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Antibiotic residues in honey: a public health issue

Resíduos de antibióticos em mel: uma questão de saúde pública

Residuos de antibióticos en la miel: un problema de salud pública

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Abstract

Honey has numerous uses and composes formulations of many food, pharmaceutical, and cosmetic products due to its chemical, biological, and sensorial characteristics. In recent years, several reports and scientific articles claim the contamination of honey by antibiotics, a fact that is a public health problem since the ingestion of them can cause several side effects. In this sense, the purpose of this work was to carry out a literature review in international circulation databases about the contamination of honey by antibiotics in the last 10 years. It is observed in the literature that several quality control methods for evaluating this product have been developed, with the main objective of detecting trace concentrations of these compounds. In this context, the chromatographic techniques, rapid tests, immunoassays, and the use of emerging technologies that are considered sustainable for sample preparation stand out. When these methods are applied to honey samples, it is clear that the contamination of honeys by antibiotic residues has been growing every year, making it evident that programs for continuous monitoring of these residues in honeys are conducted. Also, not only has the amount of antibiotics increased, but also the diversity of assets being used. Regarding the residues present, the main antibiotics found in the evaluated samples belong to the class of tetracyclines, sulfonamides, aminoglycosides, macrolides, and amphenicols. However, few countries report maximum residue limits (MRL) allowed for these substances in honey, and in Brazil, as well as in the European Union, no MRL has been established for antibiotics in this product so far, which indicates that it is extremely important that MRL be adopted to ensure food safety for consumers.

Keywords: Medicine residues; Animal product; Health risk.

Resumo

O mel possui inúmeros usos e compõe formulações de muitos produtos alimentícios, farmacêuticos e cosméticos devido às suas características químicas, biológicas e sensoriais. Nos últimos anos, vários relatórios e artigos científicos afirmam a contaminação de méis por antibióticos, fato que consiste em um problema de saúde pública uma vez que a ingestão dos mesmos pode causar diversos efeitos colaterais. Nesse sentido, a proposta desse trabalho foi realizar uma revisão na literatura em bases de dados de circulação internacional acerca da contaminação do mel por antibióticos nos últimos 10 anos. Observa-se na literatura que diversos métodos de controle de qualidade para avaliação deste produto têm sido desenvolvidos, com o objetivo principal de detectar concentrações traço destes compostos. Neste contexto, destacam-se as técnicas cromatográficas, testes rápidos, imune ensaios e o emprego de tecnologias emergentes e consideradas sustentáveis de preparo de amostra. Quando estes métodos são aplicados a amostras de mel, é notório que a contaminação de méis por resíduos de antibióticos vem crescendo a cada ano, tornando evidente que sejam conduzidos programas de monitoramento contínuo destes resíduos em méis. Além disso, não somente a quantidade de antibióticos tem aumentado, mas também a diversidade de ativos que vem sendo empregados. O que diz respeito aos resíduos presentes, os principais antibióticos encontrados nas amostras avaliadas pertencem a classe das tetraciclinas, sunfonamidas, aminoglicosídeos, macrolídeos e anfenicóis. Porém, poucos países reportam limite máximo de resíduos (LMR) permitidos para estas substâncias em mel, sendo que no Brasil, bem como na União Europeia não é estabelecido nenhum LMR para antibióticos neste produto até o momento, o que indica que é de extrema importância que sejam adotados LMR para garantir a segurança dos alimentos aos consumidores.

Palavras-chave: Resíduos de medicamentos; Produto de origem animal; Risco à saúde.

Resumen

La miel tiene numerosos usos y compone formulaciones de muchos productos alimenticios, farmacéuticos y cosméticos debido a sus características químicas, biológicas y sensoriales. En los últimos años, varios informes y artículos científicos denuncian la contaminación de la miel por antibióticos, hecho que constituye un problema de salud pública ya que la ingestión de los mismos puede ocasionar varios efectos secundarios. En este sentido, el propósito de este trabajo fue realizar una revisión de la literatura en bases de datos de circulación internacional sobre la contaminación de la miel por antibióticos en los últimos 10 años. Se observa en la literatura que se han desarrollado varios métodos de control de calidad para evaluar este producto, con el objetivo principal de detectar concentraciones traza de estos compuestos. En este contexto, destacan las técnicas cromatográficas, las pruebas rápidas, los inmunoensayos y el uso de tecnologías emergentes que se consideran sostenibles para la preparación de muestras. Cuando se aplican estos métodos a las muestras de miel, es evidente que la contaminación de las mieles por residuos de antibióticos ha ido creciendo año tras año, evidenciando que se realizan programas de seguimiento continuo de estos residuos en las mieles. Además, no solo ha aumentado la cantidad de antibióticos, sino también la diversidad de activos que se utilizan. En cuanto a los residuos presentes, los principales antibióticos encontrados en las muestras evaluadas pertenecen a la clase de tetraciclinas, sunfonamidas, aminoglucósidos, macrólidos y anfenicoles. Sin embargo, pocos países reportan límites máximos de residuos (LMR) permitidos para estas sustancias en la miel, y en Brasil, así como en la Unión Europea, hasta el momento no se han establecido LMR para antibióticos en este producto, lo que indica que es extremadamente importante. que se adopten LMR para garantizar la seguridad alimentaria de los consumidore.

Palabras clave: Residuos de medicamentos; Producto animal; Riesgo de salud.

1. Introduction

Bees are indicators of the conservation of an ecosystem. They carry out pollination and production services for honey and other beehive products, which contributes to the improvement of biodiversity and world food security (Affek, 2018). In this sense, beekeeping plays a fundamental role in the sustainable development of rural areas. In Brazil, beekeeping has an important economic and social role for many peasant families since this activity generates jobs and fixes men to the countryside (Sousa, 2013).

