

Selected mechanisms of the antimicrobial effect of honey in the aspect of drug resistance of bacteria

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Received 09.07.2022

Accepted 29.07.2022

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Summary

The aim of the study was to review the available literature on the significance of factors and mechanisms of the antimicrobial effect of honey and the possibilities of using them to stabilize or inhibit the growth of selected Gram-positive and Gram-negative pathogenic bacteria particularly capable of developing drug resistance. It can be concluded from data in the literature that the factors and mechanisms of the antimicrobial effect of honey are numerous and diverse. Honey can be effective in fighting microbes due to its physical, chemical and biological properties. The mechanisms of the antimicrobial effect of honey include, above all, high osmotic pressure, low pH caused by the presence of organic acids and hydrogen peroxide, high content of phenolic compounds, lysozyme (to a limited extent) and methylglyoxal in manuka honey and defenazine-1 in Revamil honey. Based on the presented research results, it was observed that the level of antibiotic activity of honey depends on its variety, geographic origin, concentration of honey solution, but also on the type of bacterial strain tested and its resistance. Bactericidal and bacteriostatic effects of various varieties of honey have been found in certain Gram-positive bacteria (manuka honey – *Staphylococcus aureus*; Scottish heather honey – *Staphylococcus epidermidis*) and Gram-negative bacteria (undiluted Thai honeys – *Escherichia coli*; 3% (v/v) solution of honey – *Salmonella enterica*; manuka honey – *Pseudomonas aeruginosa* biofilm). The high level of antibiotic activity of honey against these bacteria species is a potential opportunity to support therapies in cases of infections caused by these microorganisms, which can help fight the problem of drug resistance. The possibility of inhibiting or limiting the development of pathogenic bacteria without resorting to antibiotics would also have a mitigating impact on the growing problem of antibiotic resistance. However, it is advisable to extend the spectrum of research in this area to include bacteria species for which the current experimental results are mutually contradictory (*Listeria monocytogenes*, *Enterococcus faecalis*) and to include in the research microorganisms that have not yet developed drug resistance. In addition, both for economic reasons and due to the limited supply, alternative varieties of honey should be researched more extensively in research, apart from manuka honey which is most often used in experiments by scientists. In addition to the unquestionable advantages of honeys, it is important to remember that they can be contaminated with spores of *Clostridium botulinum* and *Bacillus cereus*.

Keywords: bee honey, antimicrobial effect, drug resistance, pathogenic bacteria, opportunistic pathogens

In the era of increasing resistance of microorganisms to antimicrobials, interest in searching for alternative substances with bactericidal and bacteriostatic properties is growing (41). The attention of scientists is directed mainly towards substances of natural origin. One of such substances is honey, as a product characterized by a strong antimicrobial effect (21, 58). Bee honey is a sweet substance produced by the *Apis mellifera* species of insects from plant nectar or honeydew (71). It is food for bees, so bee colonies must make it perfectly wholesome in both nutritional and microbiological terms. In case of honey contamination with numerous

colonies of bacteria, there is a high risk that the entire bee family will get sick. Bee honey, however, has many properties that protect it against the development of not only bacteria but also viruses, fungi or protozoa. An increasing number of studies on the stabilization and inhibition of growth of various pathogenic strains of Gram-positive and Gram-negative bacteria with honey show that this bee product can potentially be helpful (3, 4, 16, 55, 66, 69). Most researchers focus on a few species of bacteria and, for the most part, this research conducts investigations into the effects of honey on *Staphylococcus aureus*, which poses a serious threat to

health (7, 17, 45, 46, 55, 60, 64), and on *Escherichia coli*, which is a model bacterium in microbiological studies (14, 31, 35, 61, 66, 69, 89). This study aims to describe important mechanisms of the antimicrobial effect of honey and the possibilities of using the antimicrobial properties of honey to stabilize or inhibit the development of selected Gram-positive and Gram-negative pathogenic bacteria particularly capable of developing drug resistance, taking into account their characteristics in terms of health risks.

The importance of factors and mechanisms determining the antimicrobial activity of honey

The antimicrobial effect of honey is a very complex feature due to its wide spectrum and dependence on many factors of physical, chemical and biological nature (42, 58). The physical factors affecting the antimicrobial properties of honey include both high osmotic pressure and low pH (42). These parameters are influenced by the chemical composition of honey, with the predominance of sugars which have a 70-80% share in the total composition (70) and constitute 95% of the dry matter of honey (15). Glucose and fructose are the most abundant – each of these simple sugars accounts for about 35% – followed by maltose and melecytosis (6). The contents of different types of sugars can vary depending on the variety of honey. Borawska et al. (10) have shown that the monosaccharide content in honeydew honey is lower than in other varieties. This high content of sugars in honey is responsible for its high osmotic pressure, up to 500 Pa, which is unfavorable for the development of microorganisms (42). A parameter related to the osmotic pressure is the activity of water (a_w) expressed by the ratio of the water vapor pressure in a given product to the pressure of distilled water molecules at the same temperature (72). Water activity, which depends largely on the concentration of monosaccharides in honey, determines how much water is available to microbes (15, 92). For pure water this value is 1 a_w , while with an increase in the concentration of soluble compounds the value of this parameter decreases (49). In the study by Gallardo-Chancón et al. (24), the water activity in honeys ranged from 0.55 to 0.60 a_w . With such values of this parameter, the growth of bacteria, yeast fungi, mold fungi and xerophilic molds is not possible (42).

The acidity of honey is another physical factor affecting its antimicrobial effect. Honey contains many organic acids including gluconic, malic, lactic, citric, propionic, succinic, tartaric, oxalic, butyric, formic and acetic (36). The low pH is the result of presence of these organic acids in the product. According to Haniyeh et al. (30), pH values of honeys range from 3.2 to 4.5. This is consistent with the results obtained by Madras-Majewska et al. (54) who determined that the average pH value for honey is 3.9, which suffices to inhibit the development of most microorganisms. According to researchers, the lowest pH tolerated by

pathogenic bacteria is in the range from 4.5 to 5.7 (65). According to Bogdanov (9), the antibacterial activity of honey correlates not with the pH value but with the acid content. Currently the theory about the effect of the pH value on this property of honey prevails among researchers. The acidity of honey is clearly dependent on its variety and origin. For example, among Polish honeys, rapeseed and acacia honeys are the least acidic and buckwheat honey is the most acidic (36).

