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Herbal Bioactive Compounds for Skin Infections and Inflammatory Conditions

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Abstract

Skin microbiota is an integral part of the human immune system. *Staphylococcus aureus* is one of the essential components of the normal flora. Approximately 20–30% of healthy individuals are persistently colonized with *S. aureus*, whereas the remainders are considered low-level intermittent carriers. Despite these natural aspects of existence, *S. aureus* can be a major opportunistic human pathogen. This versatile microorganism can infect a variety of anatomical sites, causing a broad spectrum of pathologies ranging from superficial to invasive infections. It developed a variety of strategies to adopt to a changing microenvironment. This attributed to the emergence of resistance to antibiotics of different classes during the past six decades. Methicillin-resistant *S. aureus* (MRSA) was originally confined to health-care settings (health-care-associated MRSA). Later on, community-acquired MRSA was identified as another source of infections. Recent figures indicate that MRSA strains have been associated with approximately 75% of all *S. aureus* infections worldwide. Several guidelines have been published to establish an adequate treatment of skin and soft tissue infections (SSTIs) caused by MRSA strains. In the first part of this review, we focus on current treatment guidelines with a focus on medical drug therapy, but drug therapy has its own limitations. Recently, the interest in herbal remedies has greatly increased. There is growing evidence of antimicrobial activity of medicinal plants and their extracts. The second part of this review is dedicated to herbal compounds to circumvent antibiotic resistance. Herbal compounds may potentiate the action of antibiotics and restore the activities of antibacterial agents against which *S. aureus* has developed a drug resistance. Part 2 focuses on the role of *S. aureus* in pathology of the two major inflammatory skin diseases, i.e., atopic dermatitis (AD) and psoriasis. Finally, Part 3 provides an overview on natural compounds with antimicrobial activity against *S. aureus* and possible use in the treatment of SSTIs,

PART I - THE SKIN MICROBIOME, STAPHYLOCOCCUS AUREUS, SKIN AND SOFT TISSUE INFECTIONS, ANTIBIOTIC THERAPY

Introduction

Skin microbiota is an integral part of the human immune system, and it comprises a wide array of biological organisms. *Staphylococcus aureus* is one of the essential components of the normal flora, which inhabits the moist squamous epithelium of the anterior nares. Other common sites of carriage include the skin, pharynx, perineum, axillae, and vagina [1]. Approximately 20–30% of healthy individuals are persistently colonized with *S. aureus*, whereas the remainders are considered low-level intermittent carriers [2]. An individual can carry a single strain of the bacterium over an extended period or multiple strains with varying frequencies at different anatomical sites [3].

Despite these natural aspects of existence, *S. aureus* can be a major opportunistic human pathogen. This versatile microorganism can infect a variety of anatomical sites, causing a broad spectrum of pathologies ranging from superficial to invasive infections. Indeed, the bacterium was first described from abscesses in a knee joint in the 1880s [4]. Subsequently, it has become a leading cause of bacteremia, osteomyelitis, prosthetic device infections, septic arthritis, pneumonia, meningitis, toxic shock syndrome, and urinary tract infections. Besides, *S. aureus* causes a range of skin and soft tissue infections (SSTIs), which can be benign (uncomplicated cellulitis and impetigo) or life-threatening infections.

Importantly, *S. aureus* has a considerable capacity to adapt to various pressures in human, and this is attributed to the emergence of resistance to antibiotics of different classes during the past six decades [5]. In particular, resistant strains of *S. aureus* were first described after the introduction of methicillin in the 1960s [6]. Subsequently, methicillin-resistant *S. aureus* (MRSA) was confined to health-care settings (health-care-associated MRSA [HA-MRSA]). In the 1990s, several reports have revealed MRSA isolation

from children and adults with SSTIs who had not been exposed to health-care risk factors [7], [8], [9]. This was associated with an increased incidence of SSTIs as a result of the community-acquired MRSA (CA-MRSA) epidemic, which has increased the burden of endemic SSTIs strains at that time. For example, the number of emergency visits due to SSTIs in the United States was estimated to be increased from 1.2 to 3.4 million cases from 1993 to 2005 [10], where molecular typing indicated that the predominant clones were USA400 and USA300 [11], [12]. Similarly, the rates of hospitalization attributable to abscesses with CA-MRSA have evidenced a 3-fold increase in England from 1991 to 2006 and a 48% increase in Australia between 1999 and 2008 [13], [14]. Therefore, recent figures indicate that MRSA strains have been associated with approximately 75% of all *S. aureus* infections worldwide [15], [16], [17].

