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Bee Products and Essential Oils as Alternative Agents for Treatment of Infections Caused by *S. aureus*

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Additional information is available at the end of the chapter

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Abstract

Bacteria of the genus *Staphylococcus* are important human and veterinary pathogens. A crucial characteristic for this group of bacteria is that they can easily acquire mechanisms of antibiotic resistance for a plethora of antibiotics currently in use for human and animal therapies. Therefore, there is a great need to find novel, non-antibiotic chemotherapeutics with marked antistaphylococcal activity. Promising but still underestimated group of potential antistaphylococcal chemotherapeutics constitute bee products: honey, pollen, royal jelly, fermented pollen and especially propolis. Another group of natural products that exhibit promising antibacterial activity is essential oils. Usefulness of bee products and essential oils in the treatment of infections caused by *S. aureus* has been confirmed by results of many investigations carried out by researches in different regions of the world. In this chapter, we have presented the review of publication in this area as well as perspectives and limitations of future applications of these two groups of natural products.

Keywords: *Staphylococcus aureus*, resistance, bee products, honey, propolis, pollen, fermented pollen, essential oils

1. Introduction

1.1. Staphylococci: important human and veterinary pathogens

Staphylococcus aureus is a species of bacteria commonly found in many ecological niches i.e., in soil, water and public places. These bacteria also colonize the skin and mucosal surfaces of humans and also several animal species. The most important site of *S. aureus* colonization is anterior nares of the nose, but these bacteria are also often isolated from the pharynx, perineum and axilla [1–3]. It was revealed in several independent studies that persistent

colonization with *S. aureus* is observed in approximately 20% of human population, about 30% carry these microorganisms transiently and 50% are non-carriers [3–5]. *S. aureus* similarly as several other species of the genus *Staphylococcus* are classified as commensal Gram-positive bacteria. Like other commensal microorganisms, staphylococci have the ability to cause disease under certain conditions. It should be noted, however, that *S. aureus* is definitely one of the most dangerous commensal bacteria which colonize any part of the human body. Its high pathogenicity is based on production of a wide array of virulence factors such as protein A, coagulase, collagenase, hyaluronidase, hemolysins, lipases, multiple toxins, adhesive proteins and also proteins involved in biofilm formation. The ability to express these virulence factors has been confirmed not only for clinical isolates of *S. aureus*, but also for strains isolated from animal sources, e.g. bovine mastitis [6, 7] and food [8, 9]). All these isolates are potentially dangerous human pathogens. *S. aureus* has also developed several mechanisms that enable them escape from protective immune responses of infected humans or animals. Among them, protein A (SpA), staphylokinase and staphylococcal binder of immunoglobulin are the most important and the best characterized [10]. Another crucial characteristic for this group of bacteria is that they can easily acquire mechanisms of antibiotic resistance for a plethora of antibiotics currently in use for human and animal therapies. Especially important problem is rapidly growing number of isolation of strains resistant to methicillin, designated as *Methicillin Resistant Staphylococcus aureus* (MRSA). MRSA isolates are resistant to all β -lactam antibiotics. Moreover, some of them are classified as multidrug resistant (MDR)—not susceptible to the antibiotics that belong to various chemical groups. The MDR phenotype is usually associated with strains recovered from medical environment—*Healthcare Associated MRSA* (HA-MRSA). The drugs of choice for treatment of MRSA infections are glycopeptides, mainly vancomycin. Unfortunately, staphylococci developed also resistance to this group of antibiotics, and prevalence of isolation of vancomycin-intermediate *S. aureus* (VISA) and vancomycin-resistant *S. aureus* (VRSA) strains is constantly growing. The number of other antibiotics, which could be used for eradication of HA-MRSA infections, is very limited. In fact, only three antibiotics recently introduced to routine medicine procedures are effective in treatment of HA-MRSA infections: from the oxazolidinone group—linezolid; streptogramin group—quinupristin/dalfopristin, and from the glycyclcycline group—tigecycline [11–13]. HA-MRSA are also usually susceptible to rifampicin, fusidic acid and co-trimoxazole; however, the last one does not give results good enough in treatment of acute infections, and in the case of two other drugs, resistance is easily acquired by treated bacteria [12–14]. The problem regarding therapy of staphylococci-related infections is additionally enhanced by the staphylococcal ability to form biofilm. Some of the previous studies proved that the biofilm-forming bacteria (including *S. aureus*) may be in fact 10–1000 times more resistant to antimicrobial agents than the same cells growing in a planktonic manner [15, 16].

Staphylococci also belong to the most important animal pathogens. Infections caused by these bacteria leads to huge economic losses in agriculture and food industry. Contamination of food products by these bacteria is a serious issue due to their completely lack of susceptibility to lysozyme and very low susceptibility to nisin, two important agents used as preservatives in food industry [17, 18].

