# Antimicrobial Properties of Tetragonula laeviceps Propolis for Methicillin-Resistant Staphylococcus Aureus: A Review

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**Abstract**

Escalating use of antibiotics has led to the development of microbial resistance, exemplified by the emergence of methicillin-resistant Staphylococcus aureus (MRSA), which displays resistance to beta-lactam antibiotics, leading to skin infections, pneumonia, and bacteremia. The ramifications of such resistant bacterial infections, encompassing prolonged hospitalization, heightened treatment costs, and increased mortality rates, underscore the critical need for judicious management of "Drug of Choice" antibiotics to avert compromised sensitivity to MRSA. Effective resource management is imperative to address the MRSA threat. Propolis, a wax product renowned for its enriched antimicrobial properties attributed to terpenoids, saponins, alkaloids, and polyphenols, is derived from various bee species, with particular emphasis on Tetragonula laeviceps, the most widely distributed species in Southeast Asia. This literature review explores the potential of Tetragonula laeviceps propolis as an alternative treatment for MRSA infections. Recognizing the efficacy of propolis in combating antibiotic resistance holds valuable implications for future research and treatment strategies.

Keywords: Tetragonula laeviceps, Methicillin-resistant Staphylococcus aureus, antimicrobial, propolis

## 1. Background

Antibiotics as curative chemotherapy for bacterial infections have been used since 1907 to the present day. Continuous antibiotic use has impacted the development of microbial resistance to antibiotics, reducing their effectiveness (Siahaan, Herman, and Fitri, 2022). Microbial defense mechanisms adapt rapidly compared to the development of antibiotic drugs, exemplified by Staphylococcus aureus. This bacterium is identified as a major contributor to the global burden of antibiotic resistance and categorized as a Level 3 disease-related cause of death in Global Health metrics standards (Aslam et al., 2018). This is related to the ability of one strain of Staphylococcus aureus to produce a cell wall-protecting protein, adapting to the use of penicillin-class antibiotics, known as MRSA (Methicillin Resistant Staphylococcus aureus).

MRSA is defined as bacteria resistant to β-lactam antibiotics with a minimum inhibitory concentration greater than 4 μg/mL (Lakhundi and Zhang, 2018). MRSA causes skin infections, pneumonia, and bacteremia with both community and nosocomial transmission (Monaco et al., 2016). With specific modalities such as virulence factors and bacterial adaptation speed to antibiotics, the incidence of MRSA bacteremia in Southeast Asia was 2.3-69.1% in 2014 (Hassoun, Linden, and Friedman, 2017). ECDC (2020) also mentions that in 2019, 25% of invasive S. aureus infections were MRSA isolates in 7 out of 29 European Union countries. The impact of bacterial infections leads to prolonged hospitalization, increased treatment

costs, and increased mortality. This issue also implies the necessity of using the "Drug of Choice" antibiotic, threatening its sensitivity to MRSA if intensively used on a massive scale. Thus, there is potential for the development of bacteria more resistant to the chosen antibiotics that can effectively combat bacterial infections. This is an indication for resource management in tackling the MRSA threat.

The threat of bacteria has been addressed through various approaches, both preventive and curative, both direct and indirect. One alternative treatment for bacterial infections is the use of propolis. Propolis is a wax substance produced by honeybees from plant extract to protect their hive (Wagh, 2013). Propolis has antioxidant, antibacterial, and immunomodulatory properties that can be applied to humans in extract form (Toreti et al., 2013). These properties result from the content of propolis, including saponins, terpenoids, alkaloids, and polyphenols (Ahangari, Naseri, and Vatandoost, 2018). The use of propolis extract as a bacterial inhibitor has been practiced since ancient Egypt as a preservative for embalming. In the 12th century, the Georgian Medical Treatise, Karabadini, documented the benefits of propolis in treating oral and pharyngeal infections (Kuropatnicki, Szliszka, and Krol, 2013). The modern implementation of propolis extract as an antibiotic leans towards a supportive or supplemental role, while conventional antibiotics remain the mainstay in infection management.