Hydrogen peroxide is considered to be one of the most important chemical factors determining the bactericidal effect of honey. This compound is formed as a result of enzymatic oxidation of glucose to gluconic acid (88). This reaction occurs during the storage of honey. It is mediated by the glucose oxidase enzyme and hydrogen peroxide is a byproduct of this reaction (87). However, the enzyme is very sensitive to environmental conditions, such as light or temperature, which is why it quickly decomposes (88). It has been noticed that this reaction progresses much faster in diluted honey, so its antimicrobial effect can be up to 220 times stronger (79). In addition, the amount of hydrogen peroxide produced varies depending on the geographic origin and botanical composition of honey (56). Dustman's research (20) has shown that honeydew and chestnut honey contain large amounts of this compound, while acacia, heather and rape honeys have a low capacity to produce hydrogen peroxide. Hydrogen peroxide has strong antimicrobial effects and acts on both bacteria (for example, *Staphylococcus aureus* or *Pseudomonas aeruginosa*), fungi (*Candida albicans*), viruses (*Herpes Simplex Virus* or *Rubella Virus*) and protozoa (*Giardia lamblia*) (42) (Tab. 1). However, this effect can be limited by the introduction of catalase from pollen. Despite this, some honeys are able to retain their antimicrobial activity, which suggests that this property is influenced by many factors (56).

Other chemical factors showing a strong bactericidal, anti-inflammatory and anticoagulant effect are thermostable phenolic compounds including phenolic acids and flavonoids. The average content of phenolic acids in honey is 20 mg/kg, while the content of flavonoids is just about 9 mg/kg (33). The phenolic acids found in honey include gallic, ellagic, caffeic and ferulic acids (88). Studies by Świetlikowska et al. (81) have shown that the content of these compounds depends on the variety of honey. In linden honey there are six times more of them than in honeydew honey (Tab. 1). There are differences not only in the quantities but also in the types of these phenolic acids, which depends on the variety and geographic region of origin of honey. In a study by Weston et al. (86) conducted on honey from New Zealand, benzoic, cinnamic and vanillic acids were found. In contrast, studies by Lihu et al. (51) have shown the presence of gallic, caffeic and coumaric acids in Australian honeys. In the research by Świetlikowska et al. (81), which found 6 phenolic

Tab. 1. Selected antimicrobial mechanisms in honeys

Selected factors (antimicrobial effect on pathogen)	Reference	Types of honey	Content
Hydrogen peroxide (<i>Staphylococcus aureus</i> , <i>Pseudomonas aeruginosa</i> , <i>Candida albicans</i> , <i>Herpes Simplex Virus</i> , <i>Rubella Virus</i>)	Dustman (20)	acacia	18-32 µg/g
		heather	29-34 µg/g
		rape	42-125 µg/g
		chestnut	120-605 µg/g
		honeydew conifer	284-663 µg/g
Metylglucosyl (<i>Staphylococcus aureus</i> , <i>Helicobacter pylori</i> , <i>Pseudomonas aeruginosa</i>)	Adamsa et al. (1)	manuka	25-709 mg/kg
	Atrotta and Henlego (5)	manuka	189-835 mg/kg
Phenolic compounds (<i>Staphylococcus aureus</i>)	Świetlikowska et al. (81)	linden	62.00 mg/100 g of honey
		honeydew	10.29 mg/100 g of honey
Lysozyme (<i>Listeria</i> spp., <i>Staphylococcus</i> spp., <i>Streptococcus</i> spp., <i>Pseudomonas</i> <i>aeruginosa</i> , <i>Escherichia coli</i> , <i>Salmonella</i> <i>enteritidis</i> , <i>Proteus vulgaris</i> , <i>Vibrio cholera</i>)	Bąkowska and Janda (8)	acacia	1.5-15.4 µg/g
	Madras-Majewska et al. (54)	linden	2.6-5.0 µg/g
		buckwheat	4.0 µg/g
	Kędzia and Hołderna-Kędzia(42)	honeydew	1.7 µg/g
		multiflorous	7.4 µg/g

acids in Polish honeys, only ferulic acid was present in all samples, and its content depended on the variety and place of origin of honey (honeys from green and conventional apiaries were tested).

Phenolic compounds also include flavonoids: substances acting as pigments and protecting plants against UV radiation and fungal contamination. Flavonoids found in honey include luteolin, kaempferol, quercetin, glycoside-3-O-quercetin and naringenin (91). The occurrence of the individual types of flavonoids depends on the variety and geographic origin of honey. In the research of Pyrzynska and Biesaga (68), kaempferol was present in most samples, regardless of origin, while, for example, galangine was characteristic of Australian and sunflower honeys. There is a very large variety of flavonoids and phenolic acids found in honeys, so it is difficult to clearly determine to what extent they affect the antimicrobial activity.

Methylglyoxal is another chemical factor influencing the antimicrobial activity of honey. This compound occurs in large quantities in the nectar of the *Leptospermum scoparium* plant from New Zealand (1). It is produced by the conversion of dihydroxyacetone (DHA) which is found in significant quantities in the nectar of the tea tree (48). The high content of methylglyoxal in manuka honey gives this variety strong antimicrobial properties, especially targeting Gram-positive and Gram-negative bacteria (40). Studies by French et al. (22) have proven the existence of antimicrobial activity of manuka honey against *Staphylococcus aureus* and *Helicobacter pylori*. The research conducted by Cooper et al. (17) has shown that this variety of honey also inhibits the growth of methicillin-resistant *Staphylococcus aureus* (MRSA), while Kilty et al. (44) have revealed its inhibitory effect on the development of the *Pseudomonas aeruginosa* biofilm (Tab. 1). The methylglyoxal content can vary significantly de-

pending on the type of honey sample obtained. In the studies of Atrott and Henle (5), the methylglyoxal content in manuka honey samples ranged from 189 to 835 mg/kg. Previous studies by Adams et al. (1) have found a similar quantity of this substance in honey of this variety: from 25 to 709 mg/kg (Tab. 1).

One of the biological factors with antimicrobial effect in honey is lysozyme. This substance is a protein found in animals, plants and microbes. Lysozyme is present in saliva, milk, egg white, tears and, of course, honey (23). This enzyme is active in a wide pH range; however, it is sensitive to light (19). Lysozyme has an antimicrobial effect primarily against Gram-positive bacteria, such as *Listeria* spp., *Staphylococcus* spp. or *Streptococcus* spp., because it decomposes bacterial cell walls (23). However, the experiment conducted by Warren et al. (85) has shown that lysozyme also causes the lysis of Gram-negative bacteria such as *Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella enteritidis*, *Proteus vulgaris* and *Vibrio cholerae*. The effect of lysozyme depends on its content in honey, which ranges from 1.5 to 16 µg/ml. Among various type of honey, linden honey is considered to be one of the most abundant in lysozyme (8) (Tab. 1). However, it should be noted that honey dilutions are usually used in medical practice, which significantly reduces the content, and also the effect, of lysozyme.