1.2. Natural products as potential antimicrobial agents

All presented above aspects (the prevalence of the staphylococci in the environment, their high virulence potential and first of all rapid increase in antibiotic resistance) clearly indicate that there is an urgent need to develop new, effective inexpensive and not covered by current existing mechanisms of resistance antistaphylococcal agents. Interesting groups of antimicrobials, which meet all these expectations and could be used for treatment of *S. aureus* human and animal infections, but also for protection of food products against contamination by these bacteria, are bee products (honey, propolis, pollen, bee bread and royal jelly) and obtained from a broad spectrum of plant sources, essential oils (EO). Both these groups of products should be classified as well-known but rarely used antimicrobial agents. For centuries, herbs, bee products, venoms of some of animals (snakes and spiders) and other natural products were the only medicines that people knew and used. In some cases, the effects of the so-called folk/traditional medicine were surprisingly good, also in the case of therapy of infections. Unfortunately, from the beginning of “antibiotic era”, they were nearly completely eliminated from clinical. One of the most spectacular examples of successful application of natural products for treatment infections is discovered by Prof. Tu Youyou, artemisinin. Artemisinin and its semi-synthetic derivatives exhibit the most rapid action against *Plasmodium falciparum* and are now standard treatment worldwide for *P. falciparum* malaria. For the discovery of artemisinin, Professor Youyou received the Prize of Nobel in 2015. The success of Prof. Youyou clearly confirms the high therapeutic potential of natural products, and it is also a good occasion for promotion of other natural products that exhibit antimicrobial activity but in fact are nearly completely forgotten, or rather eliminated, from clinical practice.

The main purpose of the preparation of this chapter is to demonstrate that bee and plant products are still interesting and promising group of antimicrobials. Their antibacterial activity is not only a matter of history from the pre-antibiotic era. Their large antimicrobial potential was confirmed with modern microbiological methods and analytical techniques and is well-documented in many scientific publications.

2. Antimicrobial activity of bee products

2.1. Honey

2.1.1. Basic information and mechanisms of antimicrobial activity

From ancient times, honey was a very valued component of the diet. This is mainly due to its characteristic sweet taste. The main uses of honey are as follows: as a spread on bread, a sweetener for tea, milk and coffee, for preparing desserts and cakes. Recently, it has been also proposed to use honey as a component of healthy, high energetic, beverages. The sweet taste of honey is apparent from its chemical composition. Sugars, mainly fructose and glucose, and minor amounts of oligosaccharides account for about 80% of its weight. The centuries of observations have also shown that regular consumption of honey is beneficial to the health of consumer. Therefore, this product was widely used as drug in traditional medicine, one

of the most important except of herbs. The research carried out during several last decades finally confirmed that honey (and also other bee products) is a beneficial agent in treatment of a wide spectrum of human diseases, such as some cardiovascular and gastrointestinal tract disorders, infections within upper respiratory tract including cough, as well as in healing of infected wounds [19]. While folk medicine was based only on tradition and experience, the achievements of modern science and medicine have found confirmation of the therapeutic potential of honey, as the consequence of its chemical composition. It has been revealed that crucial for therapeutic properties of honey are its non-sugar components such as: enzymes, peptides, free amino acids, vitamins, organic acids, flavonoids, phenolic acids and other phytochemicals, minerals [20]. Especially interesting subject seems to be its antimicrobial activity. As it was mentioned above, this product was successfully used in treatment of skin and soft tissue infections and for elimination of pathogens infecting mucosal of respiratory tract. In all these situations, physical contact of honey (its components) with infected tissue (microorganisms infecting the tissue) is possible. The antimicrobial action of honey is based on several mechanisms: the acidity (low pH, usually in the range from 3.4 to 6.1), osmotic pressure of sugars present in honey and the presence of bacteriostatic and bactericidal substances such as H_2O_2 , antioxidants, lysozyme, polyphenols, phenolic acids, flavonoids, methylglyoxal and bee peptides [21–24]. The crucial component that is responsible for the antimicrobial activity of majority of the honey types is hydrogen peroxide, which is formed as a side product of the oxidation of glucose by glucose oxidase—the enzyme which is introduced to the honey from the salivary glands of bees. Interestingly glucose oxidase is inactive in non-diluted honey [25]. The inhibition of growth of bacteria in honey, at the original concentration of this product, is mainly caused by high concentration of sugars (high osmotic pressure) coupled with high acidity [21, 22]. Therefore, it can be stored for long period of time (at least 2 years) without any additional treatment or supplementation with preservatives. When honey is diluted to certain extent, its antibacterial activity is shifted from osmotic- and pH-dependent to peroxide-dependent mechanism of action based on the generation of H_2O_2 [26]. This scenario takes place when honey is used for treatment of infections, e.g. infected wound. On the basis of current state of knowledge, it can be said that other mentioned above components of honey, mainly phytochemicals, seem to only support the antibacterial effect of generated H_2O_2 . The dominant role of the enzyme in the antimicrobial activity of several polish unifloral honeys was recently confirmed in the researches that were carried out in our group. Preincubation of honeys solutions in 80°C for only 10 min resulted in complete loss of antibacterial activity of all tested honeys. The same effect was observed when suspensions of tested bacterial cells in solutions of honeys were supplemented with catalase. In both cases the observed, complete losses of activity of honeys were the consequence of lack of possibilities for H_2O_2 generation. Heat treatment resulted in denaturation of the enzyme, and in the presence of catalase, the generated hydrogen peroxide was immediately decomposed [27]. On the other hand, antimicrobial activity of honey also depends on the botanical source which was used by bees to collect the nectar. Buckwheat, thyme and cornflower honeys usually exhibit high antimicrobial activity, whilst produced in Poland in large amounts rape honey do not affect the growth of neither Gram-positive nor Gram-negative bacteria [27, 28]. Thus, the types of phytochemicals as well their concentration is important for final antimicrobial potential of honey. The issue of role of phytochemicals in the antimicrobial potential of honey is still not clear