Currently, agricultural resources in the form of bee cultivation are extensive in Indonesia, with 2 species of honey bees, 46 species of stingless bees, and 2 species of wasps (Kahono, Chantawannakul, and Engel, 2018). The CBD reports that 48% of honey production in the agricultural sector is dominated by stingless bee breeders, especially Tetragonula laeviceps (Commission On Genetic Resources for Food and Agriculture, 2018). This species has the largest population distributed in Southeast Asia (Buchori et al., 2022). With this condition, the utilization of Tetragonula laeviceps products, especially propolis, is more accessible and researchable. This species also has specific enzymes that function to process antimicrobial substances such as alkaloids, saponins, and polyphenols when consuming propolis (Sanpa et al., 2015). This study aims to test the antimicrobial properties found in the extract of Tetragonula laeviceps propolis against Methicillin Resistant Staphylococcus aureus. To test the antimicrobial properties, propolis needs to be transformed into extract form. The extraction method has an influence on the types of substances that can be isolated. Antimicrobial substances such as terpenoids, saponins, alkaloids, and polyphenols can be easily extracted with ethanol-based solvents, so propolis needs to be transformed into Ethanol Extract of Propolis (EEP) form (Grecka et al., 2020). Thus, the use of Tetragonula laeviceps ethanol extract of propolis is expected to be a method for combating MRSA infections during the widespread development of microbial antibiotic resistance.

## 2. Introduction to Staphylococcus aureus

Staphylococcus aureus is a Gram-positive bacterium that is spherical and arranged in clusters resembling grapes. On nutrient-rich agar media, S. aureus forms colonies that are yellow or golden due to the presence of carotenoid pigments. This organism can thrive aerobically or facultatively anaerobically at temperatures between 18°C and 40°C. Typical biochemical identification tests, such as catalase, coagulase, mannitol fermentation, and novobiocin susceptibility, yield positive results and help differentiate it from other Staphylococcus species. The bacterium is also hemolytic on blood agar medium, producing alpha, beta, gamma, and delta hemolysins. Mannitol fermentation on mannitol-salt agar indicates its salt tolerance. S. aureus is commonly found in the nasal cavity, living as a commensal bacterium (Taylor and Unakal, 2018). According to Davis et al. (2019), the taxonomy of Staphylococcus aureus is as follows:

- Kingdom: Bacteria
- Subkingdom: Posibacteria

- Phylum: Firmicutes
- Class: Bacilli
- Order: Bacillales
- Family: Staphylococcaceae
- Genus: Staphylococcus
- Species: Staphylococcus aureus

## 3. Virulence Factors of Staphylococcus aureus

Staphylococcus aureus possesses numerous virulence factors that enable it to thrive as a pathogen, resulting in a broad spectrum of human diseases. According to Gnanamani et al. (2017), S. aureus virulence factors are divided into three categories based on their functions in the pathogenesis process:

a. **Adhesins:** Adhesins are substances produced by an organism to facilitate adherence. S. aureus has Microbial Surface Components Recognizing Adhesive Matrix Molecules (MSCRAMM) and Extracellular Adherence Protein (Eap). MSCRAMM is a surface protein that interacts with collagen, fibronectin, and fibrinogen from host tissues, facilitating tissue attachment. Staphylococcal protein A, fibronectin-binding protein, collagen-binding protein work synergistically to produce the function of MSCRAMM. Meanwhile, Eap is a structural exoprotein that binds to endothelial tissue plasma proteins. Both adhesins have the ability to suppress the immune system by binding to the Fc portion of immunoglobulins, thereby limiting the immune response.

b. **Exoproteins:** Most S. aureus strains secrete exoproteins that function to convert host tissues into nutrients. These exoproteins include enzymes such as protease, lipase, nuclease, hyaluronate lyase, phospholipase C, metalloprotease, staphylokinase. Additionally, S. aureus produces exoproteins with cytolytic activity, such as alpha-hemolysin and Panton-Valentine leukocidin (PVL). Alpha-hemolysin damages cell membranes through oligomerization, forming pores and causing osmotic cytolysis, particularly in platelet and monocyte cells. Meanwhile, PVL, a bicomponent exoprotein often found in MRSA, targets leukocyte cells using a mechanism similar to alpha-hemolysin.