As a consequence, several guidelines have been published to establish an adequate treatment of SSTIs caused by MRSA strains. However, the aforementioned observations demonstrate that the *S. aureus* can virtually resist all introduced antibiotics, causing a significant health burden and substantial economic costs to eliminate the associated infections [18]. Such negative consequences require immediate attention by the scientific community to find novel alternatives for the prevention and treatment of MRSA. The proposed solutions entail a multipronged approach that includes vaccination to prevent the infection, monitoring, and the development of novel therapies [19]. The latter seems to be the most reasonable approach to control the vast burden. The use of herbal remedies is one of these solutions, although it belongs to the traditional systems of medicine. This chapter reviews the current treatment of resistant *S. aureus* infections, the mechanisms of resistance of the bacterium, and the efficacy of herbal bioactive compounds in MRSA control.

Current Recommendations for the Treatment of SSTIs Attributable to MRSA

The relevant guidelines for the management of SSTIs were published in 2005 [20] and revised in 2014 [21] by the Expert Panel of the Infectious Diseases Society of America (IDSA). The authors of the revised version reported a lack of prospective studies to support and validate the guidelines [21]. In general, incision and drainage is the mainstay treatment of the mild cases with purulent SSTIs, including furuncles, carbuncles, and abscesses. Antibiotics are recommended for patients with concurrent systemic signs of infection, such as fever, as well as patients with immunosuppression, rapidly progressive cellulitis, or patients at the extremes of age. Trimethoprim-sulfamethoxazole (TMP-SMX) can be used as both an initial empiric therapy and a defined treatment for patients with a MRSA purulent infection with moderate signs of systemic inflammation. In those who have failed the surgical intervention plus oral antibiotics or patients with a profound fever (>38°C), vancomycin, linezolid, clindamycin, daptomycin, ceftaroline, and tetracycline (doxycycline or minocycline) are recommended (Table 1) [21].

Table 1: The recommended antimicrobial agents for MRSA SSTIs infections

Antibiotic	Dosage*	Limitations
Vancomycin	Adults: 30 mg/kg/d (IV) in two divided doses Children: 40 mg/kg/d (IV) in two divided doses	VISA and VRSA emergence
Linezolid	Adults: 600 mg/12 h (IV), or 600 mg bid (oral) Children: 10 mg/kg/12 h (IV or oral)	Costly, limited clinical evidence, toxic, resistance by mutations in the rRNA methyltransferase and the 23S rRNA, cross-resistance with other PTC antibiotics
Clindamycin	Adults: 600 mg/8 h (IV), or 300–450 mg qid (oral) Children: 25–40 mg/kg/d (IV), or 30–40 mg/kg/d (oral); both regimens are given in three divided doses	Resistance by mutations in the 23S rRNA, inducible resistance
Daptomycin	Adults: 4 mg/kg/d (IV) od	Myotoxicity, interaction with pulmonary surfactants, and a trend of resistance by genetic mutations that increase the positive charge of the microbial cell membrane
Ceftaroline	Adults: 600 mg (IV) bid	Leukopenia with long-term treatment, resistance mediated by *mecA*-and non-*mecA*-dependent mechanisms.
Doxycycline, minocycline	Adults: 100 mg (oral) bid Children: not recommended in children aged<8 years	Clinical experience is still limited

* The listed dosages in children are not appropriate for neonates. IV: intravenous; PTC: peptidyl transferase center; VISA: vancomycin intermediate resistant *S. aureus*; VRSA: vancomycin-resistant *S. aureus*.

Vancomycin

Vancomycin is a broad-spectrum glycopeptide antibiotic acting by interfering with cell wall synthesis through binding to the D-alanyl-D-alanine residues; hence, it inhibits the synthesis and polymerization of the N-acetyl-glucosamine and N-acetylmuramic acid subunits within the peptidoglycan (PGN) layer in the cell wall (Figure 1). Eventually, the cell wall becomes weaker, causing leakage of the intracellular content and cell death [22]. Vancomycin is the most reliable antibiotic for MRSA treatment in the United States and China, and it can be used safely in penicillin-allergic patients [21], [23]. Besides, it has been considered the last line of defense against MRSA infections.

