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Review Article

A Review on the Antimicrobial Effect of Honey on *Salmonella* and *Listeria monocytogenes*: Recent Studies

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ABSTRACT

Foodborne pathogens like *Salmonella* and *Listeria monocytogenes* are microbial agents capable of causing severe illnesses, and they pose a perpetual menace to the food industry. Given their potential to jeopardize human health, both food producers and consumers hold significant concerns regarding these pathogens. The quest for novel strategies and natural preservatives in the domain of food safety holds paramount importance in the effort to curtail the dissemination and contamination of these pathogens. In this context, honey stands out as a notable natural product with substantial potential. Honey, through its bioactive constituents, including phenolic compounds, specialized enzymes, and particularly the production of hydrogen peroxide, can serve as an efficacious tool in combatting microorganisms. This review undertakes an exploration of the antimicrobial impacts of honey on *Salmonella* and *Listeria monocytogenes* by conducting a comprehensive assessment of existing literature and consolidating available data. The existing data strongly indicates the potential of honey's antimicrobial components to hinder the proliferation and dissemination of these pathogens. This review's principal aim is to outline a path for future research and applications, acknowledging the essential need for additional data and thorough investigations before efficiently deploying honey as a countermeasure against these pathogens.

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INTRODUCTION

Honey was used as a medicine by the Egyptians before 1900-1250 BC and is one of the oldest known foods. The scientist and philosopher Aristotle described honey as a good ointment for sore eyes and wounds, while Dioscorides recommended the use of honey for various ailments such as bruises, ulcers, coughs, sore throats and sunburn. Ancient Greeks employed honey to address exhaustion, as noted by (Khan et al., 2018). Ibn Sina, a renowned Muslim physician from the 10th century, featured numerous remedies using honey to alleviate a wide range of health conditions in his work, Al-Kanun fitTıb. Additionally, he utilized a paste composed of honey and flour to manage wounds. Hippocrates, the Greek physician often regarded as the pioneer of modern medicine, made significant contributions to medical knowledge. He argued that honey was effective in treating pain, fever and wounds. Honey has important effects on human health such as antimicrobial, antioxidant and anti-inflammatory. These effects are due to the bioactive components it contains (Bahar, 2022; Cinar, 2020; Ali et al., 2023). Extensive research has unveiled the diverse spectrum of antimicrobial qualities present in honey, encompassing antibacterial, antifungal, antimycobacterial, and antiviral effects. These characteristics have been validated through a multitude of in vitro investigations and a limited selection of clinical trials (Israili, 2014; Asma et al., 2022a). Nonetheless, its utilization within the contemporary pharmaceutical sector remains limited, primarily because of insufficient scientific inquiry and investigation (Diğrak, 2006). The existence of propolis and pollen in honey raises the possibility that some of the honey's antibacterial efficacy may be attributed to the antibacterial agents found in these elements (Redzic et al, 2011; Viuda-Martos et al., 2008). Examinations of honey's antibacterial potential reveal that honey can, in certain instances, entirely impede the proliferation of specific bacterial strains frequently responsible for infections, even when substantially diluted (Balázs et al., 2021; Dunford et al., 2000; Molan, 1999).

Manuka honey, derived from the *Leptospermum* plant, is a highly prized type of honey for its broad-spectrum antimicrobial activity. Recent research also suggests that honeydew honey may have even greater antimicrobial potential than monofloral honey, highlighting the critical role of geographical origin of honey. The specific geographical location and prevailing climatic conditions can affect the composition of bioactive compounds in plant nectar. All these elements together contribute to the overall quality of honey and consequently to its antimicrobial efficacy. Manuka honey is known to have antibacterial effect against about 60 different bacterial species including gram positive and negative, anaerobic, aerobic ones (Cokcetin et al., 2016; Pita-Calvo & Vázquez, 2017; Silici et al., 2010, Asma et al., 2022b). The studies have shown that honey has inhibitory properties not only against bacteria, but also against parasites, fungi and viruses (Hossain et al., 2020; Karadal, 2012; Nainu, 2021; Sekar et al., 2023). One of the studies found that a 10% honey solution applied to the Ecinococccus granulosus, which causes hydatid cysts, had a lethal effect within three minutes. The antibacterial activity of honey is due to factors such as hydrogen peroxide production, high sugar concentration, low pH and low water activity. Hydrogen peroxide is produced by the glucose oxidase enzyme in the hypopharyngeal glands of bees, which oxidises the glucose in honey and is the main antibacterial component of honey. It was observed that antibacterial effect persisted in honeys in which catalase enzyme from pollen of some plants inactivated hydrogen peroxide. Phenolic acids and their derivatives, flavonoids, polyphenols, aromatic acids, non-dissociated organic acids such as gluconic acid and recently Maillard reaction products have also been shown to be effective in the antibacterial activity of honey. It was found that the antibacterial activity in dark coloured honeys was higher than in light coloured honeys and it was stated that this result was related to the antibacterial activity of phenolic compounds found more in dark coloured honeys (Brudzynski, 2020; Bucekova, et al., 2014; Karadal, 2012).