c. **Toxinosis Inducers:** Toxinosis by S. aureus is caused by the production of enterotoxins, Toxic Shock Syndrome Toxin-1 (TSST-1), and exfoliative toxin. Among these three toxins, the PTSAgs (Pyrogenic Toxin Superantigen) group includes TSST-1 and enterotoxin. Both toxins have the ability to stimulate T-lymphocyte cell proliferation, leading to food poisoning and toxic shock syndrome events. On the other hand, exfoliative toxin is secreted in cases of skin infections. This toxin is a serine protease that selectively hydrolyzes desmosome proteins in the skin.

## 4. History, Evolution, and Epidemiology of Methicillin-resistant Staphylococcus aureus

The use of penicillin since the 1940s has marked two revolutions in the field of medicine: the treatment of bacterial infections and the adaptability of the microbiota. As penicillin became the preferred treatment for bacterial infections, the phenomenon of antimicrobial resistance became more prevalent, leading to the discovery of Staphylococcus aureus resistant to penicillin. This strain of Staphylococcus aureus produces β-lactamase, which breaks down the β-lactam ring, thus disabling the antibacterial properties of antibiotics. This

issue prompted the development of new antibiotics, such as semi-synthetic β-lactams resistant to β-lactamase, known as methicillin, introduced in 1959 (Enright et al., 2002).

The use of Methicillin led to the discovery of Methicillin-Resistant Staphylococcus aureus (MRSA) in the 1960s, following the introduction of Celbenin, an early type of methicillin used to treat penicillin-resistant Staphylococcus aureus. The first reports came from the UK and were followed by other countries such as Japan, Australia, and the United States. These reports indicated that the isolates still exhibited an average minimum inhibitory concentration of more than 4 μg/mL when tested with methicillin. Global cases of MRSA infections have been reported to be associated with healthcare facilities or post-medical procedures. Many studies also characterize MRSA as well-adapted to the healthcare environment, strengthening these reports (Harkins et al., 2017).

MRSA strains possess the mecA gene on the bacterial chromosome, part of the broader Staphylococcal Chromosomal Cassette mec (SCCmec) region, providing resistance to various antibiotics based on SCCmec types. The mecA gene encodes Penicillin-Binding Protein 2A (PBP-2A), which catalyzes peptidoglycan formation in the bacterial cell wall. PBP-2A has a lower affinity for binding β-lactams and other penicillin derivatives than other PBP types, allowing bacterial cell wall formation to continue despite exposure to several antibiotics. Therefore, Staphylococcus aureus strains producing PBP-2A can proliferate in the presence of many antibiotics, such as MRSA strains resistant to multiple drugs. Methicillin, nafcillin, oxacillin, and cephalosporins are generally ineffective against MRSA. SCCmec is a genetic element that can be mobilized through the adaptation process of Staphylococcus aureus. There are two theories explaining the origin and evolution of MRSA. The single clone hypothesis suggests that SCCmec A entered the S. aureus population at a specific time, forming a single clone of MRSA that spread worldwide. The theory widely accepted globally is the hypothesis of repeated horizontal transfer of SCCmecA to vulnerable methicillin precursor strains of S. aureus (Ippolito et al., 2010).

## 5. Pathogenesis of Methicillin-resistant Staphylococcus aureus

Staphylococcus aureus, particularly MRSA strains, can cause various organ-specific infections, primarily affecting the skin and subcutaneous tissues. The most common infections include skin and soft tissue infections caused by Community-Acquired MRSA (CA-MRSA), associated with conditions such as cellulitis, necrotizing fasciitis, and diabetic foot ulcers. CA-MRSA infections are increasingly resistant to various drugs, leading to frequent relapses, increased hospitalizations, and mortality (Hu et al., 2022).

Bone and joint infections, often caused by Staphylococcus, are frequently associated with antibiotic resistance, making drugs like oxacillin less effective due to their difficulty penetrating bone or joint tissues. MRSA can lead to osteomyelitis in the spine and long bones of the upper and lower extremities, either as a result of wound infection or part of a hematogenous infection. MRSA can also cause septic arthritis in native and prosthetic joints (Qiao et al., 2020).