and is a subject of many interesting research. Hydrogen peroxide is also a known cytostatic agent; however, its concentration in honey is on a very low level which is safe for humans and animals. According to Lusby et al. [29], the concentration of H_2O_2 in honey is thousand times lower than in the common 3% antiseptic solution available in pharmacies; however, its constant production causes prolonged activity which can be considered as an advantage. The concentration of hydrogen peroxide on a non-toxic and stable level is probably regulated by antioxidants and pollen-derived catalase which destroy excess amounts of H_2O_2 [30].

2.1.2. Honey as a potential antistaphylococcal agent

The results of many investigations, carried out in different geographical regions of the world, revealed especially high efficiency of honey in treatment of infections caused by Gram-positive microorganisms, e.g. staphylococci. The mentioned above investigation carried out in our group revealed that honeys obtained from some species of plants, namely cornflower (*Centaurea cyanus* L.), buckwheat (*Fagopyrum esculentum* Moench) and thyme (*Thymus vulgaris* L.) were able to inhibit the growth of reference strain *S. aureus* PCM 2051 at the concentration of 3.12 or 6.25% (v/v). Satisfactory activity (minimal inhibitory concentration—MIC $\geq 6.25\%$) was observed for honeys obtained from linden tree (*Tilia* spp.), heather (*Calluna vulgaris* L.), savory (*Satureja hortensis* L.) and coriander (*Coriandrum sativum* L.). Other tested bacteria *Staphylococcus epidermidis* PCM 2118 and especially *Pseudomonas aeruginosa* ATCC 27853 and *Escherichia coli* K12 were less susceptible [27]. Quite similar results were obtained in the research carried out by the other polish group that investigates antimicrobial potential of bee products. Buckwheat, linden tree and heather honeys were found very active. Moreover, the authors also revealed high activity of goldenrod honey (*Solidago* spp.)—that was not observed in our research and honeydew honey—not investigated in our research. The latter is a specific kind of honey produced by bees, which collect the honeydew, the sweet secretions of aphids or other plant sap-sucking insects [28]. The observed in our studies activity against *S. aureus* was similar to the activity exerted by Slovenian chestnut, fir honeydew and forest honey (MIC = 2.5%, v/v) reported by Kuncic and coworkers [31] as well as Chilean Ulmo tree honey (MIC = 3.1%, v/v) reported by group of Sherlock [32]. The MIC values (against *S. aureus*) in the range of concentrations from 3.12 to 12.5% (v/v) were also observed by Anthimidou and Mossialos, who investigated a collection of 31 Greek and Cypriot honeys, they also revealed that Gram-negative bacteria *P. aeruginosa* revealed a bit higher resistance with MICs values in the range from 6.25 to 25% (v/v) [33]. Lallam and coworkers investigated antibacterial potential of 32 samples of honey (14 monofloral and 18 multifloral) collected from the Algerian Sahara Desert against four bacteria; *Bacillus subtilis*, *Clostridium perfringens*, *Escherichia coli* and *S. aureus*. The research confirmed high antimicrobial activity of honeys collected from this region; however, only disc diffusion method was used by the authors. All floral origins of honey showed antimicrobial activity against *S. aureus* but with rather similar reactions (9–10.5 mm), except with *P. persica*-based honey, whose activity was only 6 mm [34]. High anti-staphylococcal potential of honeys was also confirmed for clinical isolates of these bacteria, including MRSA strains. Effective inhibition of growth of MRSA isolates has been revealed in the case of mentioned above Chilean honey obtained from Ulmo tree [32], Malaysian melaleuca honey [35], some Thai honeys, especially from longan flower [36], Finland [37] and also