The presence of hydrogen peroxide in honey is extensively documented and is considered one of honey's fundamental antimicrobial constituents. Honeybees (*Apis mellifera*) naturally produce hydrogen peroxide as a byproduct during the nectar collection process. Hydrogen peroxide is a well-defined antimicrobial agent. Hydrogen peroxide is an oxidative biocide that eliminates microorganisms by extracting electrons from their chemical configurations. This impedes the proliferation of microbes and induces irreversible DNA harm through the creation of hydroxyl radicals (Bernard et al., 1997; Brudzynski et al., 2012; Finnegan et al., 2010; Saranraj & Sivasakthi, 2018).

Scientists have undertaken comprehensive scientific studies to explore the antimicrobial attributes of honey and various bee-derived substances, aiming to consider their potential application in healthcare and the pharmaceutical sector. These research efforts are positioned to establish the foundation for future inquiries centered on pinpointing the precise elements that bestow antimicrobial characteristics upon honey and its related products (Asma et al., 2022a; Segueni et al., 2023).

Early examinations of honey have identified several key factors contributing to its antimicrobial properties. These include its high sugar content, low pH levels, the presence of polyphenolic compounds, hydrogen peroxide, and the discovery of an inhibin (Maddocks & Jenkins, 2013). Moreover, examinations have shed light on the plausible antimicrobial function of a protein originating from bees, known as bee defensin-1, present within honey (Albaridi, 2019; Dustmann, 1979; Molan, 1992). This implies that honey specimens contain an array of antimicrobial components, and their efficiency cannot be ascribed to a singular antimicrobial agent. Additionally, honey is composed of numerous constituents that work in synergy to enhance its antimicrobial potency (Nolan et al., 2019).

Additionally, hydrogen peroxide (H_2O_2) plays an important role in the antimicrobial activity of honey. It is produced during the ripening of honey under the action of the glucose oxidase enzyme. Glucose oxidase is secreted by the bees' pharyngeal glands. This enzyme breaks down glucose into H_2O_2 and gluconic acid. Exposure of honey to heat and light can reduce the level of H_2O_2 because glucose oxidase is sensitive to heat and light. The resulting H_2O_2 inhibits microorganisms from spoiling ripened nectar. It is important to note that it is only during the ripening stage of the honey that H_2O_2 is produced. The glucose oxidase enzyme is inactivated and H_2O_2 production ceases as its production increases the acidity of the honey. Increased acidity causes H_2O_2 to break down into water and oxygen. The activity of H_2O_2 has been observed to be increased by diluting honey and reducing its acidity. When honey is diluted with water in proportions ranging from 15% to 50%, the maximum H_2O_2 activity has been observed. Some honeys still showed antimicrobial properties even when glucose oxidase was found to be inactive (Majtan et al., 2021; Özmen & Alkın, 2006).

Phenolic compounds and flavonoids, which are transferred from nectar to honey, have been demonstrated to play a role in the antimicrobial activity of honey. The antimicrobial activity of honey is maintained even after the decomposition of hydrogen peroxide (H2O2). This persistence of antimicrobial activity is attributed to the presence of phenolic compounds and flavonoids. (Çoban, 2022; Faúndez, 2023). Studies have reported that honey has an antibacterial effect on around 70 species of bacteria, including both anaerobic and aerobic, gram positive and gram negative bacteria (Hunter et al., 2021; Ilia et al., 2021; Majtan et al., 2021; Nayaka et al., 2020).

Honey bee defensin-1 is an antimicrobial peptide found in the honey bee's circulatory system and hypopharyngeal glands. Honey bee defensin-1 is an antimicrobial peptide that is innate in bees and plays an important role in the immune system against various microorganisms such as yeasts, fungi, protozoa, Gram-positive and Gram-negative bacteria. The most significant activity of this antimicrobial peptide is observed against Gram-positive bacteria such as *Staphylococcus aureus, Paenibacillus larvae* and *Bacillus subtilis*. However, its efficiency against multidrug-resistant strains is relatively limited. Discrepancies in the levels of honey bee defensin-1 among different honey samples likely stem from variations in the production of antimicrobial peptides by bee glands. Current studies on the mechanism of action of honeybee defensin-1 argue that it induces pore formation in bacterial cell membranes and thus causes cell death (Ganz, 2003; Ilyasov et al., 2012; Kwakman et al., 2011; Oryan et al., 2016).

Honey's exceptional antimicrobial potential, particularly in the face of antibiotic-resistant bacteria, has spurred research into its synergistic interactions with antibiotics. Numerous investigations have delved into various combinations of antibiotics and honey, yielding encouraging outcomes. can be applied Honey can be applied in wound treatment as a topical or as honey-impregnated dressings. As an illustration, the amalgamation of Manuka honey with tetracycline has exhibited heightened antimicrobial effectiveness against pathogens such as *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Leveraging tetracycline's wide-ranging antimicrobial attributes and the enhanced efficacy achieved through the inclusion of Manuka honey, this pairing stands as a promising contender for advancing wound healing and therapeutic interventions (Homaeigohar and Boccaccini, 2020; Jenkins & Cooper, 2012). In a similar scenario, the simultaneous use of sub-inhibitory levels of Medihoney with rifampicin revealed the absence of rifampicin resistance among clinical *Staphylococcus aureus* isolates. These results offer substantial support for contemplating the application of honey in clinical environments, particularly when dealing with prolonged or recurring infections (Müller et al., 2013). Moreover, the interplay between honey and antibiotics has demonstrated synergistic and additive impacts when dealing with biofilms. This is well illustrated by the synergy of vancomycin with Manuka honey in the context of tackling *Staphylococcus aureus* biofilms and the complementarity observed with gentamicin and Manuka honey in addressing biofilms produced by *Pseudomonas aeruginosa* (Asma et al., 2022c; Campeau & Patel, 2014; Combarros-Fuertes et al., 2020). These findings underscore the promising possibilities of utilizing honey and emphasize the importance of additional research to fully exploit the synergistic benefits for clinical purposes.