Honey consists of a food product produced by bees (*Apis mellifera*) from flower nectar (floral honey) or secretions from living parts of plants or excretions of plant-sucking insects found on them (honeydew honey). Bees collect them, transform them, combine them with specific substances, store and let them mature in the hive combs resulting in the honey itself (Brasil, 2000; Codex Alimentarius, 2001; European Commission, 2002a).

The product in question may have different physicochemical, biological, and sensorial characteristics (Bergamo, Seraglio, Gonzaga, Fett, & Costa, 2019; Carvalho et al., 2020) and it is dependent on several factors such as the composition of the nectar or honeydew; climate conditions; soil composition; management and species of the bee involved in the production; colony physiological state; state of maturation of honey; weather conditions at harvest time; among others (Liberato & Morais, 2016).

Honey is a functional food (Battino et al., 2019) possessing several biological activities, for example, antioxidant (Gheldof & Engeseth, 2002; Stagos et al., 2018), antimicrobial (Kwakman & Zaat, 2012), antihypertensive (Erejuwa et al., 2011), anti-inflammatory (Sipahi et al., 2017), probiotic, and prebiotic (Abdel Wahab, Saleh, Karam, Mansour, & Esawy, 2018; Mohan, Quek, Gutierrez-Maddox, Gao, & Shu, 2017). Besides, protective effects on cardiovascular, nervous, respiratory, and gastrointestinal systems have been observed (Cianciosi et al., 2018).

Most consumers are unaware of the dynamics of beekeeping, so they neglect the possibility that honey may contain residues harmful to health, such as antibiotics. The misuse of such drugs to prevent and control bacterial diseases that affect bees poses potential risks to human health. Antibiotics such as streptomycin, tetracyclines, and sulfonamides are often used in beekeeping to control the American and European foulbrood, which are bacterial diseases that are extremely dangerous for bees and can destroy an apiary completely and quickly. These bacteria can, moreover, contaminate many beehives easily and are a potential danger to honey producers (Saleh, Mussaed, & Al-Hariri, 2016). Additionally, the contamination of honey with antibiotics may also result from intensive agricultural and industrial activities (Bonerba et al., 2021).

Honey has numerous uses and composes formulations of many pharmaceutical, cosmetic, and food products (Kumar, Gill, Bedi, & Kumar, 2018). In recent years, several reports claim the contamination of honey by antibiotics (Galarini, Saluti, Giusepponi, Rossi, & Moretti, 2015; Kivrak, Kivrak, & Harmandar, 2016; Korkmaz, Kuplulu, Cil, & Akyuz, 2017). The presence of antibiotic residues in honey represents a danger to the health of consumers due to the occurrence of allergic reactions, anaphylactic shocks in susceptible individuals, imbalance of intestinal flora, development of bacterial resistance, and the transfer of multi-resistance between bacteria by plasmid (Belas, 2012). Therefore, these facts give a warning signal to the potential risks of consumption and use of contaminated honey and, consequently, of the need for monitoring and action by regulatory and inspection organizations.

In Brazil, the competence to establish the acceptable daily intake and the maximum residue limits (MRL) of veterinary drugs in food is the National Health Surveillance Agency (Brasil, 2019), while the Ministry of Agriculture, Livestock, and Supply is responsible for the registration of veterinary products and their inspection (Brasil, 2019), where the presence of a wide range of veterinary drugs authorized and prohibited in Brazil in various foods, including honey, are investigated. Internationally, the main organizations that carry out the risk analysis of residues of veterinary drugs are the European Medicines Agency (European Union), Food and Drug Administration (United States), Food Safety Commission (Japan), Australian Pesticides and Veterinary Medicines Authority (Australia), and Health Canada (Canada).

Therefore, considering the importance of this theme in the current scenario, this work aimed to summarize and discuss data in the literature regarding the contamination of honey by antibiotics.

2. Methodology

The search of literature data on Food Science and Technology Abstracts, Science Direct, Scopus, Web of Science, and Google Scholar electronic databases was conducted using the following keywords together or differently combined: (“antibiotic” OR “residue” OR “contaminant”) AND (“honey”) AND (“public health”). The research of the articles was done considering the period referring to the last 10 years of publication.

Full texts were accessed and carefully revised. Articles that determined antibiotics residues in honey samples collected from different markets, different continents, different beehives, and/or survey processes, as well as that developed and validated analytical methods and/or techniques to determine antibiotics residues in honey were considered relevant and included in this review

3. Results and discussion

3.1 The use of antibiotics in beekeeping

The use of chemical substances to control pests of animal and vegetable origin in agricultural cultivation has been of great concern to environmentalists and biologists who study beekeeping. In this sense, there is a global concern with the widespread use of pesticides in the agricultural sector, and more than 150 substances used for pest control have already been identified in bee colonies. These substances cause irreparable damage in beekeeping, interfering in the production chain of both honey and agriculture itself. The toxic effect of these chemical agents promotes the death of bees mainly during the stage of larval development, in which it is most sensitive, and the symptoms of poisoning will depend on the type of chemical compound applied (Al Naggar et al., 2015; El-Nahhal, 2020).

In addition to the substances used to control pests during agricultural cultivation, since the 1940s chemical substances with antibiotic action have been used to prevent and combat bacterial diseases in bees, which are extremely dangerous and can destroy a complete apiary quickly. These bacteria can also easily contaminate beehives, which is why they are also considered a potential danger to beekeepers and the local bee production chain (Bargańska, Namieśnik, & Ślebioda, 2011; Ortelli, Edder, & Corvi, 2004).