Linezolid

Linezolid, another antimicrobial agent that belongs to the oxazolidinone class, was approved for the treatment of MRSA two decades age [24]. Linezolid acts by the inhibition of bacterial protein synthesis. This occurs by interaction with the ribosome cluster through binding to the 23S subunit of the 50s ribosome [25]. Recent meta-analyses showed significantly better clinical and microbiological cure rates in MRSA patients

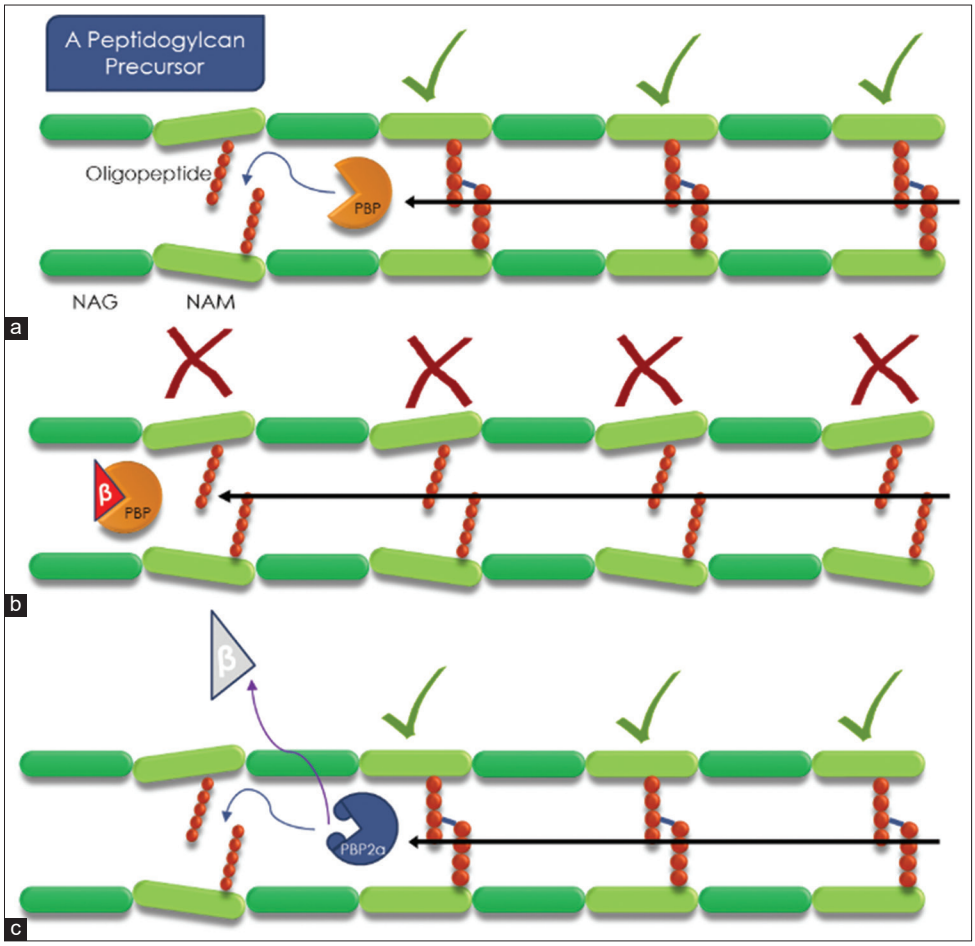

Figure 1: A schematic representation of the biological implications of peptidoglycans in the cell wall of S. aureus. (a) Normal bacterial cell wall synthesis involves a successful cross-linking of the peptidoglycan precursors, which are composed of N-acetyl muramic acid alternating with N-acetyl glucosamine and a chain of five amino acids (oligopeptide). This can be mediated by two enzymes known collectively as penicillin-binding proteins (PBPs). (b) The β-lactam ring of penicillins and cephalosporins (red triangle) binds to PBPs, interferes with cross-linking, inhibits cell wall synthesis, and ultimately leads to cell wall rupture. (c) New PBPs, namely PBP2a, are expressed by the acquisition of genomic data through horizontal DNA transfer and subsequent clonal spread. This confers the resistance to β-lactam antibiotics in MRSA leading to a successful cross-linkage of peptidoglycans

receiving linezolid as compared to those receiving vancomycin without major differences in the safety outcomes [26], [27]. Nevertheless, the daily costs of inpatient treatment were significantly higher with linezolid than vancomycin. Besides, the available relevant meta-analyses [26], [27] were based on nine comparative randomized studies with a high risk of bias. There is also an evidence of potentially adverse reactions, which may be serious (thrombocytopenia) or permanent (optic neuritis and peripheral neuropathy) [28]. Therefore, these limitations may preclude the wide spread use of linezolid, particularly in resource-limited health-care settings.