This review examines deeply antimicrobial potential of honey against pathogenic microorganisms such as *Salmonella* and *Listeria monocytogenes* and presents the latest research findings in the literature. Enhancing our comprehension of honey's antimicrobial characteristics has the potential to open doors to fresh applications in both the food industry and the healthcare domain.

Antimicrobial Effects of Honey on *Salmonella*

Salmonella pertains to a bacterial genus capable of inducing salmonellosis, an alimentary disease. Manifestations of salmonellosis encompass abdominal discomfort, diarrhea, elevated body temperature, vomiting, headaches, and fatigue. Typically, these symptoms manifest within a span of 6 to 72 hours following exposure and may endure for multiple days (Kim et al., 2018). *Salmonella* bacteria have the potential to be transmitted to individuals through the ingestion of tainted meat, water, or edibles from animals carrying the infection. Common reservoirs of this bacterium encompass uncooked or insufficiently cooked poultry, turkey, eggs, beef, and seafood (Acaröz et al., 2018). Additionally, the bacterium can be present in contaminated fruits, vegetables, and various food items. Preventing salmonellosis necessitates strict adherence to food safety guidelines when handling and cooking food, rigorous maintenance of high hygiene standards, and the avoidance of consuming undercooked food items. In addition, probiotic bacteria and their metabolites can diffuse into the gut lumen and modify the community, resulting in the elimination of enteric pathogens such as *Salmonella*. *Salmonella* continues to pose a significant challenge in the domains of food safety and public health, thereby prompting the enforcement of diverse measures for its control, encompassing the examination and oversight of food products (Acaröz et al., 2018; Ehuwa et al., 2021; Hardy, 2004; Zhu et al., 2023).

Recent research has brought to light that specific natural foods, honey among them, have the potential to obstruct the growth and transmission of harmful bacteria such as *Salmonella*. This section offers a thorough interpretation of the most recent studies delving into the antimicrobial effects of honey against *Salmonella*, accentuating its role as a potential natural antimicrobial agent.

Akyalçın & Süerdem, (2017) collected honey samples from diverse botanical sources, encompassing floral, multifloral, chestnut, oak, and thyme varieties, examined their efficacy against *Salmonella* Typhimurium. The assessment was carried out utilizing the agar well diffusion technique on Mueller-Hinton agar. Among these, oak honey exhibited the most substantial antioxidant capacity, surpassing the other honeys. Notably, all of the honey samples displayed antimicrobial properties against *Salmonella* Typhimurium. Interestingly, some earlier research has indicated that chestnut honey boasts a superior antioxidant capacity when compared to other honey varieties (Sagdic et al., 2013; Saral, 2018). Nevertheless, in contrast to these conclusions, the investigation at hand revealed that the antioxidant capacity of oak, thyme, and floral honeys surpassed that of chestnut honey. These discrepancies may be attributed to geographical variations in the plant life within the regions where the honey is produced (Bican & Akyalçın, 2018). Additionally, Russo et al., (2023) evaluated the antimicrobial potential of honey samples obtained from five different floral sources, namely eucalyptus, thyme, chestnut, citrus, and sulla produced in the Sicilian regions, against *Salmonella* Typhimurium. Both undiluted and diluted (100%, 75%, 50% and 25%) honey samples showed a wide range of inhibition activity. Interesting antagonistic properties were observed especially for thyme honey. In addition, it was observed that the inhibitory effect of dark-coloured honeys was more pronounced than that of light-coloured honeys. The honey samples were able to inhibit *Salmonella* Typhimurium in both diluted and undiluted formulations. The inhibition zone diameters ranged from 7 mm to 28 mm. In addition to, Wadi, (2022) evaluated the antibacterial activity of a variety of natural and commercial honey samples collected from 32 different countries against *Salmonella* Typhimurium. A total of 32 honey samples of different floral origin (both natural and commercial) were tested *in-vitro* for their antibacterial activity against *Salmonella* Typhimurium using disk diffusion technique in Sudan. All natural and commercial honey samples were equally susceptible to *Salmonella* Typhimurium. Commercial honey showed the same antibacterial activity as raw, unprocessed honey against the tested clinical isolates. In this context, honey could be used as a proven alternative to traditional antibiotics for the treatment of clinical isolates.

Cilia et al., (2020) evaluated 41 honey samples from different regions of Ukraine for antibacterial activity against *Salmonella* Typhimurium. Consistent with other studies, *Salmonella* showed a wide range of responses to the honeys. Also, Postali et al., (2022) evaluated the antibacterial activity of honey samples collected in Greece against *Salmonella* Typhimurium. The results showed that *Salmonella* was susceptible to honey samples only at concentrations above 6.25% (v/v).