The bacterial diseases that can affect the *Apis mellifera* bee usually cause a severe decrease in the bee population and, consequently, less production of honey and other beehive products, causing significant damage to the ecosystem as well as to the honey processing industry, and the like. Two of the most important bacterial diseases affecting the larvae of the bee in question are *American foulbrood* and *European foulbrood*, which are caused by the *Paenibacillus larvae* and *Melissococcus pluton* bacteria, respectively (Evans, 2003).

The threat of bacterial diseases and the growing demand for honey have probably led many beekeepers to randomly use antibiotic cocktails in doses that are often grossly inappropriate and at varying periods resulting in some cases in severe honey contamination. Worldwide, antibiotics are often used in relatively high doses by beekeepers as a preventive or therapeutic treatment to protect an apiary from bacterial diseases (Reybroeck, Daeseleire, De Brabander, & Herman, 2012). Veterinary medicines should be used to treat diseases and not as a prevention. For this, it is recommended an adequate feeding of the bees and proper management of beehives, which includes proper hygiene practices and regular health checks of the hives. It is worth mentioning that there are several veterinary drugs authorized for the treatment of bee diseases, including antibiotics, synthetic, and organic acaricides (Belas, 2012; Codex Alimentarius, 2010).

Moreover, the administration of antibiotics may cause bacteria resistant to many drugs and spread antibiotic-resistant strains (Bargańska et al., 2011). In this sense, the application of antibiotics in beekeeping practices is only allowed under specialized veterinary supervision and with the application of long periods of abstinence (Reybroeck et al., 2012). However, in practice, many beekeepers fail to comply with the correct actions (Manimekalai, Sheshadri, Kumar, & Rawson, 2016).

Antibiotics are usually administered by spraying the hive frames or added in sucrose syrup. Antibiotics administered through syrup delivery have a higher risk of being transferred to honey compared to spraying, mainly due to the rapid spread by the colony (Martel, Zeggane, Drajnudel, Faucon, & Aubert, 2006). The application of these substances through spraying is not so desirable for bees, besides, it is deposited in the upper part of the nest frames (Thompsson et al., 2006).

In most countries, few antibiotics are allowed to be used to fight infections that can affect beehives. In this sense, it is of fundamental importance that there is strict control of the presence of antibiotics in honey since they are considered contaminants of the final product even in low concentrations. Also, it is known that such residues can cause several adverse effects to human health, and that, when their presence on honey is found, it may prevent the commercialization of this product (Bargańska et al., 2011; Sheridan, Policastro, Thomas, & Rice, 2008). In this context, about quality control and determination of these contaminants in samples with complex matrix, as is the case of honey, few methods of sample preparation and determination techniques at trace levels are available, making the evaluation of honey quality a big challenge for analysts (Bargańska et al., 2011).

In 2006, the European Union established a trade embargo banning the export of Brazilian honey to the European market because it found persistent flaws in the product's waste monitoring system, alleging non-compliance with the implementation deadlines of the National Waste Control Program (NWCP) by the Brazilian government. The end of the European embargo on Brazilian honey occurred in 2007, with the implementation of the National Plan for the Control of Residues and Contaminants (NPCRC), which establishes the monitoring of the product, the increase in the number of samples to be examined, and a greater commitment to inspection and control of waste. Consequently, Brazil was the world's fifth-largest exporter of honey in the first half of 2008. After meeting the requirements regarding compliance with the implementation of the NWCP and NPCRC, Brazilian warehouses now comply with the Hazard Analysis and Critical Control Points system.

Producers and exporters of Brazilian beehive products have also started to employ, among other strategies, Good Beekeeping Practices, which guarantee safe production with the application and registration of hygienic and sanitary principles throughout the production process, from the field to extraction and shipping from honey to the warehouse. Good Manufacturing Practices also started to be used, which refer to a set of principles, rules, and procedures for the handling and processing of the food product. Brazil also has the National Beekeeping Chain Standardization Program, which guarantees the quality of the products and processes developed.

Today, Brazil is one of the largest exporters of honey in the world, with honey being the 11th most exported product, corresponding to almost 0.2% of all products exported by the country, second only to major commodities such as soy, corn, and coffee and products in general such as spices and vegetables, fresh and chilled. Still, according to the Ministry of Economy and Foreign Trade, in the period between January and September 2020, Brazil exported more than 35 thousand tons of honey, representing an increase of almost 70% in the amount of honey exported when compared to the same period of 2019. In this sense, it is possible to verify the relevant importance that honey has for the Brazilian economy, making it

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essential that the product produced has a quality suitable for human consumption concerning the presence of contaminants, mainly of antibiotic origin (Brasil, 2020).

3.2 Antibiotic residues identified in honey

Antibiotics are used in beekeeping to prevent or treat bacterial diseases in beehives. For this, several types of antibiotics can be used, such as those belonging to the classes of sulfonamides, tetracyclines, macrolides, quinolones, and nitrofurans. However, even in concentration at trace levels in food, they can cause harmful effects to human health when consumed (Kümmerer, 2009; Zhang et al., 2019).

In this sense, the importance of monitoring these contaminating residues in food, including honey, becomes evident, which has led to the need for the definition of MRL referring to these contaminants by several countries. According to the European Union Regulation No. 37/2010, which deals with residues of pharmacological substances in foods, only a few antiparasitic agents have established limits. Therefore, there is no established MRL for antibiotic residues (European Union, 2010). In Brazil, Normative Instruction No. 51 of December 19/2019, also establishes MRL in honey only for Cumafós and Amitraz, which are used to control mites, flies, and fleas (Brasil, 2019). However, even though the presence of antibiotics in honey is prohibited, some countries adopt MRL suitable for the presence of antibiotic residues in honey.