The last known antimicrobial factor found in honey is defensin-1 peptides found e.g. in Revamil honey, which is obtained in isolated greenhouses in the Netherlands (42). Defensin-1 was initially found in bee hemolymph and in royal jelly and only later it was detected in honey (48). The group of defensins is responsible for immunity in insects and, in the case of *Apis mellifera*, these are defensin-1 and defensin-2, of which the former has stronger antibacterial properties (27). The research by Kwakman et al. (47) has shown that such peptide can completely destroy the cells of many pathogenic bacteria occurring in a concentration of 10⁶-10⁷ specimens per milliliter within 24 hours. Bacteria susceptible to defensin-1 include methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant *Enterococcus faecalis* and

β -lactamase *Escherichia coli*. This peptide also has a strong effect on *Paenibacillus larvae*, the bacteria causing American foulbrood (48).

Indeed, the factors and mechanisms influencing the antimicrobial activity of honey are not only numerous but also very diverse. The role of some of them is still difficult to determine and their effect is difficult to measure. There is no doubt, however, that the physical, chemical and biological factors found in honey make it potentially very effective in combating various microorganisms. This feature of honey can be particularly valuable today, a time when the problem of antimicrobial resistance is significantly aggravating.

Bee honey is therefore equipped with many mechanisms that protect it against the activity of microorganisms. Honey is a substance with bactericidal properties, but nevertheless some microorganisms can be observed in this product (29). Honey microflora is variable and it is influenced by the chemical composition of honey, which in turn depends on the climatic, environmental and anthropogenic conditions (54). Honey can be microbiologically contaminated by pollen, dust, air, water or soil, i.e. primary sources of contamination that are difficult to eliminate (75). Also, microorganisms in the microflora of the digestive system of bees can be transported to honey (72). Secondary sources of contamination occur during honey processing and depend on hygiene practices (3). When extracting and processing honey, particular attention should be paid to the maturity of the honey, as unripe honey may contain pathogenic microorganisms. The number and type of microorganisms say a lot about the quality of honey (54). In honey we can primarily observe spore-producing bacteria, such as *Clostridium perfringens* or *Bacillus spp.* (75). However, the growth of a significant part of bacteria, primarily staphylococci and streptococci, is inhibited by antimicrobial factors found in honey (79). This effect applies to both Gram-positive and Gram-negative bacteria; however, it is diverse, and depends on the species and group (29).

Activity of selected Gram-positive bacteria in the honey environment

In the study of Sinacori et al. (72), the presence of bacteria of the genus *Clostridium perfringens* was observed in about 40% of the samples. They were able to produce enterotoxin at the end of the sporulation stage, which means that in case of unfavorable conditions it will release the toxin along with endospores (75). However, this toxin is not as strong as that produced by *Clostridium botulinum*, bacteria of the same genus (59). According to Wojtacka et al. (90), honey contaminated with spores of *C. botulinum* is one of the causes of botulism in children up to 1 year of age. Infections of infants with *C. botulinum* spores contained in honey have been reported in Europe, including in Portugal, Spain and France (32, 52, 74). Spores of *C. botulinum* introduced with honey into the immature

gastrointestinal tract of infants and young children find favorable conditions for germination and production of neurotoxin (77). In an adult they do not germinate, do not produce toxins and leave the body in feces. The frequency of occurrence of spores of anaerobic bacilli *C. botulinum* in honey largely depends on the country of its origin and on the geographic region of the country. It has been shown that honeys from China and Argentina are the most contaminated with *C. botulinum* spores (12% and 20% of positive tests, respectively) (54). Due to the physicochemical properties of honey, that is low pH and high concentration of sugars, it is not a product in which they can sporulate.

Bacteria of the genus *Bacillus* are aerobic bacilli that are quite common in the natural environment. The optimum temperature for their growth is in the range of 2-50°C, depending on the strain (18). These microorganisms are able to produce spores, i.e. vegetative forms thanks to which they can survive in adverse conditions, such as drought or high temperature. These endospores are most often found in soil and can get to the surface of plants, and from there to the bodies of bees. The insects then transport them to the hive and the spores eventually end up in honey (62). Spores of the genus *Bacillus* are often found in honey (39). Honey can contain *Bacillus cereus*, which in higher doses can cause food poisoning (72), but rarely because the content of these bacteria in honey is small. *Bacillus cereus* produces enterotoxin, but not under all conditions. A pH value between 6 and 8 and a temperature between 6 and 21°C are required for enterotoxin production (75). Such conditions do not occur in honey. Besides, the properties of honey completely inhibit the production of toxins.

Staphylococcus aureus is a bacterium belonging to the genus *Staphylococcus* (64). Two subspecies are distinguished within this species: *Staphylococcus aureus aureus* and *Staphylococcus aureus anaerobius*. This is due to the fact that some bacteria are able to grow only in anaerobic conditions (45). *Staphylococcus aureus* is currently one of the biggest threats to public health, as the bacterium has developed a strong resistance to many antimicrobials. Two main strains can be distinguished within *Staphylococcus aureus*: methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Staphylococcus aureus* (VRSA) (53). The phenomenon of drug resistance contributes to the ever-increasing number of infections with this bacterium, which can cause such diseases as inflammation of the respiratory system or the heart muscle, and even lead to sepsis. *Staphylococcus aureus* have developed several defense mechanisms against antimicrobials. First of all, these bacteria enzymatically inactivate antimicrobials and, in addition, rebuild their cell walls so that the drug cannot penetrate them (60). Further, these bacteria have many virulence factors, the most important of which are enterotoxins which cause, among others, food poisoning (46). Cooper et

al. (17) have showed that manuka honey inhibits the growth of many bacteria, including MRSA, and that these bacteria do not develop resistance to honey. Studies led by George and Cutting (25) have demonstrated that the growth of all strains of *Staphylococcus aureus* (including MRSA) is inhibited by a 4% (v/v) concentration of honey. In the Basualdo et al. (7) study, the growth of this bacterium was inhibited in all the samples. However, subsequent studies show that, despite honey acting against the bacterium, this effect is not strong enough. The growth inhibition diameter was just 12-13 mm in the study by Rahman et al. (69) and almost the same (13.3 mm) in the study by Mahendran and Kumarasamy (55), the latter carried out on honey with the strongest antimicrobial activity from India. A slightly higher value (17.3 mm) was obtained by Nzeako and Hamdi (61). These values are far too small to expect that honey will be effective against *Staphylococcus aureus* in clinical trials. It should be taken into account that honey applied in the treatment of infections is additionally diluted on contact with tissues.