CA-MRSA is linked to Staphylococcal pneumonia in the post-influenza era, presenting as life-threatening necrotizing pneumonia in otherwise healthy individuals across the United States. Severe respiratory symptoms, high fever, hemoptysis, and hypotension characterize this disease, which can quickly progress to sepsis and septic shock, accompanied by leukopenia and elevated C-reactive protein (over 350 mg/dL). In this clinical situation, multilobar cavitating alveolar infiltrates are consistent with CA-MRSA infection. MRSA is also the most common cause of hospital-acquired and ventilator-associated pneumonia, both of which have a poor prognosis (Bien, Sokolova, & Bozko, 2011).

Bacteremia caused by MRSA infection has been associated with mortality rates ranging from 15% to 60%. MRSA bacteremia is common in patients with central line insertion in intensive care units and can lead to

complications such as infective endocarditis. Individuals with reduced response to vancomycin have a worse prognosis than other MRSA infections (Lounsbury et al., 2019).

MRSA is a leading cause of bacterial endocarditis, with a fatal outcome in 30-37% of infected patients. Intravenous drug use and intravenous catheters are often associated with right-sided MRSA endocarditis. Septic pulmonary emboli can cause nodular infiltrates and cavitary lesions in patients with tricuspid valve vegetations. Similarly, patients with involvement of the mitral and aortic valves may develop secondary infections in distant sites such as bones and joints, kidneys, brain, and other organs. Obtaining a complete medical history, individual examination, appropriate laboratory tests, and radiological testing are crucial for accurate diagnosis and management (Siddiqui & Janak Koirala, 2018).

## 6. Treatment for Methicillin-resistant Staphylococcus aureus Infection

In general, the treatment of MRSA infections currently relies on a pharmacological therapy approach. The selection of therapy is based on the type of infection, clinical symptoms, comorbid conditions, patient age, and the need for hospitalization. In cases of skin infections such as impetigo, folliculitis, erythrasma, furunculosis, and traumatic wounds, management may involve the use of topical medications such as mupirocin and fusidic acid. Mupirocin, as a monoxycarboxylic acid class drug, exhibits antibacterial properties by binding to isoleucyl t-RNA synthetase to inhibit protein synthesis. Some reports suggest that mupirocin can be used for the decolonization of commensal Staphylococcus aureus. On the other hand, fusidic acid, a chemically tetracyclic triterpenoid, works by binding to bacterial EF-G, disrupting the DNA translocation process (Nandhini et al., 2022).

In cases of systemic MRSA infections, vancomycin antibiotics are often the primary choice. Vancomycin, a glycopeptide, inhibits the synthesis of bacterial cell walls, primarily preventing the joining of N-acetylmuramic acid subunits with N-acetylglucosamine in the peptidoglycan matrix. This antibiotic is generally indicated for Gram-positive bacterial infections and is administered intravenously. Although vancomycin has been the preferred treatment for MRSA infections since 1958, its sustained use has led to an increase in the average minimum inhibitory concentration against MRSA, approaching the upper limit of susceptibility, which is 2 μg/L. Confronting this phenomenon, optimizing treatment regimens and adjusting vancomycin doses to achieve effective concentrations in the bloodstream are crucial steps to maintain the clinical utility of vancomycin (Yu et al., 2020).

## 7. Introduction to Propolis

Propolis, commonly known as "bee glue," is a general term for resinous substances collected by bees from various plant sources. Terminologically, the term "propolis" originates from the Greek words "pro," meaning "defense," and "polis," meaning "city" or "community." Bees gather propolis from various plant secretions such as sap, gum, and resin from several plant species. These substances are then combined with saliva and enzymes to form a natural substance resembling wax. Due to the variety of bee species and plants used in the synthesis process, propolis comes in various colors and different ratios of content. The natural use of propolis can be found in beehives to seal open areas and cracks. In addition to its structural function, the application of propolis can provide antiseptic functions and regulate the hive's climate. Bees live in colonies, making the role of propolis crucial for their survival, including protecting larvae, storing honey, and preventing infections (Anjum et al., 2019).