Park et al., (2020) evaluated the antimicrobial activity of Korean Hovenia monofloral honey (*Hovenia dulcis*) against *Salmonella* Typhimurium. Hovenia monofloral honey exhibited minimum inhibitory concentration (MIC) values ranging from 25% to 50% (w/v). Hovenia monofloral honey has strong antibacterial activity and can be used to preserve food from foodborne pathogens. Furthermore, Otmani et al., (2021) used agar diffusion tests and MIC measurements to determine the *in-vitro*. antibacterial activity of 26 honey samples in Algeria. It has been determined that these honeys were sensitive to 9 types of monofloral honey and 9 types of polyfloral honey. Furthermore, the study showed that honey's dark colour was an indicator of its high bioactive and antibacterial content.

Various factors can affect the quality of stingless bee honey. The properties of this honey may vary according to different seasons of the year. Therefore, the aim of Mahmood et al., (2021a) evaluated the antimicrobial properties of honey from stingless bees from various multifloral sources. Honey was collected from hives located in two different regions in Besut, Terengganu, Malaysia. The difference between these fields is that one is planted with only two flowering species (Stevia) while the other is planted with more than two flowering species. The foodborne pathogen *Salmonella* Typhimurium was analyzed for antimicrobial properties. The results showed that honey samples collected during both dry and rainy seasons exhibited antimicrobial properties against to all tested foodborne pathogens. However, dry season samples showed higher antioxidant properties compared to honeys harvested in the rainy season. This study underlines the significant impact of seasonal changes on antioxidant properties.

Oğur et al., (2022) aimed to determine the antimicrobial activity of natural honey obtained from Bitlis, Turkey against *Salmonella* Enteritidis, a foodborne pathogen. The hollow agar method was used to determine the antimicrobial activity of natural honey samples at different concentrations (10%, 25%, 50% and 100%). 50% concentrate of natural honey showed significant antimicrobial activity against *Salmonella* Enteritidis by creating an inhibition zone of 22.50±2.73 mm. As a result, natural honey from this region has been found to have the potential to develop new drugs for the treatment of certain infectious diseases using its active compounds that exhibit antimicrobial activity.

Adeyemo et al., (2017) conducted a study on the evaluation and comparison of the antibacterial activities of different types of honey in southwestern Nigeria. They also aimed to provide information about a more affordable and effective natural-based antibacterial product and to confirm the antibacterial activity of honey in the region by comparing the antibacterial activity of honey with the standard antibiotic streptomycin. In this study, honey inhibited the growth of *Salmonella* Typhimurium when diluted with sterile distilled water to a 50% (v/v) concentration. Zones of inhibition exhibited by honey ranged from 6.0±0.6 mm to 30.7±1.2 mm. Super dark amber honey has been shown to have the highest antimicrobial activity, comparable to the antibiotic streptomycin. It demonstrated potent broad-spectrum antibacterial activity against a wide range of bacteria. However, more research is needed to assess its feasibility for clinical use.

Wang et al., (2021) investigated the antimicrobial activity of Qinling *Apis cerana* honey, a traditional Chinese honey, against *Salmonella* Typhimurium. The extract caused significant shrinkage and collapse of *Salmonella* Typhimurium cell membranes in vitro. The extract significantly reduced *Salmonella* Typhimurium levels in mice. This research provides valuable insights into the antimicrobial properties of Qinling *Apis cerana* honey against *Salmonella* Typhimurium and its antibacterial mechanism.

Ejaz et al., (2023) isolated 20 highly drug-resistant *Salmonella* Typhimurium strains from pediatric sepsis patients. PCR was used to identify the antimicrobial resistance genes carried by these strains. The antimicrobial activity of five Pakistani honeys, namely Sidr (*Ziziphus spina-christi*), Neem (*Azadirachta indica*), Mustard (*Brassica nigra*), Beri (*Ziziphus mauritiana*), and Orange (*Citrus sinensis*) honey, was evaluated using the agar diffusion assay. Notably, among the five honeys, Beri honey showed an inhibition zone ranging from 7 to 15 mm, while Neem honey showed an inhibition zone ranging from 7 to 12 mm. These results indicate that these five honeys from Pakistan have the potential to effectively contribute to the management of extensively drug-resistant *Salmonella* Typhimurium.

Ayub et al., (2020) investigated the effect of sub-inhibitory concentrations of locally available Marhaba brand honey and non-branded honey extracted from the *Ziziphus mauritiana* plant on *Salmonella* Typhimurium in Pakistan. The results showed that the growth of the pathogenic bacteria tested was effectively controlled by both Marhaba honey and *Ziziphus mauritiana* honey. However, before suggesting honey as a superior antibacterial agent in food production facilities where low pH and high temperatures are frequently used, it is recommended that further research be conducted to understand the development of tolerance to adverse conditions such as pH 2.5 and temperature of 60°C.

Ghifari Alhadz et al., (2021) investigated the antibacterial activity of five different types of honey against the growth of *Salmonella* Typhimurium. Results showed Kupang and Sumba honey had low antibacterial activity (<6mm). In addition, Lombok white honey (7.74 mm), Gunung Kidul-Lanceng honey (7.37 mm) and Lombok black honey (6.74 mm) were found to have moderate antimicrobial activity at 100% concentration. In addition, Kalidasan et al., (2017) evaluated the antimicrobial activities of three different honey samples, namely Kombu honey, Malan honey and Commercial honey, against *Salmonella* Typhimurium. All honeys were used at a concentration of 75 µl and Kombu honey showed an inhibition zone of 24 mm, Malan honey 21 mm and Commercial honey 19 mm. Consequently, it was concluded that Kombu honey had higher antimicrobial activity against bacterial pathogens and was considered more effective compared to Malan and Commercial honey in the scientific article.