many other geographical regions. Moreover, some authors revealed high activity of honey in eradication MRSA infections using *in vivo* models [38]. Considering honey as a therapeutic, antimicrobial agent the honey produced from the manuka bush (*Leptospermum scoparium*) indigenous to New Zealand and Australia deserves special attention. In contrast to majority of other nectar and honeydew honeys, the crucial factor responsible for the bactericidal activity of manuka honey is high concentration of 1, 2-dicarbonyl compound—methylglyoxal (MGO) in this product. High acidity and sugar concentration as well as hydrogen peroxide generation play in this case supporting roles [39, 40]. As a consequence, antimicrobial activity of this honey is not affected by heat treatment, catalase or proteolytic enzymes (hydrolysing glucose oxidase) [37, 40]. Several investigations *in vitro* confirmed high bactericidal, including antistaphylococcal activity of this honey [e.g. 27, 33]. Jenkins and Cooper revealed synergistic action between manuka honey and some antibiotics against MRSA and *P. aeruginosa* strains isolated from wounds [41]. Moreover, manuka honey has been successfully used for treatment of chronic wound infections caused by MRSA [42].

2.1.3. Perspectives and limitations of treatment staphylococcal infections with honey

From the point of view of possibilities of exploiting of the therapeutic potential of honey especially promising, and most realistic, seem to be application of this product as a component of wound dressing materials. Using honey to eliminate pathogens from infected wounds has a long tradition, which can be counted in hundred or even thousands of years. Currently, several companies specialize in production of dressings containing honey. However, many technical problems still have to be solved: (1) elimination of indigenous flora of honey, especially spores, without thermal or chemical treatment—results in deactivation of glucose oxidase and loss of antimicrobial activity, (2) inhibition of natural process of crystallization of honey, (3) large diversity of antimicrobial activity of honey—only the honeys with certified antibacterial activities should be used for medical applications. These problems could be partly solved by using manuka honey. However, research of many investigators revealed that many “classical honeys”—which activity is based mainly on the generation of hydrogen peroxide, exhibit even higher activity in comparison to manuka honey with a high content of methylglyoxal—550 mg/L [27, 28, 33]. Thus, they are also good candidates to be used in clinical practice as a component of dressings, ointments and creams, or direct application on the surface of infected skin, sores, diabetic foot, or mucous, e.g. in the oral cavity or genital tract.

2.2. Propolis

2.2.1. Basic information

The bees collect four products: nectar and honeydew for production of honey (source of carbohydrates), pollen (source of proteins) and propolis. Propolis is not a component of bees' diet; however, it is absolutely necessary for the proper development of bee colonies. This product, which is also called bee glue, is a natural resinous substance produced from plants' buds and exudates, modified by addition of bees' salivary secretions and wax. Similarly, as in the case of honey, propolis is a product of complex chemical composition. Some of its ingredients mainly polyphenols and flavonoids exhibit high antimicrobial activity. As a consequence, it

is used as a hive disinfectant. Bees use propolis for elimination of pathogenic microorganisms from the walls of hive and cells of honeycomb, in which larvae develop (nest wells) and honey is stored [43]. It is important for prevention of development of such dangerous pathogens as *Paenibacillus larvae*, the bacteria responsible for American foulbrood, one of the most important diseases of bees [44]. Some authors also suggest that components of propolis, at least partially, affect the growth of *Varroa destructor*—most damaging parasite affecting honeybee colonies [45]. Because of its antimicrobial activity, propolis is also used by bees for mummification of larger pests such as mice invading the hive, which were killed as a result of the sting. Mummification prevents the decomposition of the body of the pest—development of pathogenic flora as well as generation of unpleasant odour. Due to its physical and chemical properties (high viscosity, low water solubility), propolis is also used as an important building material for sealing the hive or natural habitats of these insects (protection against wind or rain, stabilization of construction), which is also important for safety of bees' colonies.