Clindamycin

Based on the IDSA recommendations, MRSA skin infections can be managed by a number of other antimicrobial agents. Clindamycin is a lincosamide bacteriostatic antibiotic that has been approved for the treatment of several infections, including lower respiratory infections, septicemia, gynecological infections, and SSTIs. It has the ability to reversibly bind to the 50S subunit of the ribosome. Therefore, it inhibits protein synthesis [29]. The rates of treatment failure when clindamycin was administered after incision and drainage of CA-MRSA skin infections were 25%; these rates were comparable to those following the use of a TMP-SMX combination [30].

Daptomycin

Daptomycin is a rapidly bactericidal lipopeptide antibiotic which has a broad-spectrum *in vitro* activity against several Gram-positive bacteria. It was approved by the Food and Drug Administration (FDA) in 2003 for the treatment of SSTIs, right-sided endocarditis, and *S. aureus* bacteremia [31]. The antibiotic is structurally similar to a group of innate antimicrobial molecules named cationic antimicrobial peptides (AMPs), particularly cathelicidin LL-37, which can inherently disrupt the microbial membrane integrity [32]. However, the mechanism of action of the antibiotic is basically dependent on the interaction with calcium and the

anionic phospholipid phosphatidylglycerol (PG) to successfully deliver daptomycin molecules to the bacterial cell membrane [33]. Consequently, owing to binding to phosphatidylglycerol headgroups, daptomycin molecules lead to membrane depolarization and permeabilization and leakage of intracellular content [34].

Clinically, daptomycin causes significant improvements in about 90% of patients with complicated SSTIs [35], [36]. In addition, despite the scarcity of comparative studies, daptomycin had superior efficacy and safety outcomes compared to vancomycin for complicated skin infections, as revealed by clinical cure rates, resolution of symptoms, and the duration of inpatient treatment [37]. However, although daptomycin is well-tolerated, it may cause myopathy and increased serum creatine phosphokinase levels [38], [39]. Besides, daptomycin may interact with pulmonary surfactants; thereby, it has a limited efficacy in MRSA-attributable pneumonia [40].

Ceftaroline

Ceftaroline is a bactericidal antibiotic of the fifth-generation cephalosporins, which has a broad-spectrum activity against multiple Gram-positive and Gram-negative bacteria. Its FDA approval was granted in 2010 for acute SSTIs caused by MRSA and the susceptible strains to methicillin [41]. It has also been approved for clinical use in Europe and Australia in 2012 and 2013, respectively [42]. The molecular basis of its activity relies on its high affinity to bind to the MRSA-associated penicillin-binding protein 2a (PBP2a), rendering a high antibacterial action to MRSA by preventing bacterial cell wall synthesis. These actions are mediated by the 1,3-thiazole ring in its molecular structure. Based on the results of phase III randomized clinical trials (RCTs), intravenous ceftaroline showed high clinical cure rates and few adverse events comparable to those implied by vancomycin plus aztreonam among patients with complicated SSTIs [43], [44]. Furthermore, a recent meta-analysis showed a mean cure rate of 74% with infrequent toxicities, although a considerable proportion of patients on prolonged courses (≥21 days) experience neutropenia [45]. Therefore, patients should be regularly monitored for leukopenia.

In addition to the absence of validating prospective studies for the use of the mentioned antibiotics in MRSA infections, there are marked deficiencies in the concordance with IDSA guidelines, particularly in the emergency settings [46]. Besides, the effects of these treatments on the quality of life of patients are still questionable. The most important limitation of treatment is the expanding rates of resistance by MRSA, which is discussed in detail in the following section.