Another representative of the genus *Staphylococcus* is the bacterium *Staphylococcus epidermidis* which colonizes human skin and supports its protective function (45, 63). In some cases, however, this bacterium can have a pathogenic effect, especially in people with immunity dysfunctions (64). In the case of healthy people, *Staphylococcus epidermidis* has an antimicrobial effect due to the bacteriocins they produce, which affect another bacterium of the genus *Staphylococcus*, namely *Staphylococcus aureus* (63). Infections with this bacterium occur in people with immunity impairments, which is a consequence of the increasing antimicrobial resistance of these microbial strains (64, 75). *Staphylococcus epidermidis* produce toxins (mainly PSM proteins) that protect the bacteria against the human immune system (53). Once these bacteria live as commensals, this feature is beneficial, but in a situation of weakened human immunity, it is one of the most important factors in the virulence of this bacterium. In addition, *Staphylococcus epidermidis* bacteria can produce biofilm for adhesion to host tissues, which also poses a risk in the event of infection (64). The effect of honey on this bacterium is relatively rarely studied, probably due to the fact that they are not strictly pathogenic microorganisms, because they only cause opportunistic infections. However, the study by Basualdo et al. (7) has shown that 60% of the tested samples of undiluted honey inhibited the growth of this bacterium, whereas the dilution of the samples resulted in a decline of the antimicrobial effect on *Staphylococcus epidermidis*. Other studies have shown that honey solutions as thin as 3% (v/v) inhibit the growth of this bacterium, depending on its strain and resistance (22). The effect of honey depends on its variety. The study by Alnaimata et al. (2) looked at 18 honeys of different varieties and geographic origins.

It was found that Scottish heather honey has the strongest antimicrobial effect against this bacterium: the growth inhibition diameter was 23.7 mm on average.

Another important, from the public health protection point of view, Gram-positive bacterium is *Listeria monocytogenes* belonging to the *Corynebacteriaceae* family (33). *Listeria monocytogenes* is relatively common in food products and it is resistant to both short-term freezing, pasteurization and some detergents (38). This bacterium is found in the digestive tract of humans, livestock, companion animals, birds, insects, fish and crustaceans (28). *Listeria monocytogenes* is an opportunistic bacterium. It causes listeriosis in people with compromised immunity, diabetes, cirrhosis of the liver and transplants (80). Four clinical forms of the disease can be distinguished: fetal infection, sepsis, central nervous system infection and gastrointestinal infection. Half of the patients with the infection of the nervous system die (49). *Listeria monocytogenes* is a bacterium sensitive to the most important groups of antimicrobials, such as penicillins or tetracyclines, and resistant strains are rare (78). Despite this, alternatives to antimicrobials are being sought. The results of the study of antimicrobial activity of honey against this bacterium are mutually contradictory. In the study by Lee et al. (50), each of the honey varieties tested inhibited the growth of this bacterium, and manuka honey was the most effective. However, in another experiment, conducted by Georgescu et al. (26), no effect of manuka honey on this bacterium was found. The study by Junie et al. (37) has demonstrated that 40% of the honeys tested did not inhibit the growth of *Listeria monocytogenes*, and the inhibition diameter in the remaining tests ranged from 14 to 16.5 mm. In this experiment, multifloral and sunflower honeys had the strongest effect. It has been observed that the antimicrobial activity of honey varies depending not only on the variety because honeys of the same botanical origin can also have different effects on the same species of bacteria.

The next described species of Gram-positive bacteria is *Enterococcus faecalis*, a fecal streptococcus belonging to the genus *Enterococcus*. This microorganism is a facultative anaerobe that retains the ability to grow in the pH range of 4.5 to 9.6 (67). In addition, it can survive at temperatures above 60°C for about 30 minutes and is resistant to high salt concentrations (11). These bacteria are abundant in the natural environment including soil, water, plants and mammalian digestive systems. In healthy people *Enterococcus faecalis* is a commensal organism that produces bacteriocins toxic to other species of Gram-positive and Gram-negative bacteria (67). However, it can cause infection in both immunocompromised and healthy people after getting from the digestive system to the urinary one. *Enterococcus faecalis* is a penicillin-sensitive bacterium but has a natural resistance to dalfopristin (80). In addition, strains of bacteria resistant to vancomycin

and fluoroquinolones, which are very important groups of antimicrobials are also often observed (11). There is little information in the scientific literature about the effect of honey on the growth of *Enterococcus faecalis*. However, the existing few studies show that this impact is not obvious. A study by Khalila et al. (43) has shown that the diameter of inhibition of the growth of this bacterium ranges from 15 to 30 mm, depending on the variety of honey used. On the other hand, Mohapatra et al. (57) did not observe any effect of honey on these microorganisms. Thus, there is a clear need for further research on this subject.

Bactericidal and bacteriostatic effects of various honey varieties against Gram-positive bacteria *Staphylococcus aureus* and *Staphylococcus epidermidis* are a fact. The results of the experiments are important and promising in the context of supporting therapies in cases of infections caused by these microorganisms and combating the problem of their drug resistance. However, the results of various studies on antimicrobial activity of honey against Gram-positive *Listeria monocytogenes* and *Enterococcus faecalis* are mutually contradictory and need to be continued.

Activity of selected Gram-negative bacteria in the honey environment

The *Escherichia coli* bacterium belongs to the *Enterobacteriaceae* family and to one of the most characteristic groups that inhabit the human digestive tract (35). They are commensals and participate in the breakdown of nutrients in the intestine and in the production of vitamins (66). In some cases *Escherichia coli* can cause infections, e.g. in the urinary system (35). Infections with *Escherichia coli* can affect not only the urinary tract but also the stomach and even cerebral meninges (31). Contact with this bacterium is not rare because it is common in the natural environment including water, soil and plants (GIS, 2018). *Escherichia coli* is very sensitive to antimicrobials, such as streptomycin or gentamycin, but in a short time this bacterium becomes resistant to drugs (69). In addition, it is a model bacterial organism, which is why the effect of various substances on *Escherichia coli* is often studied. Chanchao et al. (14) investigated the antimicrobial activity of Thai honey against this bacterium depending on the concentration of honey in the solution. Experience has shown that undiluted honeys have the strongest effect and April honey produces the largest growth inhibition zone: 40.3 mm. Another study by Chanchao (12, 13) has demonstrated that a 25% solution of Thai honey has no inhibiting effect on the growth of *Escherichia coli*. This effect appeared only in a 50% solution and the inhibition radius was 21.4 mm. The studies by Wilkinson and Cavanagh (89) have shown that a 10% honey solution produced a growth inhibition zone of 21.1 mm. In contrast, in the studies of Nzeako and Hamdi (61) no inhibitory effect was observed at a 10% honey concentration, and

for undiluted honeys the growth inhibition zone was 18.2 mm on average.

