibiotics in the Egyptian Bee Honeys Collected from Different Governorates

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## Abstract

**Background and Objective:** Bee honey as one of the most complete natural food for humans due to its therapeutic effect. Antibiotic residues in bee honey are still a significant problem in a wide range of the world. This study was conducted to determine three antibiotics residues, tylosin, chloramphenicol and tetracycline in the Egyptian bee honeys to give short brief about the Egyptian honeys status. **Materials and Methods:** Sixty-four bee honey samples (52 bee honey samples produced in different seasons and regions +12 samples from Egyptian supermarket) were collected from different types and governorates to determine the antibiotics residues by using high performance liquid chromatography. **Results:** Egyptian bee honey samples had contaminated by residues of 89 tylosin, 47% chloramphenicol and 31% tetracycline. Although, commonly used the antibiotics tylosin in the most tested samples, chloramphenicol recorded highest estimated residue. **Conclusion:** The chloramphenicol residues recorded the highest mean value comparing with tylosin and tetracycline residues in all types of bee honey except citrus and banana bee honeys. Natural bee honey should be free from any antibiotic residues and its presence due to wrong practices and lack of awareness of beekeepers, which negatively affected on human nutrition and health. Moreover, need to encourage beekeepers and urge them to transfer their colonies to newly reclaimed areas.

Key words: Egyptian bee honey, antibiotics residue, tylosin, chloramphenicol, tetracycline

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Competing Interest: The author has declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

## INTRODUCTION

Bee honey is the most important colony product of honey bees (*Apis mellifera*). It is the natural sweet substance produced by honey bees from the nectars of plant flowers and honey dew[1]. The bee honey has been considered as nutritive and therapeutic substances because it provided energy (80-85% carbohydrate) and monosaccharides which are easily digestible as those in many fruits[2-4]. In addition to many important substances such as organic acids, phenolyde compounds, flavonoids, flavonoids, antioxidants, pigments, vitamins, enzymes, etc[5]. The bee honey compositions depend on many factors such as geographical, floral origin, season, environmental and practices of beekeepers[6,7].

Currently, bee honeys are produced in a polluted environment with many pollutant means (pesticides, heavy metals, antibiotics, bacteria and radioactivity) whether environmental or specific practice of beekeepers[8,9]. The honey contaminated from the beekeepers' erroneous practices is due to use of varroa parasites control and insecticides to control wax worms. Diseases and pests can be infected honey bee colonies, such as American, European foulbrood and nosemosis[8]. Sometimes antibiotics are used as a precaution to prevent infection[10,11]. Beekeepers are using tetracyclines, streptomycin, sulfonamides, tylosin, nitrofurans and chloramphenicol antibiotics for controlling diseases[12,13]. Additionally, these antibiotics are highly harmful for human health even it recorded with trace level in honey[14]. Antibiotic residues have a relatively long half-life period, which may have effects on consumers are directly toxic, allergies, may result in resistance to antibiotics in humans and damage to the central nervous system. Also, some antibiotics, such as chloramphenicol, which is known to cause tissue destruction, blood anemia, nitrofuran and possibly carcinogenic or causing fetal mutagenic[15-17]. Resistance of antibiotic is one of the 3 greatest threats to human health[18], especially, a long-term exposure to antibiotics in humans, livestock, horticulture and food preservation. Oxytetracycline and chloramphenicol residues have been found above the regulatory standards in honey. Thus, determination of antibiotic residues in the bee honey was rigorous and should be free from organic or inorganic strange materials[19-21].

In some EU countries, using antibiotics are illegal. There are no maximum residue limits established for antibiotics in honey according to European community regulations, this mean that honey contained antibiotics residues do not allowed for sale[22-24]. According to the previous studies, it is important to determine the antibiotics residues in the Egyptian bee honeys. Therefore, this current study aimed to determine residues of the most common three antibiotics (tetracycline, chloramphenicol and tylosin) in the Egyptian bee honeys.

## MATERIALS AND METHODS

This investigation was carried out in the laboratory of the apiary yard, Experimental Station, Faculty of Agriculture, Cairo University at Giza. During 2017 year.

**Honey samples:** Sixty-four samples of Egyptian bee honey were collected from different Egyptian governorates as shown in Table 1.