2.2.2. Possibilities of application of propolis in therapy of bacterial infections

Similarly to honey, propolis was widely used in traditional medicine. The detailed history of using propolis in medicine and a discussion of perspectives of its future application have been recently presented by Silva-Carvalho and coworkers [46]. The chemical composition of this product depends on many factors: the geographical region where it was collected (the species of plants which were available for bees), season, weather conditions and many other. Some significant correlations were found primarily in the case of chemical composition of propolis and place of its isolation. On this basis, several different types of propolis have been proposed, e.g. poplar propolis, birch, green, red, "Pacific" and "Canarian". This classification still evaluates and new, different types of propolis are being recognized, e.g. Mediterranean or Portuguese [46]. At least, 13 different types of propolis have been identified in Brazil [47]. Because of differences in chemical composition the biological, including antibacterial, activity of different propolis samples may vary significantly. However, the carried out to date studies revealed that staphylococci and other Gram-positive bacteria are usually highly sensitive to this product collected in many different geographical locations. Propolis remains especially popular in the non-conventional medicine in Brazil as well as in other tropical countries, thus the product obtained in this region is well-characterized. The global market size of propolis was about 2300 tons in 2015 (it is established that it will increase to 2900 tons in 2021), and Brazil is the largest production and exporting country of this product (<https://www.whatech.com/market-research/materials-chemicals/125806-world-propolis-industry-trends-share-size-2021-forecast-report>). High anti-staphylococcal activity of propolis sourced from State of Paraná, in Brazil, was observed by Pamplona-Zomenhan and coworkers, the MIC₅₀ and MIC₉₀ for the 210 strains (162 MSSA and 48 MRSA) were both 1420 µg/mL [48]. The results of investigations performed by the groups of Fidoralisi [49] and Santana [50] indicated that propolis extracts might be effective against mastitis-causing *S. aureus*. The group of Fidoralisi observed reduction in *S. aureus* growth on average, 1.5 and 4 log₁₀ times at concentration of propolis 200 and 500 µg/mL, respectively. At concentrations of 1000 µg/mL, all tested propolis samples reduced bacterial growth to zero [49]. The same effect was observed by Santana and coworkers, but only

in BHI (Brain Heart Infusion) medium. The authors noticed that in milk the bactericidal dose was at least 20-fold greater [50]. Interesting results were also published by Suleman and colleagues [51]. Some of 39 South Africa propolis samples exhibited much higher anti-staphylococcal activity in comparison to three tested samples of Brazilian propolis with MIC and MBC values of only 6 µg/mL [51]. Al-Waili and coworkers revealed that ethanolic extracts of propolis collected from Saudi Arabia (EEPS) and from Egypt (EEPE) effectively inhibited the growth of antibiotic resistant *E. coli*, *S. aureus* and *C. albicans* in single and polymicrobial cultures [52]. Strong antioxidant and antibacterial activity of propolis sourced from three different areas of Sonoran Desert in northwestern Mexico were confirmed in the research of Velazquez group [53]. The MIC against *S. aureus* of the most active sample (coming from Ures) was 100 µg/mL [53]. An antimicrobial effect of propolis harvested from honeybees in subtropical eastern Australia was investigated by Massaro and coworkers. The two tested propolis crude, ethanolic extracts showed bactericidal effects against *S. aureus* ATCC 25923 reference strain at the concentrations of 0.37–2.04 mg/mL [54]. Propolis produced in many Asian and European apiaries is also effective in elimination of staphylococci. The number of publications presenting results of biological properties of propolis sourced from this region has evidentially increased during the last decade. Because of limited size of this chapter only selected investigations can be described herein. Some promising results regarding antistaphylococcal activity of ethanolic extract of Polish propolis (EEPP) against methicillin-sensitive *S. aureus* (MSSA) and methicillin-resistant *S. aureus* (MRSA) clinical isolates have been recently revealed by Wojtyczka and coworkers. The investigated EEPP displayed varying effectiveness against twelve *S. aureus* strains, with MIC in the range from 0.39 to 0.78 mg/mL, determined by broth microdilution method, and minimal bactericidal concentration (MBC) of the EEPP ranged from 0.78 to 3.13 mg/mL. The disk diffusion assay revealed also that EEPP-enhanced antistaphylococcal activity of eight classical antimicrobial antibiotics, namely: cefoxitin, clindamycin, tetracycline, tobramycin, linezolid, trimethoprim+sulfamethoxazole, penicillin and erythromycin [55]. The same authors also investigated EEPP activity against biofilm-forming coagulase-negative *Staphylococcus* strains. The biofilm formation ability of all tested *S. epidermidis* strains was inhibited at EEPP concentrations ranging from 0.39 to 1.56 mg/mL [56].

Due to its health-promoting properties, propolis is widely used as a component of cosmetics, some food and beverages. In our opinion, these applications are not adequate to the biological properties of this product. The results of many presented above studies clearly indicate that propolis is a promising antimicrobial agent. Moreover, many other biological activities of this product have been described, including antioxidant, antiviral, antitumor, antifungal and immunomodulatory properties [46]. Large diversity of its chemical composition and consequently its biological activity eliminate propolis from clinical applications. Nevertheless, this limitation should be considered in therapy of serious, life-threatening infections (or other diseases), where the exact amount of biological active agent has to be used. *Staphylococci* are often responsible for many, not really serious, but bothersome infections. We would suggest more frequent use of propolis for treatment of this type of diseases. Currently, most of these infections are treated with antibiotics, which should be rather reserved for serious infections. It leads to the overuse of antibiotics and development of resistance phenomenon. In contrast

to antibiotics, propolis is a mixture of many antimicrobial components, thus development of resistance against this product (parallel resistance to all active constituents) is not possible. We also agree with other authors that it is necessary to continue research on correlation of biological activity and chemical composition of this product. Identification of crucial ingredients or their compositions could be used also for treatment serious, life-threatening diseases.