Bacteria of the *Salmonella* genus belong to the family *Enterobacteriaceae*, a group of bacteria sharing similar morphological and physiological features (35). The *Salmonella* genus comprises two species: *Salmonella enterica* and *Salmonella bongori* (34). *Salmonella* is present mainly in the digestive tract of both humans and animals but its presence has also been noted in reptiles and insects (4). An infection with *Salmonella* can lead to serious health disorders, so antimicrobial treatment is used in such cases. Currently, however, the resistance of the bacteria to antimicrobials from the third generation cephalosporin group, which are most often used for salmonellosis, has become a serious problem (35). Due to this, interest in other substances that can fight this type of microorganism is gradually increasing. Honey could be one of such substances. *Salmonella* bacteria require an environment with a water activity ratio of more than 0.93 a_w to grow (4). Honeys have a much lower water activity, so they can significantly inhibit the multiplication of these bacteria. In the study by Mandalla (56), the effect of inhibiting the growth of *Salmonella enterica* bacteria appeared in a honey solution of just 1% (v/v), while 3% (v/v) solutions turned out to be fully effective inhibitors of the growth of this bacterium.

The bacterium of the *Pseudomonas aeruginosa* species is a mobile aerobic bacillus with low nutritional requirements (84). This bacterium is considered an opportunistic pathogen causing infections in people with weakened immunity, such as transplant patients, for example. The blue pus bacillus produces numerous toxins and enzymes that slow down the immune response, destroy host cells and tissues, as well as weaken the microflora of the attacked organism (83). The most important from the point of view of the pathogenicity of this bacterium is exotoxin A, which works by inhibiting protein synthesis (73). The blue pus bacillus is resistant to numerous antimicrobials, such as aminopenicillins, cephalosporins, tetracyclines and chloramphenicol (83). Their resistance is based on mucus produced by the bacteria, which protects them from antimicrobials and disinfectants (82). The resistance of this bacterium to antimicrobials is not only based on natural resistance but also on acquired resistance. Therefore, *Pseudomonas aeruginosa* acquire resistance to subsequent antimicrobials over time, and this in turn can lead to infections that are impossible to cure. Studies on honey show that this main bee product can prove effective in the fight against the blue pus bacillus. A study by Kilty et al. (44) has demonstrated that manuka honey has an inhibitory effect on the biofilm of *Pseudomonas aeruginosa*, as well as on its planktonic forms. In the experiment of Mandalla (56), honey in a concentration of 1.5% (v/v) showed an inhibitory effect on the growth of this bacterium, while a 4% (v/v) honey solution caused complete inhibition of its growth. Research

by George and Cutting (25) has shown that the effect of inhibiting the growth of all strains of *Pseudomonas aeruginosa* occurs with a honey concentration of 8% (v/v). The zone of inhibition of growth of these bacteria was 18.3 mm for undiluted honey (61) and null for 10% solutions. In contrast, research by Cooper et al. (16) has proven that natural honey is 2.5-2.8 times more effective than artificial honey in inhibiting the growth of *Pseudomonas aeruginosa* and, in addition, manuka honey and honey obtained from pastures had an inhibitory effect even after thinning to 10%.

It has been proven that honeys are effective against all these gram-negative bacteria and the effect largely depended on honey variety, geographic origin and concentration. Indeed, the mechanisms of the antimicrobial effect of honey can be beneficial in the context drug resistance of bacteria of the *Escherichia coli*, *Salmonella enterica* and *Pseudomonas aeruginosa* species.

The foregoing literature review shows that bee honey is equipped with many mechanisms protecting it against the growth of bacteria. The mechanisms of the antimicrobial effect of honey definitely include the high osmotic pressure, which is a result of the high content of sugars, as well as low pH caused by the presence of organic acids. Also lysozyme, the antimicrobial substance found in honey, contributes to this effect. However, it is hydrogen peroxide (a compound with strong antimicrobial properties) formed as a result of the enzymatic reaction of glucose oxidase that adds most to the strong antimicrobial activity of this bee product. An additional factor preventing the development of microorganisms in some honeys is the high content of phenolic compounds. Based on the current state of knowledge, it can also be said that another important antimicrobial factor is methylglyoxal which is found in the largest amounts in manuka honey. The last of the known mechanisms protecting honey against the growth of bacteria is defensin-1, a peptide found, for example, in Revamil honey. From the literature data, it can be concluded that the mechanisms and factors of the antimicrobial effect of honey are very diverse. The physical, chemical and biological features of honey make it very effective in combating a number of microorganisms. Antimicrobial factors of honey are crucial for the high bactericidal and bacteriostatic potential of this product. Therefore, the growth of both Gram-positive and Gram-negative bacteria can potentially be inhibited by the effect of honey, which is particularly valuable in the context of the growing problem of drug resistance of bacteria and the limitations of antimicrobial therapies.

Based on the presented research results, it was observed that the antimicrobial effect of honey differs depending not only on its variety, geographic origin (sometimes honeys of the same botanical origin can have different effects on the same species of bacteria) and the concentration of honey solution, but also on the type of strain tested and bacterial resistance.

Bactericidal and bacteriostatic effects of various varieties of honey on certain Gram-positive bacteria have been confirmed. The strongest antimicrobial effect against *Staphylococcus aureus* was shown by manuka honey. In contrast, Scottish heather honey inhibited the growth of *Staphylococcus epidermidis*. The antimicrobial activity of honey towards Gram-negative bacteria has also been demonstrated. Antimicrobial effects of undiluted Thai honey against *Escherichia coli* bacteria have been observed. The effect of complete inhibition of the growth of bacteria of the *Salmonella enterica* species was obtained in 3% (v/v) solutions of honey. Manuka honey has also been found to have an inhibitory effect on the biofilm of *Pseudomonas aeruginosa*. The high level of antimicrobial activity of honey against these bacteria species is a potential opportunity to support therapies in cases of infections caused by these microorganisms, which can help fight the problem of drug resistance. However, further research on this subject is required to confirm the foregoing conclusions. It is also important to expand the spectrum of studies of the impact of honey on bacteria species where the results of the study of antimicrobial activity of honey are mutually contradictory, e.g. for Gram-positive *Listeria monocytogenes*, *Enterococcus faecalis*. The spectrum of research should also take into account microbes that have not developed drug resistance yet. The possibility of inhibiting or limiting the development of pathogenic bacteria without resorting to antimicrobials would also have a mitigating impact on the growing problem of antimicrobial resistance. In addition, it should be noted that New Zealand manuka honey is currently considered to be the honey with the strongest antimicrobial effect and this variety is most often studied by researchers. However, alternative varieties of honey with a different geographic origin should be sought, both for economic reasons and for the limited supply of manuka honey. Yet, without questioning the benefits of honey, it is important to remember that it can be contaminated with *Clostridium botulinum* or *Bacillus cereus* spores.