**Chemical analysis:** Determinations of antibiotics (Tylosin, chloramphenicol and tetracycline) residues in each source of bee honey were done at food safety and quality control lap. Fac. Agric., Cairo Univ., Giza, Egypt. Using high performance liquid chromatography (HPLC), bee honey samples were analyzed according to Manual of Methods of Analysis of Food, Antibiotic and hormones residue[25].

**Statistical analysis:** Data were analyzed using SAS software (SAS[26]). One-way analysis of variance (ANOVA) with unequal number of replications (Table 1) was used to compare among residues of three antibiotics within each types of honey and to compare among 7 different sources of honey within each antibiotic. When significant differences at $p \leq 0.05$ were noted, Duncan's[27] multiple range test was used to separate means.

## RESULTS

**Differences among sources of bee honey in antibiotics residues:** Results in Table 2 showed that significant difference ($p = 0.0058$) among all sources of Egyptian bee honeys in its content of chloramphenicol antibiotics residue. The significant higher values of chloramphenicol residue were appeared in market samples

Table 1: Sources of bee honey, number of samples and Egyptian governorates

| Sources of honey | Samples number | Governorates |
|---|---|---|
| Medical plants | 9 | Faiyum, Minya, Beni-Suef, Asyut |
| Clover | 15 | Faiyum, Beni-Suef, El-Dakahlia, El-Sharqia, Minya |
| Citrus | 8 | El-Beheira and El- Sharqia, El-Menofia, Sina |
| Cotton | 8 | El-Dakahlia, El-Menofia, Faiyum, Beni-Suef |
| Banana | 2 | El-Beheira |
| Sugar feeding | 10 | Giza, El-Beheira |
| Market | 12 | Markets |

All collected samples were stored at 20±2°C in the laboratory until the chemical analysis

Table 2: Mean and range of three antibiotic residues in different sources of Egyptian bee honeys

| Sources of bee honeys | Number of tested samples | Antibiotic residues in honey: Tylosin (mg kg$^{-1}$) | Chloramphenicol (mg kg$^{-1}$) | Tetracycline (mg kg$^{-1}$) | p-value |
|---|---|---|---|---|---|
| Medical plants | 9 | 0.0434±0.0193$^{y}$ (0.0-0.19) | 13.7511±5.809$^{ab,x}$ (0.0-54.9) | 0.0014±0.0007$^{y}$ (0.00-0.0047) | 0.0102 |
| Clover | 15 | 0.0215±0.0083$^{y}$ (0.0-0.13) | 2.0967±0.7753$^{b,x}$ (0.0-9.80 ) | 0.0012±0.0006$^{y}$ (0.0-0.066) | 0.0020 |
| Citrus | 8 | 0.0411±0.0111$^{x}$ (0.0-0.086) | 0.0000±0.0000$^{b,y}$ (0.0-0.00) | 0.0023±0.0011$^{y}$ (0.004-0.008) | 0.0002 |
| Cotton | 8 | 0.0368±0.0080$^{y}$ (0.018-0.089) | 12.4213±5.7820$^{ab,x}$ (0.0-44.00) | 0.0000±0.0000$^{y}$ (0.00-0.00) | 0.0220 |
| Banana | 2 | 0.0845±0.0265$^{x}$ (0.058-0.111) | 0.0000±0.0000$^{b,y}$ (0.0-0.0) | 0.0026±0.0025$^{y}$ (0.0-0.0051) | 0.0485 |
| Sugar feeding | 10 | 0.0972±0.0249$^{y}$ (0.022-0.242) | 1.3200±0.690$^{b,x}$ (0.0-5.500) | 0.0005±0.0004$^{y}$ (0.00-0.0048) | 0.0481 |
| Markets | 12 | 0.1237±0.0684$^{y}$ (0.0-0.16) | 16.0642±4.992$^{a,x}$ (0.0-59.2) | 0.0066±0.0037$^{y}$ (0.000-0.046) | 0.0003 |
| p-value | | 0.2985 | 0.0058 | 0.2092 | |
| Grand mean | | 0.0619±0.0142$^{y}$ (0.0-0.242) | 6.5219±2.7160$^{x}$ (0.0-54.90) | 0.0022±0.0008$^{y}$ (0.0-0.066) | 0.0120 |

$^{a,b,x,y}$Means followed by the same letters are not significantly different ($p \leq 0.05$)

(16.06417±4.992 mg kg$^{-1}$) and ranged (0.0-59.2 mg kg$^{-1}$) compared to all the other different sources of bee honey samples followed by medical plants and cotton bee honey samples with no significant differences (13.75111±5.809 and 12.42125±5.78200 mg kg$^{-1}$) and (0.0-54.90 and 0.0-44.00 mg kg$^{-1}$) ranges, respectively.