Sulaiman & Sarbon, (2022) conducted a study to investigate the effect of heat treatment on the antimicrobial properties of Tualang, Acacia and Kelulut honey against *Salmonella* Typhimurium. As the temperature of the heat treatment increased, the antimicrobial efficacy of the treated honey decreased. Only Kelulut honey showed an inhibition zone at all temperatures in the agar well diffusion test. In general, there was a decrease in the antimicrobial properties of the heat-treated honeys. This decrease is related to the reduction of antibacterial elements in honey, such as hydrogen peroxide, due to the application of elevated temperatures. Kelulut and Tualang honey showed superior antimicrobial properties compared to Acacia honey. Also, Shalsh et al., (2021) conducted a study to investigate the antimicrobial activity of commercially available local *Malaysian Trigona* sp. honey against the pathogen *Salmonella* Typhimurium. The disc diffusion and agar well diffusion tests were used to test different concentrations of honey. The raw form of Kelulut honey showed better antibacterial results than the diluted honey based on the results of the agar well diffusion test. Clear zones of inhibition against *Salmonella* Typhimurium of 7.2±0.3 mm and 8.1±0.4 mm were obtained from two separate samples of Kelulut honey. These results demonstrate that *Trigona* sp. honey has antibacterial properties. The different concentrations of honey (%20, %40, %60 and %80) play a significant role in its efficacy.

Bhalchandra & Joshi, (2021) conducted a study to investigate the antibacterial properties of raw honey from three species of bees found in the Kannad taluka of Aurangabad district. The Agar Well Diffusion method was used to test the honey samples against *Salmonella* Typhimurium. The results revealed the susceptibility of the honey samples to *Salmonella* Typhimurium. Significantly, the observation of inhibition zones distinctly pointed out that honey sourced from *Apis florea* and *Apis cerana indica* manifested the most elevated antibacterial efficacy, whereas honey from *Apis dorsata* displayed comparatively lower antibacterial activity.

Akyalçın & Suerdem, (2017) carried out an evaluation involving six honey samples sourced from Kosovo to investigate their antibacterial potential against *Salmonella* Typhimurium. The methodology employed was the agar well diffusion method on Mueller Hinton agar. Notably, all six honey samples displayed robust antibacterial properties, showcasing inhibition zones that spanned from 16 to 40 mm. These findings hint at the potential utility of honey as a promising alternative to antibiotics, particularly in the context of managing bacterial skin and soft tissue infections.

Agar diffusion assessments targeting *Salmonella* underscore the substantial antimicrobial efficacy of honey, as underscored by the emergence of inhibition zones. The inhibition areas of honey obtained from various flowers and in various concentrations against different *Salmonella* types are shown in Table 1. These conclusions lend weight to the application of honey as a natural solution for addressing *Salmonella* infections. In the context of this investigation, the manifestation of inhibition zones serves as tangible proof of honey's antimicrobial attributes. These outcomes emphasize the potential of honey to function as a natural deterrent against pathogenic bacteria within the human organism. Consequently, further research is essential to delve into the prospects of utilizing honey in *Salmonella* management and prevention.

Table 1. *Inhibition areas of honey obtained from various flowers and in various concentrations against different Salmonella types*

Honey Types	***Salmonella* Types**	**Concentration of Honey**	**Inhibition Zone (mm)**	**References**
Floral	*S.* Typhimurium ATCC 51812	100%	32	Akyalçın & Süerdem, 2017
Highland			30	
Chestnut			30	
Oak			26	
Thyme			14	
Chestnut	*S.* Typhimurium ATCC 14026	100%	27±1.15	Russo et al., 2023
		75%	27±0.00	
		50%	17±0.58	
		25%	15±0.00	
Eucalyptus		100%	20±0.58	
		75%	18±1.00	
		50%	16±0.58	
		25%	8±0.58	
Sulla		100%	28±1.00	
		75%	27±0.58	
		50%	24±1.73	
		25%	14±2.08	
Thyme		100%	30±0.00	
		75%	27±1.53	
		50%	19±1.00	
		25%	11±0.00	
Citrus		100%	23±0.00	
		75%	17±1.15	
		50%	16±2.00	
		25%	8±0.00	
Blossom honey (*Arbutus andrachne L.*)	*S.* Typhimurium DT193	75%	9.33±1.15	Postali et al., 2022
Honey from stingless bee (*Heterotrigona itama*)	*S.* Typhimurium ATCC 13311	100%	Dry Season 1: 8.67±0.58	Mahmood et al., 2021a
			Dry Season 2: 11.33±0.58	
			Rainy Season 1: 13.67±1.15	
			Rainy Season 2: 17.33±0.58	
Honey Sample 1	*S.* Enteritidis ATCC 13076	100%	13.00±1.10	Oğur et al., 2022
Honey Sample 2			14.00 ± 1.10	
Honey Sample 3			15.00 ± 0.00	
Dark amber	*S.* Typhi	50%	6.0±0.0	Adeyemo et al., 2017
Super light			18.0±0.6	