Some countries such as China, Switzerland, Belgium, the United Kingdom, and France adopt MRL between 10 and 50 μg kg^{-1} for several classes of antibiotics, among which are sulfonamides, aminoglycosides, nitrofurans, fluoroquinolones, macrolides, among others. Even though the use of antibiotics is prohibited, the establishment of these MRL is done mainly to monitor the presence of these contaminants in products originating from imports, seeking to ensure their quality (European Commission, 2002b; European Union, 2003; European Union Reference Laboratory, 2007; Hammel, Mohamed, Gremaud, LeBreton, & Guy, 2008; Zhang et al., 2019).

Antibiotics are divided into classes according to their mechanism of action, among which are sulfonamides (sulfathiazole, sulfamerazine, sulfamethazine, sulfamethoxazole, sulfadiazine, sulfamethoxypyridazine, sulfadoxine, sulfadimidine, sulfanilamide), aminoglycosides (streptomycin, dihydrostreptomycin), tetracyclines (tetracycline, oxytetracycline, chlortetracycline, doxycycline), amphenicols (chloramphenicol), macrolides (tylosin, myrosamine), beta-lactams (penicillins), and nitrofuran metabolites (3-amino-2-

oxazolidinone, semicarbazide). According to Bogdanov (2006), these are the main classes of antibiotics that can be found in honey.

In general, the consumption of honey containing antibiotic residues represents a real danger to human health, considering that the indiscriminate consumption of these compounds is the main cause of bacteria resistance to antibiotics, especially in the case of aminoglycosides. Also, antibiotics can cause allergic reactions in people sensitive to certain classes of compounds and can cause hypersensitivity in up to 5% of individuals exposed to these substances (Brum, 2018; Ford, 2017).

In the case of beta-lactam antibiotics that act by impairing cell wall synthesis, the main adverse effects are diarrhea, nephritis, neurotoxicity, and hematological problems. Aminoglycosides are antibiotics that act by interfering with the replication of bacterial DNA by blocking protein synthesis. This class of antibiotics causes serious toxic adverse effects on the kidneys and the auditory system and, when used in high concentrations, can cause a serious risk of neuromuscular paralysis. Regarding tetracyclines, these cause adverse effects to the gastrointestinal system, causing irritation and may lead to deficiency disorders in the absorption of some vitamins. Besides, they cause kidney damage and bone marrow disorders, and can also cross the placental barrier and deposit in the bones (Brum, 2018; Ford, 2017).

Sulfonamides, better known as sulfas, are antibiotics that act by inhibiting the metabolism of bacterial cells by inhibiting folic acid, making the cells unable to grow and multiply. Regarding the adverse effects caused by these substances, these can range from itchy skin, hypersensitivity reactions, liver problems to problems related to blood cells. Finally, nitrofurans are prohibited in some countries due to their serious adverse effects, among which the carcinogenic, mutagenic, and genotoxic effects stand out. Mainly affect the pulmonary, cardiac, reproductive, peripheral, and hepatic systems, causing serious damage to human and animal systems (Brum, 2018; Claussen, Stocks, Bhat, Fish, & Rubin, 2017; Ford, 2017; Omidi, Niknahad, & Mohammadi-Bardbori, 2016).

Usually, these contaminants are present in honey in extremely low concentrations, which makes quantification an analytical challenge as it requires efficient sample preparation and techniques with high sensitivity. For this reason, it is very important to develop reliable analytical methods capable of identifying and quantifying these contaminants in honeys, as well as their use in the continuous monitoring of antibiotics in honeys (Bonta, Marghitas, Dezmirean, & Bobis, 2009). Only based on this information is it possible to know and monitor the situation regarding the presence of these contaminants in honey and to subsidize actions to control and prevent contamination of these products.

The European Union stresses the importance that laboratories that perform quality control have the definition of minimum performance limits required (MPLR). It is important from an analytical point of view, ensuring that the quality control methods employed are capable to determine the presence of prohibited or unauthorized substances in products with adequate sensitivity. In this sense, several products are contemplated, among which stand out milk, meat, eggs, and also honey, which according to the Decision Commission 2003/181/EC establishes an MPLR for the analytical method employed, represented by chloramphenicol, of 0.3 µg kg^{-1} (Bonerba et al., 2021; European Commission, 2002b; Valério, 2012).

Thus, to monitor residues in honey, several studies have been conducted to develop analytical methods that can be used with adequate sensitivity and precision (Correia, 2008; Mohamed et al., 2007; Sheridan et al., 2008). In this context, Table 1 shows studies performed in the last 10 years regarding the antibiotic's investigation on honeys.

Table 1. Classes of antibiotics evaluated in honeys in the last 10 years.

Class of antibiotics	**Reference**
Tetracyclines	(Alla, 2020; Bonerba et al., 2021; Galyautdinova et al., 2020; Kivrak et al., 2016; Korkmaz et al., 2017; Kumar, Gill, Bedi, & Chhuneja, 2020; Kumar, Gill, Bedi, Chhuneja, & Kumar, 2020; Louppis, Kontominas, & Papastephanou, 2017; Mahmoudi, Norian, & Pajohi-Alamoti, 2014; Santana, Santana, & Pereira, 2018; Thanasarakhan et al., 2011)
Sulphonamides	(Baeza Fonte, Castro, & Liva-Garrido, 2018; Chiesa, Panseri, Nobile, Ceriani, & Arioli, 2018; Galarini et al., 2015; Kivrak et al., 2016; Korkmaz et al., 2017; Kümmerer, 2009; Louppis et al., 2017; Mujić et al., 2011; Santana et al., 2018; von Eyken et al., 2019)
Aminoglycosides	(Aksoy, 2019; Barrasso et al., 2018; Bonerba et al., 2021; Ji et al., 2013; Kumar, Gill, Bedi, & Chhuneja, 2020; Li, Li, Wang, & Ji, 2018; Mahmoudi et al., 2014; Perkons, Pugajeva, & Bartkevics, 2018; Wang et al., 2015)
Macrolides	(Aksoy, 2019; Alla, 2020; Bonerba et al., 2021; Du et al., 2018; Kumar, Gill, Bedi, & Chhuneja, 2020; Mahmoudi et al., 2014;