The lowest values of chloramphenicol residue were observed in samples of sugar feeding honey (1.32±0.69 mg kg$^{-1}$ with range of 0.0-5.50 mg kg$^{-1}$) and clover bee honey (2.0967±0.775 mg kg$^{-1}$ and range of 0.0-9.80 mg kg$^{-1}$) with no significant differences. Moreover, no chloramphenicol residue values were shown in citrus and banana bee honeys.

Also results presented in Table 2 revealed no significant differences among all types of Egyptian bee honeys contaminated with tylosin ($p = 0.2985$) or tetracycline ($p = 0.2092$) residues.

**Differences among antibiotics residues:** Concerning the difference among antibiotics residues in bee honey, results in Table 2 show that grand mean of chloramphenicol residue was significantly the highest (6.521±2.7160 mg kg$^{-1}$) with range (0.0-54.90 mg kg$^{-1}$). However, the differences between residues of tylosin and tetracycline antibiotics were the lowest (0.0619±0.0142 and 0.002±0.0008 mg kg$^{-1}$ and ranged (0.0-0.242 mg kg$^{-1}$) and ranged between 0.0-0.066 mg kg$^{-1}$, respectively) with no significant differences as affected by sources of bee honey.

**Practice of beekeepers:** Figure 1 showed that the percentage of the Egyptian bee honeys containing one, two or three kinds of antibiotic residues reached 89, 52 or 13%, respectively. This may be due to the wrong practice of beekeepers in treating colony by antibiotics. Beekeepers usually use high doses for treating infection of bacterial brood and low doses to prevent infection. The obtained results are not accepted by The Quality Control of Egyptian Honey Specifications[28] which states the bee honeys must not contain any antibiotics residue.

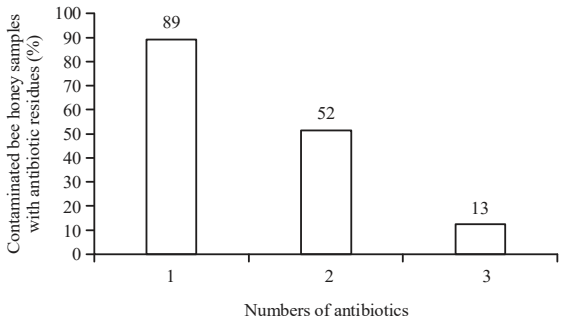


Fig. 1: Percentage of bee honey samples that contaminated with 1, 2 or 3 antibiotic residues

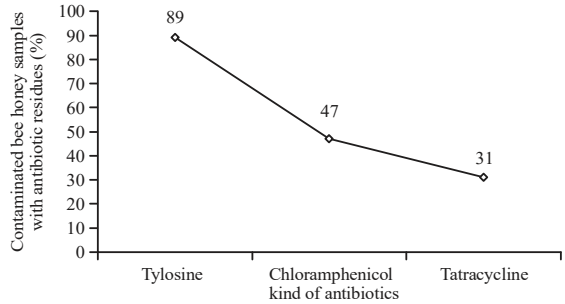


Fig. 2: Percentage of each antibiotic residue in the Egyptian bee honeys

**Antibiotics residues survey:** Results of the antibiotics residues survey which were appeared in different sources of bee honey samples are illustrated in Fig. 2. The highest contamination percentage (89%) for bee honey samples was with tylosin residue during different seasons. Except 11% of honey samples were not contaminated with the tylosin residue. The chloramphenicol residue was detected at 47% of bee honeys samples during different seasons and not detected in 53% samples. On the other hand, 31% of bee honey samples were contaminated with tetracycline residue but 69% of honey samples were not contaminated.