2.2.3. Other bee products: pollen and royal jelly

Investigation of antimicrobial activity of pollen, fermented pollen (bee bread) and royal jelly is not as popular as in the case of honey and propolis. However, several authors confirmed that these products also reveal some antimicrobial potential. Boukraa and coworkers revealed that MIC value for royal jelly against *S. aureus* is about 2% (v/v) [57], and the group of Gunaldi [58] when using this product obtained promising results in the preservation of implant-related infection in rats. The activity of royal jelly is probably a consequence of presence within composition of this product some peptides that inhibit growth of bacteria including members of the genus *Staphylococcus* [59].

Antimicrobial, antimutagenic, antioxidant and even anti-inflammatory activity of bee pollen (collected in Portugal and Spain) has been revealed in the research of Pascola and colleagues [60], and *S. aureus* was found as the most sensitive microorganism to the activity of these products. High antimicrobial activity of methanolic and ethanolic extracts of several monofloral Slovakian bee pollens was observed by the group of Fatracova-Sramkova. *S. aureus* was found as most sensitive to the poppy pollen ethanolic extract. The most sensitive bacteria of rape bee pollen methanolic extract and sunflower ethanolic extract was *Salmonella enterica* [61].

2.2.4. Honey and other bee products as a source of bacteriocinogenic bacteria with bactericidal antistaphylococcal activity

However, bacteriocins are usually active against bacteria closely related to producing strain, but there are also many exceptions to this general rule, e.g. nisin, which is active against broad spectrum of Gram-positive bacteria [62]. Recently carried out research of the group of Prof. Worobo from Cornell University revealed that honey should be considered as a potential source of microorganisms producing promising antimicrobial compounds, especially bacteriocins [63]. The mentioned authors analyzed two Manuka honeys from New Zealand and six domestic honeys from US. The 2217 isolates out of 2398 strains (92.5%) exhibited activity at least against one of the tested microorganisms. Among all the bacterial indicator strains, *Listeria monocytogenes* had highest susceptibility (69%) to the various antimicrobial compounds produced by all active bacterial isolates (1655 out of 2398), whereas *S. aureus* showed the second highest susceptibility of all indicator microorganisms tested. Growth of this bacterium was inhibited by 66.9% of all the isolates (1605 out of 2398) [63]. The bacterial isolate TH13 was found as an efficient producer of peptide compound with high activity against *P. larvae* spp. *larvae*, which are etiological agents of American Foulbrood. It is a disease of honeybees that results in the annihilation of the honeybee colony [64]. Another isolate, identified as *Bacillus thuringiensis* SF316, was shown to efficiently produced thuricin H,

a bacteriocin which strongly inhibits the growth of *Bacillus cereus* [65]. Thus, it was shown that antimicrobial activity of honey is attributed not only to hydrogen peroxide (formed by glucose oxidase originating from honeybees), antioxidant components or dimethylglyoxal (in the case of Manuka honey), but also to microorganisms which are present in this product.

However, results of some successful investigations have been published the trials of isolation of bacteriocinogenic bacterial strains from honey and other than honeybee products were rarely carried out to date. In our opinion, especially, promising source of bacteria producing interesting bacteriocins could be fermented pollen—it is pollen which is collected by bees for the winter and early spring. The high antimicrobial activity of fermented pollen is the consequence of lactic acid bacteria (LAB) presence in this product, and the products of LAB metabolism—lactic acid, as well as bacteriocins.

3. Antibacterial activity of essential oils and plant extracts against *S. aureus*

Essential oils (EO) and their components are becoming increasingly popular as antimicrobial agents. They belong to the group of secondary metabolites that are enriched in compounds based on an isoprene structure and are called terpenes. They occur as di-, tri-, tetra-, hemi- and sesquiterpenes. The compounds that contain additional elements, usually oxygen, are termed terpenoids. Terpenes, terpenoids, as well as essential oils containing these substances exhibit antibacterial activity against broad spectrum of microorganisms including staphylococci [66].