The human use of propolis has been documented since 300 BC. Due to its connection to agriculture, propolis is classified as an exotic medicinal substance. The use of propolis as medicine and a preservative has been applied in Roman, Greek, and ancient Egyptian cultures. Scientists such as Dioscorides, Galen, Aristotle, and Pliny described propolis's functions as an antiseptic and wound healer. Similarly, during the industrialization era, doctors used propolis as an anti-inflammatory and antipyretic during the Anglo-Boer War and World War II (Rojczyk et al., 2020).

Each bee species has a different composition of propolis. However, these differences are limited to the ratio of the main components of propolis due to variations in the dietary patterns of bee species. Generally, propolis is composed of resin (50%-70%), beeswax (30%-50%), pollen (5%-10%), and components such as amino acids, minerals, flavonoids, phenols, and vitamins (Ahangari, Naseri, & Vatandoost, 2018). Resin is the tree sap collected by bees, modified into a polishing and nest-cleaning substance. Beeswax, on the other hand, is a yellowish, soft, and highly absorbent substance produced by bees, containing esters, acids, high-fat alcohols, and even free hydrocarbons. While beeswax is stable and highly resistant to moisture, it is not resistant to heat or mechanical pressure.

Pollen from flowers has high nutritional value and consists of more than 96 different elements. The content of bee-collected pollen is determined by specific flowers, containing a high concentration of vital amino acids, vitamins, minerals, and hormones. Flavonoids, a type of polyphenol found in propolis, serve as a measure to assess its quality. Flavonoids are classified into various types, including flavones, flavonols, flavanones, flavanonols, chalcones, dihydrochalcones, isoflavones, isodihydroflavones, flavans, isoflavans, neoflavonoids, and flavonoid glycosides. The proportion of these substances varies based on their chemical structure and location. Hydrocarbons such as alkanes, diesters, and aromatic esters can also be found in propolis. Lastly, terpenes, secondary metabolites produced by plants, contribute to the distinct odor of propolis, serving as a benchmark for determining its authenticity (Santos et al., 2019).

The complex structure of propolis necessitates an extraction process to harness its benefits. Antibacterial components in propolis can be dissolved using water, methanol, ethanol, chloroform, dichloromethane, and acetone. The extraction method also influences the percentage of pharmacological components that can be extracted. A traditional method for propolis extraction is maceration. This involves mixing propolis with water or alcohol and allowing it to stand for 72 hours at room temperature. Another method is the Soxhlet extraction, where propolis is extracted using a mixture of water and alcohol for 2-24 hours at a temperature of 60 degrees Celsius (Bankova, Trusheva, & Popova, 2021).

## 8. Pharmacological Effect of Propolis

The phenolic content of propolis, consisting of coumarin, quinine, and flavonoids, provides antioxidant, anti-inflammatory, and antibacterial effects. Flavonoids can chelate metal ions such as iron and copper, inhibiting the production of free radicals. They exhibit antibacterial effects by inhibiting DNA or RNA synthesis in bacteria and anti-inflammatory activities by inhibiting nitric oxide synthesis, glycosidase, lipoxygenase, protein kinase, and prostaglandin. Flavonoids are more effective in inhibiting Gram-positive bacteria than Gram-negative bacteria. However, the effectiveness of antibacterial properties depends on the propolis extraction technique. Terpenes of the carvacrol group can activate TRPA1 and TRPV3 ion channels, which mediate inflammatory pain. They can also inhibit COX-2, producing analgesic effects (Miyata et al., 2020).

## 9. Conclusion

The antimicrobial properties of propolis extracts from the Tetragonula laeviceps species show promising potential for the development of a novel MRSA agent. This not only serves as an alternative regimen for MRSA treatment but also has the added benefit of encouraging the local economies of South-East Asian bee farmers engaged in bee cultivation and the production of bee-related products.

## Acknowledgements

The authors acknowledge the contribution of Putu Bagus Dharma Permana in providing valuable comments and informal review for the manuscript.

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