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	Orso et al., 2016; von Eyken et al., 2019; Wang et al., 2018)
Amphenicols	(Alla, 2020; Barrasso et al., 2018; Bonerba et al., 2021; Campone et al., 2019; Kivrak et al., 2016; Kumar, Gill, Bedi, & Chhuneja, 2020; Louppis et al., 2017; Mahmoudi et al., 2014)
Quinolones	(Barrasso et al., 2018; Bonerba et al., 2021; Galarini et al., 2015; Kumar, Gill, Bedi, & Chhuneja, 2020; Wang et al., 2018)
Penicillins	(Kumar, Gill, Bedi, & Chhuneja, 2020; Louppis et al., 2017)
Fluoroquinolones	(Louppis et al., 2017; Mahmoudi et al., 2014)
Lincosamides	(Aksoy, 2019; Bonerba et al., 2021)
Cephalosporins	(Barrasso et al., 2018; Bonerba et al., 2021)
Nitrofurans	(Bailone, Fukushima, & Roça, 2016; Shendy, Al-Ghobashy, Gad Alla, & Lotfy, 2016)
Nitroimidazoles	(Galarini et al., 2015)
Ionophores	(Orso et al., 2016)
Diamino-pyrimidine	(Louppis et al., 2017)
Diterpenes	(Louppis et al., 2017)
Streptogramins	(Bonerba et al., 2021)

Source: Authors.

According to the research shown in Table 1, in most cases, the determination of antibiotic content in honeys was made after the development of analytical methods, with the main objective of evaluating the accuracy and other parameters of merit of these methods. However, the evaluation of honey samples in these studies gives an idea about the type and concentration of antibiotics in honeys.

Several classes of antibiotics were evaluated, with tetracyclines, sulfonamides, aminoglycosides, macrolides, and amphenicols being the most investigated (Bogdanov, 2006). Also, it is possible to verify that the methodology used is quite diversified, but for the most part, were used chromatographic techniques for detection after sample preparation by extraction techniques.

As mentioned, chromatographic techniques are highlighted in the determination of antibiotics in honeys, and high-performance liquid chromatography (HPLC) coupled with mass spectrometry (MS) has been widely used. Recently, a method was developed for the determination of 27 antibiotics belonging to the classes of sulfonamides, quinolones, and nitroimidazoles in honey samples by HPLC-MS/MS (Galarini et al., 2015). The developed method was applied to 74 commercial honey samples and, according to the authors, 12% presented antibiotics in concentrations of up to 2 µg kg^{-1} (Galarini et al., 2015). In another study using the same technique, 43 honey samples were evaluated for the presence of 13 types of antibiotics (Orso et al., 2016). In this study, only two contaminated samples were found, one of which presented erythromycin and the other salinomycin, in concentrations of 5.9 and 3.5 µg kg^{-1}, respectively (Orso et al., 2016). According to the authors, the method developed proved to be adequate, effective, and with a great sustainable appeal, given that it allows the evaluation of honey samples regarding the presence of antibiotics and pesticides using low volumes of solvent combined with low execution cost (Orso et al., 2016).

To develop a fast and effective method for the determination of antibiotic contaminants in honeys, a study conducted by von Eyken et al. (2019) was performed. In this study, honey samples were evaluated to determine the presence of residues of tylosin, sulfamethazine, and sulfadimethoxine by direct injection into HPLC coupled to time-of-flight (TOF) and MS. According to the data, residues of tylosin A, tylosin B, sulfamethazine, and sulfadimethoxine were found in 6, 9, 6, and 23% of the evaluated honey samples, respectively. However, it is worth noting that even though it is present in the samples, according to the authors, the concentration was below that stipulated by regulatory agencies (Von Eyken et al., 2019).

A multiclass method for determining antibiotic residues in honey was developed using HPLC as a means of detection, in which 98 commercial samples were evaluated (Bonerba et al., 2021). In this, which is the most recent study, several types of antibiotic residues were found, such as quinolones, ceftiofur, chloramphenicol, streptomycin, tylosin, tetracycline, spiramycin, apramycin, bacitracin, neomycin, tobramycin, tylosin B, spectinomycin, amikacin, lincosamide, erythromycin, and virginiamycin (Bonerba et al., 2021). According to this study, the concentration of these residues was relatively high, being between 1.1 to 18.6 µg kg^{-1} (Bonerba et al., 2021). Besides, when looking at the wide range of substances found, that not only has the concentration of antibiotics increased but also that, over time, more types of antibiotics have been used by beekeepers (Bonerba et al., 2021).

Still employing chromatographic techniques, an analytical method was developed and validated using *QuEChERS* as a form of sample preparation and subsequent determination by HPLC-MS/MS of nitrofurantoin, furazolidone, nitrofurazone, and their metabolites (Shendy et al., 2016). In this work, 30 honey samples were evaluated and none of them presented contaminants in values above the limits of quantification (LQ) (Shendy et al., 2016). In a similar study, using liquid-liquid extraction (LLE) and subsequent determination by HPLC coupled to electrospray ionization (ESI) and MS, the concentration of tetracyclines, amphenicols, fluoroquinolones, penicillin G, trimethoprim, tiamulin, and sulfonamides were determined in 20 honey samples from Greece (Louppis et al., 2017). Three honey samples showed concentrations above those established for oxolonic acid, sulfathiazole, and sulfadimethoxine (Louppis et al., 2017).