One of the best characterized EO, which effectively inhibits growth of *S. aureus*, including MRSA isolates, is tea tree oil (TTO) derived mainly from the Australian native plant *Melaleuca alternifolia*. The antimicrobial activity of TTO is attributed mainly to its major component—terpinen-4-ol, and α -terpineol which is present in a lower concentration [67]. Several groups of researchers have evaluated the activity of TTO against MRSA. Carson et al. examined 64 MRSA isolates from Australia and the United Kingdom and showed that the MIC and MBC for the Australian isolates were 0.25 and 0.5% (v/v), respectively, while for the United Kingdom isolates were 0.312 and 0.625%, respectively [68]. The TTO has been also evaluated as an alternative decolonization agent for MRSA. The ointment and body wash containing 4 and 5% of TTO, respectively, were found as more effective in eradication of MRSA carriers than classical therapies with using 2% mupirocin nasal ointment and triclosan body wash [69]. Carson and coworkers revealed that TTO and its main components compromise the cytoplasmic membrane of *S. aureus* [70]. The most important consequences of the damages of lipid bilayer are as follows: leakage of important cytoplasmic components, inhibition of respiration (leakage of potassium ions), loss of sodium chloride tolerance and some changes in cell morphology [70, 71]. TTO, terpinen-4-ol and α -terpineol showed strong activity against biofilm formed by *S. aureus* on biomaterials. Partial destruction of 24-h-old biofilms was achieved in the concentration 4–8 times greater than MIC after 1 h, whereas 2–4 \times MIC was adequate to obtain 90% reduction metabolic activity of biofilm after 4 h of treatment [72]. The research of some authors showed transient decreases in antibiotic susceptibility in several bacteria that had been exposed to TTO. It raises concerns that TTO may hinder the effectiveness of conventional antibiotics and influence the development of resistance [73–75].

However, recently Hammer et al. showed that the presence of TTO or terpinen-4-ol resulted in only minor changes in antibiotic susceptibility of *S. aureus* isolates that were serially sub-cultured with sub-inhibitory TTO or terpinen-4-ol [76].

Promising results were also obtained in the investigation of antimicrobial potential of lavender oil (LO). The oil obtained from *Lavandula angustifolia* demonstrated *in vitro* activity against MRSA at concentration of <1% [77]. Several chemically characterized lavender oils were assessed for their antibacterial activity using the disc diffusion method. All tested lavender oils inhibited growth of both MSSA and MRSA with inhibition zones ranged from 8 to 30 mm in diameter at oil doses ranging from 1 to 20 μ L, respectively [78]. Some significant differences in the chemical composition and antibacterial activity of LO, which mainly depend on the origin of the lavender samples, were observed in research carried out by different authors. For example, it was demonstrated that oil from lavender of Bulgarian origin, which contains 51.1% linalool and 9.5% linalyl acetate as main components, was more effective against bacteria than oil originated from lavender sample of French origin containing 29.1% linalool and 43.2% linalyl acetate. It is believed, however, that lavender oil may be useful, first and foremost, as a prophylactic or topical application for surface infection [79].

Another interesting, from the point of view of its antimicrobial properties, is *Thymus* essential oils (TOs). Its main chemical components are α -thujone, α -pinene, camphene, β -pinene, p-cymene, α -terpinene, linalool, borneol, β -caryophyllene, thymol and carvacrol [80]. Different chemotypes of the essential oil from the genus *Thymus* were distinguished based on the presence of chemical components [81]. The antimicrobial properties of TOs are related to their high content of carvacrol and thymol, which were identified as the most efficient against bacteria [80]. Using an agar dilution method, the MIC values for MSSA and MRSA were reported for carvacrol (0.015–0.03%, v/v) followed by thymol (0.03–0.06%, v/v) [82]. The *Thymus* essential oils blended, in which the principal components were thymol, linalool, terpinen-4-ol and α -terpinene, exhibited significant inhibitory and bactericidal effects against strains of epidemic MRSA. The mean MIC and MBC values for the oil blend was 0.3 and 0.6%, respectively, whereas for the linalool chemotype thyme oil the MIC and MBC values were 0.4 and 0.8%, respectively. In the disc diffusion assay, the essential oils blended resulted in the formation of mean zone of inhibition size of 34.8 mm, while linalool chemotype produced a mean zone of 20.7 mm [83].

Recently research of many authors showed also the antimicrobial activity of geranium oil (GO) against MRSA [84, 85]. Among 67 components of geranium oil from *Pelargonium graveolens* Ait, citronellol, geraniol, nerol, citronellyl formate, isomenthone and linalool are the main constituents responsible for its biological activity. The research based on agar dilution method showed that the geranium oil had very strong activity against the clinical *S. aureus* strains, including MRSA strains, exhibiting MIC values of 0.25–2.50 μ L/mL [85]. Moreover, Rosato et al. showed the occurrence of a synergism between geranium oil and norfloxacin against reference *S. aureus* strains [86].