In general, 89% of different bee honey samples collected from different Egyptian governorates was contained with one antibiotic residue at least but 11%were free from any antibiotics residue. Tylosin antibiotic was the most commonly used followed by chloramphenicol and tetracycline. However, the values of chloramphenicol residues were the highest.

## DISCUSSION

This study was investigated to determinate the three common antibiotic residues (tylosin, chloramphenicol and tetracycline) in the Egyptian bee honeys. Results indicated that from all samples 89% were contained with one antibiotic residue, 52% were contaminated with two kinds of antibiotics and 13% were contaminated with the three kinds. The bee honeys should be free from any antibiotic residues, presence of these substances, even in small quantities which affecting on marketing according to the quality control of Egyptian bee honey specifications[28] and European Commission (EC) Directive[24] with annexes states. The chloramphenicol residues were the highest values comparing with tylosin and tetracycline residues in all sources of bee honey except citrus and banana honeys. This is may be due to these honeys are produced in newly reclaimed land, which they depend on wells water as they are characterized by low relative humidity in this region. Consequently, these factors lead to reduce the spread of diseases and antibiotics in reclaimed land. So, we should encourage beekeepers and urge them to transfer their colonies to newly reclaimed areas. Furthermore, antibiotic residues in honey have become a major concern for consumers[19]. The reason of antibiotic residues presence in bee honeys is wrong practices of beekeepers not from the environment. Residue of antibiotics have a relatively long life span causing direct toxic effects on consumers such as allergic reactions to individuals with hypersensitivity and blood clotting disorder or indirect effect by stimulating bacterial strains to resistance[15]. Similar trends were observed by Payne *et al.*[29] and Reybroeck[30] whom reported that repeated exposure of bee honey antibiotic residues had toxic effect on blood, liver and bone marrow in human.

In Switzerland from 75 commercial honey samples, 34 samples were contaminated with originated materials[31]. Another study in Greece using HPLC analysis of 251 bee honey samples, 29% of the samples had antibiotic derived residues contamination. Most of them contained from 0.018-0.055 mg $kg^{-1}$ tetracycline residues and some others derivatives[32] exceed 0.100 mg $kg^{-1}$.

In correspondence and parallel study, Sunay[33] recorded that 25% of the samples contained sulfonamide and tetracycline group of antibiotics. Hammel *et al.*[34] reported that in a limited survey of bee honey collected from different geographic origins showed that positive honey samples were often contaminated with more than one category of medicine .

In Switzerland, Ortelli *et al.*[35] obtained that 75 commercially honey samples were tested, 13 samples (17%) including chloramphenicol residues from 0.4-6.0, 6 samples from 0.8-0.9 mg $kg^{-1}$ and 2 samples 5mg $kg^{-1}$. In Iran, a small amount of tylosin residues ($0.3\pm0.1$ ng $g^{-1}$) were detected in summer samples[36]. Barrasso *et al.*[37] revealed that among 66 Apulian honey analyzed, 40% was detected by antibiotics (39 samples tylosin and 36 samples tetracycline residues). Finally, Antibiotics are not a component of bee honey but are the result of the misbehavior of beekeepers and the desire to maximize financial benefits without being aware of the effects on consumers. Beekeepers should be constantly aware of the adverse effects of the wrong practices in collecting, storing and trading the bee honey. Be careful when using antibiotics and use in the minimum, excluding the colonies that are treated from harvesting and collecting honey. Continuous encouragement to produce clean, healthy bee honey.

## CONCLUSION

It concluded that under the Egyptian condition no significant differences in the Egyptian bee honeys contaminated with tylosin and tetracycline residues. However, a significant difference attributed to chloramphenicol residue contamination. This is referring to the use of beekeepers in large doses for the treatment of diseases without examining the effects on the presence of residuals on the health of consumers. Therefore, we should be monitoring the residues of antibiotic by beekeepers awareness to produce a healthy bee honey foods that free form antibiotics derivatives.

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## SIGNIFICANCE STATEMENT

This study discovers there are residues of antibiotics in bee honey that can be dependent on one kind of antibiotic and it's possible more than one kind together. That can be beneficial for reserve later from the use of antibiotics in the honeybee colony which honey is harvested. This study will help the researcher to uncover the critical areas in sources of contaminations and cheating of honey that many researchers were not able to explore. Thus a new theory on How to get bee honey without contamination? May be arrived at.

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