References

1. Adams C. J., Boulton C. H., Deadman B. J., Farr J. M., Grainger M. N., Manley-Harris M., Snow M. J.: Isolation by HPLC and characterization of the bioactive fraction of New Zealand manuka (*Leptospermum scoparium*) honey. *Carbohydr. Res.* 2008, 345, 651-659.
2. Alnaimat S., Wainwright M., Al'Abri K.: Antibacterial potential of honey from different origins: a comparison with manuka honey. *J. Microbiol. Biotechnol. Food Sci.* 2012, 5, 1328-1338.
3. Al-Waili N. S., Salom K., Butler G.: Honey and microbial infections: a review supporting the use of honey for microbial control. *J. Med. Food* 2011, 14, 1079-1096.
4. Andino A., Hanning I.: *Salmonella enterica*: Survival, Colonization, and Virulence Differences among Serovars. *Sci. World J.* 2015, 1-16.
5. Atrott J., Henle T.: Methylglyoxal in Manuka Honey – Correlation with Antibacterial properties. *Czech J. Food Sci.* 2009, 27, 163-165.
6. Basista K.: Właściwości biologiczne i prozdrowotne miodu oraz jego zastosowanie jako zdrowa żywność. *Gazeta Farmaceutyczna* 2013, 3, 26-28.
7. Basualdo C., Sgroy V., Finola M. S., Marioli J. M.: Comparison of the antibacterial activity of honey from different provenance against bacteria usually isolated from skin wounds. *Vet. Microbiol.* 2007, 124, 375-381.