The antibacterial activity of essential oils from oregano (*Origanum vulgare*) against multiresistant bacteria, including MRSA, was analysed by Costa et al. [87]. The MIC values were determined by the microdilution method. MRSA were inhibited by the essential oil at the concentration of 0.125%. Nostro et al. investigated activity of essential oils from oregano

against biofilm-grown *S. aureus* and the effects of the oil on biofilm formation. The biofilm inhibitory concentrations (0.125–0.500%, v/v) and biofilm eradication concentrations (0.25–1.0%, v/v) were twofold or fourfold greater than the concentration inhibitory planktonic growth. Sub-inhibitory concentrations of the oils from oregano prevented biofilm formation by *S. aureus* strains [82].

Nigella sativa is a herbaceous plant cultivated in many countries in the world [88]. Crude extract and seed essential oil possess antibacterial activity against several bacteria [89]. The antibacterial effect may be due to the presence of the two important active compounds of *N. sativa*, thymoquinone and melanin [90]. The activity of *N. sativa* extract against clinical isolates MRSA was investigated by Hannan et al. [91]. They showed that all MRSA isolates were sensitive to *N. sativa* extract at a concentration of 4 mg/disc and MIC was in the range of 0.2–0.5 mg/mL. On the other hand, the multidrug resistant *S. aureus* strains isolated from nasal and milk samples of cows and buffalo were completely inhibited by *N. sativa* extract at concentration of 40 µg/mL on disc and MIC values were between 0.3 and 2.5 mg/mL [92].

Essential oil of cinnamon and cinnamaldehyde, which is main chemical constituent of this oil, also showed activity against MRSA. Essential oil from *Cinnamomum osmophloeum* (clone B) had an excellent inhibitory effect with the MIC of the essential oil and cinnamaldehyde against MRSA from human stand at 250 µg/mL [93]. The antimicrobial activity of cinnamon essential oil and *trans*-cinnamaldehyde against *Staphylococcus* spp. from clinical mastitis of cattle and goats was not dependent on the antibacterial susceptibility profile. However, the best antimicrobial activity was showed with *trans*-cinnamaldehyde and this compound could be used in the treatment of mastitis [94].

The number of research on antibacterial properties of extracts from medicinal plants against MRSA increased in recent years. These researches are conducted in different countries and show that extracts of plants are rich source of unique phytochemicals with activity against MRSA. Among recently investigated plant was *Schinus areira* L., which grows naturally in Argentina, Peru, Bolivia and Northern Chile. The essential oil from leaves and fruits of two specimens of *S. areira* differ in chemical profile. The limonene-rich oil isolated from the leaves and fruits had potent antibacterial effect on MRSA. When using 3.2 and 15 µL/mL (MICs value) of essential oil from leaves and fruits, respectively, the complete inhibition of MRSA growth was observed. Leaves and fruits oils showed bactericidal action after incubation for 24 h with 20 and 40 µL/mL, respectively. On the other hand, the α -phellandrene-rich fruit oil, having a lower content of limonene, was inactive against MRSA [95].

According to research of Endo and Dias Filho [96], MRSA is also sensitive to berberine (plant alkaloid) which is used in Chinese medicine. MICs values of berberine ranged from 62.5 to 250 µg/mL and MBC values were the same or twofold above the MIC. Highly potent anti-MRSA activity with MIC values in range of 25–50 mg/mL was detected among Libyan medicinal plants such as *Cistus salviifolius*, *Salvia officinalis*, *Pistacia atlantica*, *Arbutus pavarii* and *Myrtus communis* [97]. Significant anti-MRSA activity was documented in many studies on extracts of plants used in traditional medicine in Brazil. A mixture of hydrolyzable tannins from *Punica granatum* and the naphthoquinones α -lapachone I and α -xyloidone II from *Tabebuia avellanedae* showed antibacterial activity against all *S. aureus* strains tested, including MRSA isolates [98].

Turnera ulmifolia L. occurs in the north and northeast Brazilian regions and ethanol extract from this plant showed synergistic effect on gentamicin and kanamycin against MRSA strains. Coutinho et al. [99] found that the presence of ethanol extract of *T. ulmifolia* in growth medium at concentration of 32 µg/mL causes a significant reduction in the MIC for these antibiotics. The other studies conducted in India reported that ethanol, methanol and acetone extracts of *Moringa oleifera*, *Elettaria cardamomum* and *Tamarindus indica* seeds from India showed antibacterial activities against multidrug resistant MRSA isolates from wound infection [100].

4. Conclusions

Staphylococci belong to the most important pathogens for both humans and animals. The number of antibiotics effective in treatment of infections caused by these pathogenic bacteria is rapidly decreasing. Many centuries of observation and the use of bee products and essential oils in folk medicine as well as the results of advanced scientific research carried out during the last several decades clearly confirm high antimicrobial, including antistaphylococcal activity of these products. We have no doubt that they are an interesting and promising alternative to classical antibiotics and should be more seriously considered as therapeutic agents.

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