Seeking to perform the simultaneous determination of six aminoglycoside antibiotics in honey samples, Perkons et al. (2018) developed a method using solid-phase extraction (SPE) and subsequent determination by HPLC-TOF-MS. In this study, 46 samples of honeys from the Georgia region were evaluated. Most of them were suitable for consumption, except for one sample that presented gentamicin at a concentration of 32 ng g^{-1} and two samples that presented streptomycin at the concentration of 117 and 35 ng g^{-1} (Perkons et al., 2018).

Regarding macrolides class, Du et al. (2018) developed a promising method for the identification and quantification of these antibiotic residues in foods of animal origin. A convenient and efficient analytical method based on Mini-SPE was established and applied to the extraction of five macrolide compounds (azithromycin, clarithromycin, erythromycin, lincomycin, and roxithromycin) in honey and milk, followed by HPLC-MS analysis. Mini-SPE allowed an automatic extraction, with enrichment and purification of the target analytes in a short time, in addition to minimizing the amount of sample and solvent used, decreasing the waste generation and cost (Du et al., 2018; Gałuszka, Migaszewski, & Namieśnik, 2013; Tobiszewski, Marć, Gałuszka, & Namieśnik, 2015).

The evaluation of Cuban honeys was carried out by Baeza Fonte et al. (2018) employing HPLC-MS/MS to identify and quantify six antibiotics belonging to the sulfonamide class. The study aimed to optimize and validate the use of an adsorbent polymer and subsequent determination of sulfonamides in honey samples (Baeza Fonte et al., 2018). A similar approach has been developed by Ji et al. (2013), which evaluated the use of a molecularly imprinted polymer as a sorbent to determine the presence of aminoglycoside antibiotics. The developed method was applied to four samples of commercial honeys, which showed relatively high concentrations for gentamicin (31.5 to 52.6 µg kg^{-1}) in all samples (Ji et al., 2013). Also, in three samples, the antibiotic spectinomycin was present in concentrations between 11.8 and 16.3 μg kg^{-1} and one sample presented streptomycin at a concentration of 9.0μg kg^{-1} (Ji et al., 2013). According to the authors, the developed method is ideal for application in analysis routines for screening due to its high sensitivity to the antibiotics evaluated (Ji et al., 2013).

A study using sorbents composed of magnetic nanoparticles was developed and evaluated as an SPE method for antibiotics of aminoglycoside class using a Fe_3O_4 core coated with polyvinyl alcohol and subsequent determination by HPLC (Li et al., 2018). The developed method was applied to evaluate four commercial samples of honey, and in one of them the presence of streptomycin and dihydrostreptomycin was detected in concentrations of 10.77 and 25.11 μg kg^{-1}, respectively (Li et al., 2018). Wang et al. (2015) also developed a hydrophilic sorbent coated with polyvinyl alcohol quite similar to that developed by Li et al. (2018) for the extraction of antibiotics of aminoglycosides class in honey samples and subsequent determination by HPLC-MS/MS (Wang et al., 2015). In this study, the authors evaluated the presence of dihydrostreptomycin, streptomycin, kanamycin, and spectinomycin in four honeys samples, and in all samples, the concentrations were below the limits of quantification (LQ) of the method (Wang et al., 2015).

The use of techniques and methods more environmentally friendly has gained prominence in the last decade (Gałuszka et al., 2013). This is due to the need for greater sustainability in all processes, thus seeking greater preservation of natural resources. Therefore, quality control methods that have greater appeal for environmental preservation are the objective of several studies. In this sense, the miniaturization of already consolidated techniques allows the reduction of the use of solvents and reagents that harm the environment and, when combined with obtaining satisfactory results, it is a sustainable alternative that can be adopted in laboratory routines (Gałuszka et al., 2013; Tobiszewski et al., 2015). Based on that, Du et al. (2018) proposed and validated the use of a miniaturized scale SPE using silica as a sorbent for the determination of macrolide antibiotics and subsequent determination by HPLC-TOF-MS (Du et al., 2018). The authors applied the proposed method to honey samples, and residues of lincomycin (17.07 μg kg^{-1}), erythromycin (65.88 μg kg^{-1}), clarithromycin (114.69 μg kg^{-1}), and roxithromycin (166.18 μg kg^{-1}) were found. Only azithromycin was not detected in any evaluated sample.

The use of alternative extraction methods for sample preparation is a trend, since, in its majority, it provides several advantages related to time, reagent consumption, and lower costs, being increasingly present in quality control laboratories. In this sense, Kivrak et al. (2016)

developed a method for determining 23 types of antibiotics in honey using ultrasound-assisted extraction and subsequent determination by HPLC-ESI-MS/MS. Antibiotics belonging to the classes of tetracyclines, amphenicols, and sulfonamides were evaluated with high sensitivity in Turkish honeys (Kivrak et al., 2016). According to the results presented, a high concentration of sulfamethazine was found in the evaluated samples, reaching a concentration of 647 μg kg^{-1} (Kivrak et al., 2016). Besides, samples with high levels of tetracyclines were found, with concentrations of up to 968, 197, 743, and 158 μg kg^{-1} of tetracycline, epitetracycline, oxytetracycline, and epioxitetracycline, respectively (Kivrak et al., 2016). According to the authors, the method showed adequate sensitivity, precision, and reproducibility, in addition to being a fast, effective, and reliable alternative to be applied in laboratory routine (Kivrak et al., 2016).