8. Bąkowska M., Janda K.: Właściwości prozdrowotne wybranych miódów. *Pomeranian J. Life Sci.* 2018, 64, 147-151.
9. Bogdanov S.: Nature and Origin of the Antibacterial Substances in Honey. *LWT – Food Sci. Technol.* 1997, 30, 748-753.
10. Borawska M., Arciuch L., Puścion-Jakubik A., Lewoc D.: Zawartość cukrów (fruktozy, glukozy i sacharozę) i proliny w różnych odmianach naturalnych miódów pszczołach. *Probl. Higieny Epidemiol.* 2015, 96, 816-820.
11. Chajęcka-Wierzchowska W., Zadernowska A., Laniewska-Trokenheim L.: Oporność na antybiotyki bakterii z rodzaju *Enterococcus* występujących w żywności. *Kosmos* 2017, 66, 67-79.
12. Chanchao C.: Antimicrobial activity by trigonalaveiceps (stingless bee) honey from Thailand. *Pak. J. Med. Sci.* 2009, 25, 364-369.
13. Chanchao C.: Properties and antimicrobial activity of *Apis dorsata* honey from Thailand. *Pak. J. Med. Sci.* 2009, 25, 313-318.
14. Chanchao C., Sintara K., Wongsiri S.: Comparison of antibiotic and organoleptic properties of honey from various plant sources in Thailand. *J. Apic. Sci.* 2006, 50, 59-63.
15. Chirife J., Zamora M. C., Motto A.: The correlation between water activity and % moisture in honey: fundamental aspects and application to Argentine honeys. *J. Food Eng.* 2006, 72, 1-22.
16. Cooper R. A., Halas E., Molan P. C.: The Efficacy of Honey in Inhibiting Strains of *Pseudomonas aeruginosa* From Infected Burns. *J. Burn Care Res.* 2002, 23, 366-370.
17. Cooper R. A., Jenkins L., Henriques A. F., Duggan R. S., Burton N. F.: Absence of bacterial resistance to medical-grade manuka honey. *Eur. J. Clin. Microbiol. Infect. Dis.* 2010, 29, 1237-1241.
18. Czubkowska A., Rola J. G., Osek J.: *Bacillus cereus* – istotny czynnik zatruc pokarmowych u ludzi. *Med. Weter.* 2013, 69, 399-402.
19. Dubiago G., Nowak A., Klimowicz A.: Wybrane właściwości miodu szczególnie przydatne w kosmologii. *Post. Fitoter.* 2018, 19, 58-64.
20. Dustman J. H.: Messung von Wasserstoffperoxid und Enzymaktivität in mitteleuropäischen Honigen. *Z. Bienenforsch.* 1967, 9, 66-73.
21. Finola M. S., Lasagno M. C., Marioli J. M.: Microbiological and chemical characterization of honeys from central Argentina. *Food Chem.* 2007, 100, 1649-1653.
22. French V. M., Cooper R. A., Molan P. C.: The antibacterial activity of honey against coagulase-negative staphylococci. *J. Antimicrob. Chemother.* 2005, 56, 228-231.
23. Gajda E., Bugla-Płoskońska G.: Lizozym – występowanie w przyrodzie, właściwości biologiczne i możliwości zastosowań. *Post. Hig. Med. Dośw.* 2014, 68, 1501-1515.
24. Gallardo-Chancón J. J., Caselles M., Izquierdo-Pulido M.: Inhibitory activity of monofloral and multifloral honeys against bacterial pathogens. *J. Apicult. Res. Bee World* 2008, 47, 131-136.
25. George N. M., Cutting K. F.: Antibacterial Honey (Medihoney™): in-vitro Activity Against Clinical Isolates of MRSA, VRE, and Other Multiresistant Gram-negative Organisms Including *Pseudomonas aeruginosa*. *Wounds* 2007, 19, 231-236.
26. Georgescu M., Dobrea M., Dobrea V. C.: Antimicrobial effect of commercial manuka honey and conventional local honey against Gram-negative and Gram-positive bacteria. *Vet. J.* 2017, 2, 133-136.
27. Gliński Z., Buczek K., Marć M.: Zjawiska i mechanizmy odporności przeciwzakaznej pszczoły miodnej – nowe osiągnięcia. *Życie Wet.* 2011, 86, 687-694.
28. Gliński Z., Kostro K.: Listerioza współczesnym zagrożeniem. *Życie Wet.* 2012, 87, 577-581.
29. Grabowski N. T., Klein G.: Microbiology and foodborne pathogens in honey. *Crit. Rev. Food Sci. Nutr.* 2017, 57, 1852-1862.
30. Haniyeh K., Seyyed M. S., Hussein M.: Preliminary study on the antibacterial activity of some medicinal plants of Khuzestan (Iran). *Asian Pac. J. Trop. Med.* 2010, 3, 180-184.
31. Harrington S. M., Dudley E. G., Nataro J. P.: Pathogenesis of enteroaggregative *Escherichia coli* infection. *FEMS Microbiology Letters* 2005, 254, 12-18.
32. Hoarau G., Pelloux I., Gayot A., Wroblewski I., Popoff M. R., Mazuet C., Maurin M., Croizé J.: Two cases of type a infant botulism in Grenoble, France: No honey for infants. *Eur. J. Pediatr.* 2012, 171, 589-591.
33. Holderna-Kędzia E., Kędzia B.: Badania nad przeciwtleniającymi właściwościami miodu pszczelego. *Acta Agrobotanica* 2006, 59, 265-269.
34. Hoszowski A., Wasyl D.: Taksonomia i nomenklatura rodzaju *Salmonella*. *Med. Weter.* 2000, 56, 75-78.
35. Jarzab A., Górska-Frączek S., Rybka J., Witkowska D.: Zakażenia pałeczkami jelitowymi – diagnostyka, oporność na antybiotyki i profilaktyka. *Post. Hig. Med. Dośw.* 2011, 65, 55-72.
36. Jasicka-Misiak I., Kafarski P.: Chemiczne markery miódów odmianowych. *Wiad. Chem.* 2011, 65, 821-837.
37. Junie L. M., Vica M. L., Glevitzky M., Matei H. V.: Physico-chemical characterisation and antibacterial activity of different types of honey tested on strains isolated from hospitalized patients. *J. Apic. Sci.* 2016, 60, 5-17.
38. Jurkiewicz A., Oleszczak-Momot W.: *Listeria monocytogenes* jako problem zdrowia publicznego. *Med. Ogólna Nauki Zdr.* 2015, 21, 29-32.
39. Kačániová M., Hleba L., Džugan M., Pasternakiewicz A., Kňazovická V., Pavelková A., Felsöciová S., Petrová J., Rovná K., Kluz M., Grabek-Lejko D.: Microbiological properties and antimicrobial effect of Slovakian and Polish honey having regard to the water activity and water content. *J. Microbiol. Biotechnol. Food Sci.* 2012, 2, 272-281.
40. Kędzia B., Holderna-Kędzia E.: Aktywność antybiotyczna miodu manuka i jego działanie na drobnoustroje chorobotwórcze dla człowieka. *Post. Fitoter.* 2015, 4, 56-60.
41. Kędzia B., Holderna-Kędzia E.: Działanie przeciwdrobnoustrojowe roślinnych pochodnych fenolu. *Post. Fitoter.* 2012, 3, 151-155.
42. Kędzia B., Holderna-Kędzia E.: Współczesne poglądy na mechanizm przeciwdrobnoustrojowego działania miodu. *Post. Fitoter.* 2017, 18, 290-297.
43. Khalil A. T., Khan I., Ahmad K., Khan Y. A., Khan J., Shinwari Z. K.: Antibacterial activity of honey in north-west Pakistan against select human pathogens. *J. Tradit. Chin. Med.* 2014, 34, 86-89.
44. Kilty S. J., Duval M., Chan F. T., Ferris W., Slinger R.: Methylglyoxal: (active agent of manuka honey) in vitro activity against bacterial biofilms. *Intern. Forum Allergy Rhinology* 2011, 5, 348-350.
45. Kmieciak W., Szewczyk E. M.: Gatunki koagulazododatnie rodzaju *Staphylococcus* – taksonomia, chorobotwórczość. *Post. Mikrobiol.* 2017, 56, 233-244.
46. Korpysa-Dzirba W., Rola J. G., Osek J.: Enterotoksyny gronkowcowe. Część I. Epidemiologia i znaczenie dla zdrowia publicznego. *Życie Wet.* 2012, 87, 695-697.
47. Kwakman P. H. S., de Boer L., Ruyter-Spira C. P., Creemers-Molenaar T., Helsper J. P. F. G., Vandenbroucke-Grauls C. M. J. E., Zaai S. A. J., te Velde A. A.: Medical-grade honey enriched with antimicrobial peptides Has enhanced activity against antibiotic-resistant pathogens. *Eur. J. Clin. Microbiol. Infect. Dis.* 2011, 30, 251-257.
48. Kwakman P. H. S., Zaai S. A. J.: Antibacterial components of honey. *Life* 2012, 64, 48-55.
49. Kwiatek K., Kukier E., Goldsztejn M.: Normy metodyczne w badaniach mikrobiologicznych łańcucha żywnościowego. *Życie Wet.* 2014, 89, 519-523.
50. Lee H., Churey J. J., Worobo R. W.: Antimicrobial activity of bacterial isolates from different floral sources of honey. *Int. J. Food Microbiol.* 2008, 126, 240-244.
51. Lihu Y., Yueming J., Singanusong R., Datta N., Raymond K.: Phenolic acids and abscisic acid in Australian Eucalyptus honeys and their potential for floral authentication. *Food Chem.* 2004, 86, 169-177.
52. López-Laso E., Roncero-Sánchez-Cano I., Arce-Portillo E., Ley-Martos M., Aguirre-Rodríguez J., García-Ron A., Mora-Navarro D., Méndez-García M., Camino-León R.: Infant botulism in Andalusia (Southern Spain). *Eur. J. Pediatr. Neurol.* 2014, 18, 321-326.
53. Lusby P. E., Coombes A. L., Wilkinson J. M.: Bactericidal activity of different honeys against pathogenic bacteria. *Arch. Med. Res.* 2005, 36, 464-479.
54. Madras-Majewska B., Rosiak E., Zajczkowska J.: Ocena aktywności mikrobiologicznej wybranych miódów. 51. Naukowa Konferencja Pszczelarska, Szczyrk 2014, 115-116.
55. Mahendran S., Kumarasamy D.: Antimicrobial activity of some honey samples against pathogenic bacteria. *Int. Lett. Nat. Sci.* 2015, 7, 15-20.
56. Mandal S., Mandal M. D.: Honey: its medicinal property and antibacterial activity. *Asian Pac. J. Trop. Biomed.* 2011, 2, 154-160.
57. Mohapatra D. P., Thakur V., Brar S. K.: Antibacterial Efficacy of Raw and Processed Honey. *Biotechnol. Res. Int.* 2011, 32, 1-6.
58. Molan P. G.: The antibacterial activity of honey. 2. Variation in the potency of the antibacterial activity. *Bee World* 1992, 73, 59-76.
59. Nevas M., Lindstrom M., Hautamaki K., Puoskari S., Korkeala H.: Prevalence and diversity of *Clostridium botulinum* types A, B, E and F in honey produced in the Nordic countries. *Int. J. Food Microbiol.* 2005, 105, 145-151.
60. Nowakowicz-Dębek B., Wlazło Ł., Kasela M., Ossowski M.: Epidemiologia wielolekoopornych szczepów *Staphylococcus aureus*. *Probl. Hig. Epidemiol.* 2016, 97, 106-112.
61. Nzeako B. C., Hamdi J.: Antimicrobial potential of honey on some microbial isolates. *Medical Sci.* 2001, 2, 75-79.
62. Olaitan P. B., Adeleke O. E., Ola I. O.: Honey: a reservoir for microorganisms and an inhibitory agent for microbes. *Afr. Health Sci.* 2007, 7, 159-165.
63. Podgórska B., Kędzia D.: Dwa oblicza bakterii *Staphylococcus epidermidis*, czyli od komensala do patogenu. *Post. Mikrobiol.* 2018, 57, 338-347.
64. Podkowik M., Bania J., Schubert J., Bystron J.: Gronkowce koagulazoujemne: nowe zagrożenie dla zdrowia publicznego? *Życie Wet.* 2014, 89, 60-66.