amber				
Light amber			20.7±1.2	
Super dark amber			27.3±1.2	
Bitter			21.3±1.2	
Sidr	*S.* Typhi	50%, 40%, 30%, 20% and 10%	7-12	Ejaz et al., 2023
Neem			6-13	
Mustard			5-9	
Orange			6-12	
Beri			7-15	
Kupang	*S.* Typhi	100%	<6±.00	Ghifari Alhadz et al., 2021
Sumba			<6±.00	
Lombok white			7.74±.16	
Gunung Kidul-Lanceng			7.37±0.52	
Lombok black			6.74±0.42	
Super Archipelago			7.29±.60	
Raw bee honey	*S.* Typhi	ND	19 ± 0.5- 25 ± 0.6	Wadi, 2022
Kombu	*S.* Typhi	75%	24	Kalidasan et.al., 2017
Malan			21	
Commercial			19	
Kelulut	*S.* Typhi	100%	7.2 ± 0.3	Shalsh et al., 2021
Kelulut	*S.* Typhi	100%	18.10 ± 0.25	Sulaiman & Sarbon, 2022
Tualang			13.10 ± 0.15	
Acacia			12.10 ± 0.15	
Mountain	*S.* Typhimurium ATCC 51812	50%	36	Akyalçın & Suerdem, 2017
Floral 1			40	
Meadow flowers			36	
Pinus			36	
Floral 2			36	
Floral 3			36	
Chestnut	*S.* Typhi	90%	18.0±2.0	Gośliński et al., 2020
		80%	16.0±2.0	
		70%	15.0±1.0	
		60%	13.0±1.0	
		50%	12.0±1.0	
		40%	12.0±1.0	
		<30%	0	
Linden		90%	12.0±1.0	
		80%	0	
		70%	0	
		60%	0	
		50%	0	
		40%	0	
		<30%	0	
Honeydew		90%	14.0±1.0	
		80%	13.5±0.5	
		70%	12.0±0.0	
		60%	12.0±0.0	
		50%	0	
		40%	0	
		<30%	0	

Antimicrobial Effects of Honey on Listeria monocytogenes

Listeria monocytogenes poses a substantial public health concern, primarily due to its widespread presence in the environment and its capability to endure adverse conditions encompassing refrigeration, freezing, heating, and desiccation. It constitutes a highly pathogenic microorganism with the potential to elicit a spectrum of human infections, inclusive of conditions like meningitis, septicemia, conjunctivitis, and skin and mucous membrane infections. While healthy adults often exhibit tolerance to low-level exposure to *Listeria monocytogenes* without symptomatic development, specific demographic groups, including children, the elderly, expectant mothers, infants, and individuals with compromised immune systems, are markedly more vulnerable to the deleterious effects associated with this bacterium (Acaröz et al., 2017; Goulet et al., 2006; Kara et al., 2019). Addition, encephalitic listeriosis is a type of listeria infection that causes inflammation of the brain and enters the body through food consumption. It is critical to take various food safety precautions, such as cooking foods well and paying attention to hygiene practices, to reduce the risk of encephalitic listeriosis. In addition, people with weakened immune systems may need to be more cautious about such infections (Hatipoğlu et al., 2022; Özdemir et al., 2021).

Because *Listeria monocytogenes* is so important, food safety experts and researchers have investigated the potential of natural antimicrobial agents. In this context, the role of honey as a potential natural antimicrobial agent against *Listeria monocytogenes* was examined. In this part, we explore contemporary research endeavors that examine the antimicrobial potential of honey in the context of combating *Listeria monocytogenes*. The results emanating from these studies hint at the promising prospect of honey serving as a natural substitute for traditional antibiotics.

Pajor et al., (2018) conducted an examination wherein they subjected honey samples originating from the northern region of Poland to a screening process aiming to identify bacteria capable of generating metabolites that impede the proliferation of *Listeria monocytogenes*. The outcome revealed the presence of highly potent bacteria, yielding 38 active strains that exhibited significant efficacy against *Listeria monocytogenes*. This study underscores the potential of honey produced in northern Poland as a valuable source of bacteria yielding antimicrobial metabolites. Additionally, Putri et al., (2020) lactic acid bacteria were extracted from honey sourced from *Apis mellifera* bee through the utilization of the coating method, yielding a total of 9 isolates. These isolates underwent thorough characterization, and their prospective antimicrobial efficacy against *Listeria monocytogenes* was assessed via the well-diffusion test. As an outcome of this investigation, two promising isolates were successfully identified.

Çakır & Dervişoglu, (2022) investigated the antimicrobial effects of honey collected from different regions of Bingöl province using the disk diffusion method. Honey samples were prepared and tested for their antimicrobial activity against *Listeria monocytogenes* at three different concentrations (500, 250 and 125 mg/ml). The results obtained showed that none of the honey samples prepared at three different concentrations had any antibacterial activity against *Listeria monocytogenes*.

Nazzaro et al., (2021) aimed to evaluate the sensitivity of bacterial biofilm formation and metabolic changes occurring in bacterial cells caused by sulla, lavender, tree of heaven, ivy and strawberry tree monofloral honey varieties. *Listeria monocytogenes* exhibited up to 72.20% sensitivity to biofilm inhibition. Additionally, Fratianni et al., (2021) evaluated the effects of different monofloral honeys of Italian origin on *Listeria monocytogenes*. *Listeria monocytogenes* was generally sensitive to the inhibitory effects of all honeys. While sensitivity reached 90% in the presence of ivy honey, it was higher than 90% in the presence of blackberry honey and snowberry honey.