Wang et al. (2018) determined 39 antibiotics by HPLC-MS after extraction using solvents in honey samples from Zheijang. Three antibiotics were found in 52 evaluated samples, with the incidence of contamination being 2.6, 8.6, and 3.9% for roxithromycin, norfloxacin, and ciprofloxacin, respectively (Wang et al., 2018). According to the authors, the concentration of antibiotics in question is high, considering that concentrations of up to 59.6~214.4, 4.7~486.3, and 5.9~215.1 μg kg^{-1} were found, respectively, for roxithromycin, norfloxacin, and ciprofloxacin (Wang et al., 2018).

An alternative approach to LLE is the dispersive liquid-liquid microextraction (DLLME), which at the same time allows the extraction of compounds using fewer solvents. In this sense, a method using DLLME as a way to prepare honey samples followed by the determination of 10 antibiotics by HPLC was developed by Santana et al. (2018). In this work, 33 honey samples were evaluated for the presence of sulfathiazole, sulfadiazine, sulfamerazine, sulfamethazine, sulfamethoxazole, sulfadimethoxine, oxytetracycline, tetracycline, chlortetracycline, doxycycline, and their metabolites (Santana et al., 2018). The authors report the presence of sulfadimethoxine in three samples at concentrations between 21.2 and 38.5 μg kg^{-1}, whereas tetracycline was found in only one sample (35.8 μg kg^{-1}) (Santana et al., 2018). According to the results presented, the proposed method was adequate in terms of linearity, precision, and LQ (Santana et al., 2018). However, it is important to note that the antibiotic levels found in the honey samples evaluated are worrying, given the high concentration of these substances found (Santana et al., 2018).

In this context, Campone et al. (2019) developed an ultrasound-assisted DLLME method coupled with HPLC-MS/MS determination for fast and accurate analysis of chloramphenicol in honey. The parameters affecting extraction efficiency were carefully optimized and the analytical method was validated and successfully applied to 66 honey samples. Previously, Ortelli et al. (2004) also developed an analytical method using the HPLC-MS/MS technique to assess the presence of chloramphenicol in honey, presenting adequate results for the analytical parameters evaluated.

Another emerging alternative for assessing the chemical quality of products is the use of enzyme immunoassays. This technique has gained prominence in recent years and, in this context, several studies using this technique are being conducted to evaluate samples of honey concerning the presence of antibiotics. In this sense, Mahmoudi et al. (2014) evaluated 135 samples of honeys from northern Iran for the presence of chloramphenicol, tetracycline, enrofloxacin, tylosin, and gentamicin(Mahmoudi et al., 2014). According to the data presented, chloramphenicol was found in 17% of the evaluated samples in concentrations between 0.4 and 6.0 µg kg^{-1}, while tetracycline was present in 14% of the samples in concentration quite similar (0.2 to 6.2 ng g^{-1}) (Mahmoudi et al., 2014). In 18.5% of the samples, residues of enrofloxacin and tylosin were found in concentrations between 0.6 to 72.1 ng g^{-1} and 1.0 to 17.3 ng g^{-1}, respectively (Mahmoudi et al., 2014). Besides, the authors point out that the presence of the highest concentrations found was in honeys from autumn blossoms, in which contamination of 18.5% of samples with gentamicin was found (Mahmoudi et al., 2014).

Korkmaz et al. (2017) evaluated 59 Turkish honey samples using an enzyme immunoassay to determine sulfonamides and tetracyclines. According to the data, the samples have a high level of contamination by these antibiotics, given that concentrations between 6 and 42 µg kg^{-1} of tetracycline were found in 35 samples and between 3 and 32 µg kg^{-1} of sulfonamides in 31 of the evaluated samples (Korkmaz et al., 2017). Thus, it was concluded that these honeys had high concentrations of these contaminants, which can be a danger to consumers' health (Korkmaz et al., 2017).

Immunoassays in general are tests with high selectivity that allow quick and efficient evaluation of samples for the presence of contaminants. In this sense, Kumar, Gill, Bedi, Chhuneja, et al. (2020) evaluated 150 samples of honey from India regarding contamination by oxytetracycline, erythromycin, and chloramphenicol. The authors report that oxytetracycline and erythromycin were present in concentrations above that established by regulations in 15.3 and 5.3% of the honey samples evaluated, respectively (Kumar, Gill, Bedi, Chhuneja, et al., 2020). Chloramphenicol was not found in any of the samples evaluated (Kumar, Gill, Bedi, Chhuneja, et al., 2020). An important fact reported in the study is that the presence of these contaminants was found in all samples that did not have certification, showing that programs for continuous monitoring of this product should be instituted, especially those from agribusinesses (Kumar, Gill, Bedi, Chhuneja, et al., 2020).

The use of microbiological tests through kits with chemiluminescent detection has been used for a range of analytes (Barrasso et al., 2018). The use of biochips allows the determination of a wide range of substances quantitatively and simultaneously, using immobilized and specific antibodies for the evaluation of the substance of interest (Barrasso et al., 2018). In this context, 66 honeys were evaluated using the Anti-Microbial Array II kit to determine the presence of six classes of antibiotics (Barrasso et al., 2018). According to the data presented, it was possible to observe that the antibiotic tylosin was present in 38 samples, tetracyclines in 36 samples, quinolones in 27 samples, thiamphenicol in 21 samples, cephalosporins in 19 samples, and streptomycin in one of the evaluated samples (Barrasso et al., 2018). Finally, it was found that 26 samples did not have any antibiotic residues evaluated, indicating that more than half of the samples contained some type of antibiotic as a contaminant (Barrasso et al., 2018). In another similar study, Aksoy (2019) used the Anti-Microbial Array IV kit to evaluate 45 honey samples for the presence of 12 classes of antibiotics. Erythromycin, streptomycin, amikacin, lincosamide, tylosin B, and neomycin residues were simultaneously detected (Aksoy, 2019). According to the results presented in the study, erythromycin was present in 91% of the evaluated samples, while streptomycin in 15%, indicating that these contaminants are widely present in honey samples (Aksoy, 2019).