65. *Popek S.*: Studium identyfikacji miódów odmianowych i metodologii oceny właściwości fizykochemicznych determinujących ich jakość. Wyd. AE w Krakowie, Kraków 2001.
66. *Półtorak K., Wieczorek K., Osek J.*: Patogenne *Escherichia coli* – mechanizmy chorobotwórczości. *Med. Weter.* 2016, 72, 352-357.
67. *Pražmo E., Godlewska R., Kwaśny M., Mielczarek A.*: Udział czynników wirulencji *Enterococcus faecalis* w rozwoju chorób miazgi i tkanek okołowierzchołkowych. *Post. Mikrobiol.* 2016, 55, 247-254.
68. *Pyrzynska K., Biesaga M.*: Analysis of phenolic acids and flavonoids in honey. *Trends Anal. Chem.* 2009, 28, 893-902.
69. *Rahman M. M., Richardson A., Sofian-Azirun M.*: Antibacterial activity of propolis and honey against *Staphylococcus aureus* and *Escherichia coli*. *Afr. J. Microbiol. Res.* 2010, 16, 1872-1878.
70. *Ratiu I. A., Al-Suod H., Bukowska M., Ligor M., Buszewski B.*: Correlation study of honey regarding their physicochemical properties and sugars and cyclitols content. *Molecules* 2020, 25, 34.
71. Rozporządzenie Ministra Rolnictwa i Rozwoju Wsi z dnia 3 października 2003 r. w sprawie szczegółowych wymagań w zakresie jakości handlowej miodu, Dz. U. Nr 181, poz. 1773.
72. *Rudnicka K., Kwiatkowska P., Gajewski A., Chmiela M.*: Mikroflora miodu jak źródło spor *C. Botulinum* i przyczyna rozwoju botulizmu niemowląt – rozważania na temat zasadności oczyszczania miodu w kontekście obowiązującego prawa. *Post. Mikrobiol.* 2015, 54, 184-194.
73. *Rusiecka-Ziółkowska J., Fleischer M., Staroszczyk J.*: Właściwości immunologiczne Gram-ujemnych pałeczek *Pseudomonas aeruginosa*. *Post. Hig. Med. Dośw.* 2007, 61, 95-98.
74. *Saraiva M., Campos Cunha I., Costa Bonito C., Pena C., Toscano M., Teixeira Lopes T., Sousa I., Calhau A.*: First case of infant botulism in Portugal. *Food Control* 2012, 26, 79-80.
75. *Silva M. S., Rabadzhiev Y., Eller M. R., Iliev I., Ivanova I., Santana W. C.*: Microorganisms in Honey, [in:] *Honey Analysis*. In Tech, London 2017, 233-258.
76. *Sinacori M., Francesca N., Alfonso A., Cruciatu M., Sannino C., Settami L., Moschetti M.*: Cultivable microorganisms associated with honeys of different geographical and botanical origin. *Food Microbiol.* 2014, 38, 284-294.
77. *Sobel J.*: Botulism. *Clin. Infect. Dis.* 2005, 41, 1167-1173.
78. *Soltysiuk M. M., Sztajn J., Wiszniewska-Łaszczyc A.*: Bakterie z rodzaju *Listeria* zagrożeniem dla zdrowia ludzi i zwierząt. *Med. Weter.* 2019, 75, 214-220.
79. *Stawiarz E., Dyduch J.*: Zastosowanie produktów pszczelich pochodzenia roślinnego w apiterapii. *Episteme, Lublin* 2014, 25, 111-127.
80. *Szczypta A., Talaga K., Bulanda M.*: Enterokoki odporne na wankomycynę jako czynniki etiologiczne zakażeń związanych z opieką zdrowotną – chorobotwórczość i metody kontroli. *Hygiene Public Health* 2016, 51, 134-140.
81. *Świetlikowska K., Hallmann E., Sławińska J., Rembiałkowska E.*: Ocena zawartości związków polifenolowych ogółem, w tym kwasów fenolowych i flawonoidów w różnych odmianach miódów ekologicznych i konwencjonalnych. *Post. Tech. Przetw. Spoż.* 2013, 2, 65-69.
82. *Tomczak H., Wierzbicka M., Okrutnik D., Bartochowska A., Luś A., Horla A., Szyfter W.*: Problematyka narastania oporności *Pseudomonas aeruginosa* u pacjentów hospitalizowanych w Klinice Otolaryngologii i Onkologii Laryngologicznej Uniwersytetu Medycznego w Poznaniu w latach 2005-2007. *Otolaryngologia Polska LXII*, 2008, 5, 593-597.
83. *Urbanowicz P.*: Skomplikowany *Pseudomonas aeruginosa* – portret niezwykle groźnej bakterii. *Aktualności Narodowego Programu Ochrony Antybiotyków* 2016, 2, 1-4.
84. *Urbanowicz P., Gniadkowski M.*: „Ciężkozbrojny” *Pseudomonas aeruginosa*: mechanizmy lekooporności i ich tło genetyczne. *Kosmos* 2017, 66, 11-29.
85. *Warren G. H., Gray J., Bartell P.*: The lysis of *Pseudomonas aeruginosa* by lysozyme. *J. Bacteriol.* 1955, 70, 614-619.
86. *Weston R. J., Brocletbank L. K., Yinrong L.*: Identification and quantitative levels of antibacterial components of some New Zealand honeys. *Food Chem.* 2000, 70, 427-435.
87. *White J. W.*: Inhibine and glucose oxidase in honey – a review. *Am. Bee J.* 1966, 106, 214-216.
88. *Wilczyńska A., Przybyłowski P.*: Charakterystyka związków fenolowych zawartych w miódach. *Zesz. Nauk. Akademii Morskiej w Gdyni* 2009, 61, 33-38.
89. *Wilkinson J. M., Cavanagh H. M. A.*: Antibacterial Activity of 13 Honeys Against *Escherichia coli* and *Pseudomonas aeruginosa*. *J. Med. Food* 2005, 8, 100-103.
90. *Wojtacka J., Gomółka-Pawlicka M., Liedtke K., Uradziński J.*: Jakość i higiena miodu. *Med. Weter.* 2014, 70, 719-724.
91. *Wybranowski T., Ziolkowska B., Kruszewski S.*: Antioxidant properties of flavonoids and honeys studied by optical spectroscopy methods. *Med. Biol. Sci.* 2013, 27, 53-58.
92. *Zamora M. C., Chirife J., Roldan D.*: On the nature of the relationship between water activity and % moisture in honey. *Food Control* 2006, 17, 642-647.

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