Fratianni et al., (2023) investigated the potential antimicrobial effects of legume honeys, especially alfalfa, astragalus, carob, indigo and sainfion, against the pathogen *Listeria monocytogenes*. With a few exceptions, all legume honeys have been found to be biofilm inhibitors. Carob honey was the most effective honey sample in inhibiting *Listeria monocytogenes* biofilm formation. The inhibition efficiency was up to 81.71%. It was also effective in the presence of glucose in the bacterial growth medium. In conclusion, the study shows that these five honeys may, in different ways, enhance certain prebiotic properties and inhibit biofilm when consumed.

Zapata-Vahos et al., (2023) examined the antimicrobial activity of honey obtained from two bee species, *Melipona eburnea* and *Apis mellifera*, against *Listeria monocytogenes* in Colombian tropical forests.

Inhibitory activity was observed against *Listeria monocytogenes*. It was observed that there was a statistically significant difference in inhibitory activity against *Listeria monocytogenes* between the rainy season and the dry season in honey obtained from *Apis mellifera* ($p \leq 0.05$), whereas there was no such difference in honey obtained from *Melipona eburnea*. The study concluded that environmental conditions, flower species and geographical location of flower sources are responsible for the variability in the antimicrobial activity of honey.
Gkoutzouvelidou et al., (2021) investigated an inquiry encompassing eight honey samples sourced from Lemnos Island, in addition to one Manuka honey sample. The objective was to assess their antimicrobial potential against *Listeria monocytogenes* bacteria. The noteworthy revelation from this study is that all the Lemnos honey samples displayed antibacterial characteristics, resulting in inhibition zones of equivalent dimensions (5.0±0.0 mm) as observed in the case of Manuka honey. Additionally, Đurović et al., (2022) assessed the antimicrobial potential of honey variants, including acacia, sunflower, and forest meadow, sourced from Serbia was scrutinized concerning their effectiveness against *Listeria monocytogenes*. The findings unveiled that forest meadow honey showcased the most robust antibacterial activity, closely followed by sunflower honey, whereas acacia honey exhibited the comparatively lower antibacterial efficacy.

Engin et al., (2022) assessed the antibacterial attributes of chestnut, citrus, sunflower, and clover honey were evaluated in the context of their response to varying storage conditions, including exposure to heat and light, as well as different durations of storage. Notably, all four varieties of honey demonstrated a moderate level of antibacterial activity against *Listeria monocytogenes* when examined at a 1:1 concentration ratio. This study suggests that storing honey in cool, dark conditions may preserve its antimicrobial activity. Additionally, Çakır et al., (2020) investigated the antimicrobial activity of honey samples from Sivas (Zara), Gümüşhane, and Rize (Anzer) provinces against *Listeria monocytogenes*. None of the honey samples exhibited antimicrobial activity against *Listeria monocytogenes*. Furthermore, Bican & Akyalçın, (2018) investigated oak honey samples were assessed for antimicrobial activity against *Listeria monocytogenes* using the agar well diffusion method. The inhibition zone for *Listeria monocytogenes* in oak honey was 14 mm.

Mahmood et al., (2021b) conducted a study to determine the antimicrobial properties of non-stinging honey from different multifloral origins in both dry and wet seasons. Two different multifloral areas (Area A and B) were used to collect honey samples. While Area A was planted with only two flowering species (including Stevia), Area B was planted with more than two flowering species. Well diffusion method was used to analyze the antimicrobial properties against *Listeria monocytogenes*. Both sets of honey samples collected in both seasons showed antimicrobial properties against *Listeria monocytogenes*, while samples from the dry season showed higher levels of inhibition.

Oğur et al., (2022b) aimed to determine the antimicrobial activities of natural honey against *Listeria monocytogenes* in Bitlis. They used the agar well diffusion method to determine their antimicrobial activity at 10%, 25%, 50% and 100% concentrations. The results showed strong antimicrobial activity; 50% concentration of honey produced a zone of inhibition of 34.00±1.10 mm on *Listeria monocytogenes*. The greatest inhibition zones were observed in 100% concentrate. No zones were observed in the 10% concentrate. Additionaly, Adeyemo et al., (2017) evaluated and compared the antibacterial activities of different types of honey in southwestern Nigeria. Their aim was also to verify the antibacterial effectiveness of honey in southwestern Nigeria and to provide information on a potentially cheaper alternative antibacterial product of natural origin. Broth tube dilution method was used to determine the minimum inhibitory concentration of honey samples. The data obtained showed that the inhibitory zone size of honey samples varied between 6.7±1.2 mm and 26.0±1.2 mm, while the zone size of streptomycin was 12.0±0.6 mm. Compared to the standard antibiotic streptomycin, this shows that honey has antimicrobial activity.