Thus, it is observed that the use of kits to determine the presence and concentration of antimicrobials in honey samples is a promising alternative for application as screening tests, allowing fast and reliable responses. Also, they can be used as a way to monitor honeys from different regions and classify honeys according to their quality (Aksoy, 2019).

A method of sequential analysis by spectrophotometry after the complexation of antibiotics with Yttrium was developed to determine tetracycline, chlortetracycline, and oxytetracycline (Thanasarakhan et al., 2011). The authors evaluated six samples of commercial honeys, in which residues of tetracycline (7.2 to 13.9 ng L^{-1}) and oxytetracycline (0.7 to 60 ng L^{-1}) were found in some samples (Thanasarakhan et al., 2011). According to the authors, because it is a method with relatively low cost, high sensitivity, reproducibility, and fast, it is ideal for application in routine laboratory analysis (Thanasarakhan et al., 2011).

There is a constant concern throughout the world in terms of contamination of natural products by pesticides, heavy metals, antibiotics, and also radioactive substances (Mujić et al., 2011). In this sense, a study was conducted to monitor environmental contamination through the analysis of honeys from regions of Bosnia and Herzegovina (Mujić et al., 2011). Forty-six honey samples were evaluated for the presence of sulfonamides using the disc plate method to evaluate the antimicrobial action of the samples (Mujić et al., 2011). According to the data presented by the authors, the honey samples evaluated did not contain sulfonamides and antibiotic residues, indicating that the evaluated site is not contaminated with these types of substances (Mujić et al., 2011). Still in this context, Chiesa et al. (2018) evaluated 95 honeys considered organic about the presence of antibiotics and neonicotinoids. The results found by the authors suggest that the honeys evaluated are of excellent quality and confirmed their organic origin, considering that no residue was found, demonstrating the absence of bee treatments (Chiesa et al., 2018).

Bailone et al. (2016) found the presence of nitrofurazone in three samples of Brazilian honey, in which values of up to 20.7 μg kg^{-1} were observed. Nitrofurans (furazolidone, nitrofurazone, nitrofurantoin, furaltadone, and nifursol) are veterinary antibiotics that can be used in beekeeping (Vass, Hruska, & Franek, 2008), but are carcinogenic compounds and of great importance for public health (Li, Li, & Xu, 2017; McNamee, Rosar, Persic, Elliott, & Campbell, 2017). In another study, in which 64 honey samples from the Egypt region were evaluated, the presence of tylosin, chloramphenicol, and tetracycline was verified in 89, 47, and 31% of the samples, respectively (Alla, 2020). The tetracycline class is the most studied by researchers and is present in honey with a higher prevalence (Galyautdinova et al., 2020). In a study conducted to evaluate 100 samples from northern India, oxytetracycline and erythromycin were found in concentrations above those stipulated in the country (Kumar, Gill, Bedi, & Chhuneja, 2020).

Based on the data evaluated in this review, it is possible to state that most honeys have residues of antibiotics as contaminants. The presence of these contaminants is more worrying concerning those belonging to the classes of tetracyclines, aminoglycosides, macrolides, and sulfonamides. According to the results found, the concentration of these contaminants reached values of 968 μg kg^{-1} in the case of tetracycline (Kivrak et al., 2016). Furthermore, in some studies, it is shown that the prevalence of contamination is extremely high, as shown by Aksoy (2019) in which 91% of the evaluated samples were contaminated with erythromycin residues. Therefore, the presence of these contaminants in honey is a concern and should be the target of greater sanitary control.

Thus, the contamination of honey by antibiotics is a public health issue and, through studies conducted to assess the presence of antibiotics in honeys, it is clear that these products present a potential risk to consumers. The presence of these contaminants in concentrations, often extremely high, is related to the lack of care of beekeepers, using exaggerated concentrations, not respecting the period of application, and using some assets not allowed for beekeeping. Still in this context, it is important to note that the bee's metabolism can change the initial concentration of the antibiotic. This was verified in the study of Martel et al. (2006) where tetracycline was degraded more quickly in honey from the beehive than from the laboratory at 35 ºC, a fact that can be explained by the enzymatic activity of bees (Argauer & Moats, 1991). Therefore, it is evident that the application of these drugs to control the diseases that affect the beehives must always be conducted with the accompaniment and the appropriate indication by professionals in the field. The correct management of beehives with antibiotics makes the residual amount present in honey minimal and only active ingredients with real effectiveness are used, making the beehive products intended for consumption suitable.

4. Final Considerations

Based on this review, it was possible to prove that the presence of antibiotic residues in honey is worrying, which evokes the need for the adoption of alternative management practices by beekeepers to control and/or prevent bacterial diseases in bees and reduce the use of antibiotics and the consequent risk of honey contamination. Also, it is evident that when antibiotics are used, there must be monitoring and the correct indication of use by qualified professionals, intending to reduce the spread to the honey produced. In this sense, the consumption of this food containing residues of antibiotics is concerning from the point of view of public health, especially for children, the elderly, and the immunocompromised. Therefore, the definition of MRL for these contaminants and the implementation of continuous monitoring programs for these residues should be promoted to ensure that actions to control and prevent the contamination of honey and other foods are taken efficiently by regulatory and health organizations, as well as to support future regulations regarding antibiotic content in honey, if necessary.

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