The findings acquired indicate that honey possesses a notable antimicrobial influence against *Listeria monocytogenes*. The inhibition areas of honey obtained from various flowers and in various concentrations against different *Listeria* types are shown in Table 2. These outcomes hint at the potential utility of honey in bolstering food safety, presenting a natural substitute for controlling pathogens like *Listeria monocytogenes*. Nonetheless, for the effective utilization of honey against *Listeria monocytogenes*, additional data concerning application conditions and quantities, as well as further exploration through clinical research, is imperative. Furthermore, more extensive studies are necessary to delve into the antimicrobial attributes of various honey varieties and the factors that influence these properties. Thus, it is incumbent upon future research to provide additional insights into the efficiency and applicability of honey as a safe preservative within the food industry.

Table 2. *Inhibition areas of honey obtained from various flowers and in various concentrations against different Listeria types*

Honey Types	*Listeria* Types	Concentration of Honey	Inhibition Zone (mm)	References
Apis mellifera-1	*L. monocytogenes*	ND	12,05+1,0	Putri et al., 2020
Apis mellifera-1			8,80+0,8	
Apis mellifera-1 (Genç)	*L. monocytogenes* NCTC 5348	500, 250, and 125 mg/mL	No activity	Çakır & Dervişoglu, 2022
Apis mellifera-2 (Kiğı)			No activity	
Apis mellifera-3 (Sancak)			No activity	
Apis mellifera-4 (Yedisu)			No activity	
Melipona eburnea and *Apis mellifera* honey	*L. monocytogenes* ATCC 9118	ND	Dry Sample: 10.49±7.76 Rainy Sample: 26.03±4.91	Zapata-Vahos et al., 2023
Chestnut, citrus, sunflower, and clover	*L. monocytogenes* ATCC 7944		9-18	Engin et al., 2022
Sample-1	*L. monocytogenes* NCTC 5348	50%	34.5	Çakır et al., 2020
Sample-2			34.0	
Sample-3			37.0	
Oak	*L. monocytogenes*	ND	14	Bican & Akyalçın, 2018
Multifloral honey from stingless bee (*Heterotrigona itama*)	*L. monocytogenes* ATCC 13932	ND	Dry Sample-1: 11.33±0.58 Dry Sample-2: 14.67±0.58 Rainy Sample-1: 5.67±0.58 Rainy Sample-2: 8.33±0.58	Mahmood et al., 2021b
Sample-1	*L. monocytogenes* ATCC 7644	100%	13.50±0.55	Oğur et al., 2022b
		50%	11.00±1.10	
Sample-2		100%	38.50±1.64	
		50%	34.00±1.10	
Sample-3		100%	12.00±0.00	
		50%	8.50±1.64	
African bee honey-1	*L. monocytogenes*	50%	7.3±0.0	Adeyemo et al., 2017
African bee honey-2			21.3±1.6	
African bee honey-3			26.0±1.2	
African bee honey-4			18.7±1.2	
African bee honey-5			7.7±1.5	
African bee honey-6			24.3±0.6	
African bee honey-7			7.3±1.2	
African bee honey-8			7.7±0.6	
African bee honey-9			24.3±1.2	
African bee honey-10			14.3±0.6	
African bee honey-11			12.0±0.0	
African bee honey-12			6.7±1.2	
African bee honey-13			9.0±0.0	
African bee honey-14			8.0±0.0	

CONCLUSION

This comprehensive review delved into the antimicrobial efficacy of honey against pathogenic microorganisms, including *Salmonella* and *Listeria monocytogenes*. Scientific investigations unequivocally confirm the robust antimicrobial properties intrinsic to honey. The innate components of honey effectively impede the proliferation and replication of pathogenic microorganisms. Research has notably demonstrated honey's effectiveness against perilous bacteria such as *Salmonella* and *Listeria monocytogenes*, which holds paramount significance concerning food safety and public health. Honey emerges as a promising alternative therapeutic avenue, especially in the face of antibiotic-resistant bacterial strains. Further exploration of honey's antimicrobial attributes is imperative. A pivotal aspect involves scrutinizing whether honey sourced from distinct floral origins or regions exerts varying antimicrobial effects. Additionally, delving deeper into the mechanistic underpinnings of honey's antimicrobial components is essential for a comprehensive understanding. There is a pressing need to encourage greater integration of honey in the medical and healthcare sectors, with particular emphasis on wound care, infection management, and alternative approaches to antibiotics. The applicability of honey in the food industry should also undergo rigorous investigation. Research focusing on honey's potential in countering foodborne pathogens like *Salmonella* and *Listeria monocytogenes* could markedly enhance food safety standards. In summation, the antimicrobial attributes and potential of honey should be accorded heightened consideration in the domains of public health and medicine. This is a pivotal step toward devising novel strategies for combatting antimicrobial resistance. In conclusion, the ongoing research into honey's antimicrobial effects underscores its versatility as a natural product with far-reaching applicability. Subsequent studies will undoubtedly enrich our comprehension of honey's antimicrobial prowess and facilitate its application across diverse sectors, encompassing medicine, food, and more.

Conflict of interest

The authors declared no conflict of interest.

Author contribution

All authors contributed equally.

Ethical approval

During the writing process of the study titled "A Review on the Antimicrobial Effect of Honey on *Salmonella* and *Listeria monocytogenes*: Recent Studies " scientific rules, ethical and citation rules were followed; No falsification has been made on the collected data and this study has not been sent to any other academic media for evaluation. Since this research is based on document analysis and descriptive analysis, there is no obligation for an ethics committee decision.

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