Restrictions to the Use of Antibiotics

MRSA antimicrobial resistance: the genetic basis

The acquisition of antibiotic resistance by *S. aureus* has been a critical clinical problem. The adaptation of *S. aureus* to various environmental stresses that confer antimicrobial resistance is mainly mediated by the exchange of genetic information between bacteria via mobile genetic elements (MGEs), including plasmids, staphylococcal cassette chromosomes (SCCs), bacteriophages, and transposons. Both SCCs and plasmids play an integral part in the resistance to vancomycin and β-lactam antibiotics [47]. Indeed, the latter group of antibiotics act inherently via binding of the β-lactam ring to two main enzymes involved in the cross-linking of cell wall PGN (Figure 1); these enzymes are termed PBPs. The earliest wave of resistance (against penicillin) was confined to hospitals, and it was predominantly conferred by the *blaZ* gene, which inactivates the β-lactam ring via encoding a β-lactamase (Figure 2) [48]. This was followed by a second wave of

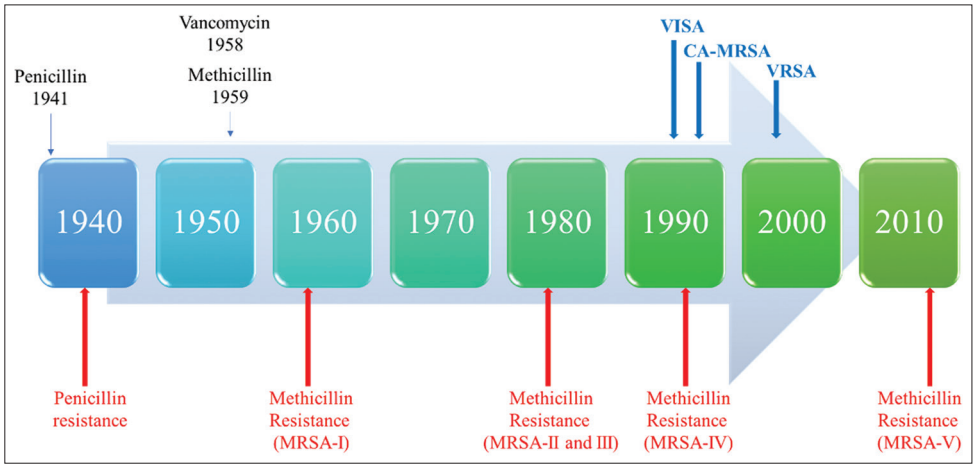

Figure 2: A timeline of resistance stages of Staphylococcus aureus following the introduction of antimicrobial agents

methicillin resistance that is mediated by the low affinity PBPs (PBP2a). These proteins are encoded by the *mecA* gene on a specific SCC (type I SCC*mec*, MRSA-I). Most CA-and HA-MRSA strains have specific SSC*mec* variants, indicating the prominent role of MGEs in the antimicrobial resistance. The third stage of resistance entailed the emergence of novel MRSA strains (MRSA-II and III), and it was still involving patients admitted to hospitals and health-care facilities (Figure 2).

As previously mentioned, vancomycin has been widely used to manage multiple MRSA strains. Nonetheless, due to the extensive prescription of vancomycin worldwide, resistant strains to vancomycin have been discovered in the mid-to-late 1990s [49], namely vancomycin intermediate-resistant *S. aureus* (VISA). These strains were acquired in the community, and they contained novel MGEs (MRSA-IV). Subsequently, completely resistant strains were reported in 2002 (vancomycin - resistant *S. aureus* [VRSA]) [50]. The minimum inhibitory concentrations (MICs) of VISA and VRSA strains are 4–8 μg/mL and ≥16 μg/mL, respectively. The VISA phenotype is acquired through a set of stepwise mutations in distinct genes that play important roles in the biosynthesis of bacterial cell walls (reviewed in [51]). This leads to a significant thickening of the cell wall. On the other hand, complete vancomycin resistance is conferred by the *vanA* operon, which is located on a plasmid [52]. The *vanA* operon is carried by a Tn*1546* element acquired from vancomycin-resistant *Enterococcus faecalis* [53].

The resistance of *S. aureus* to other antibiotics was also evident in the clinical practice. Mutations in PBP proteins, exclusively outside the penicillin-binding domain, seem to correlate with resistance to ceftaroline [54]. Interestingly, Kelley *et al*. [55] stated that ceftaroline resistance could be mediated by missense mutations that might had already been established before the introduction of the antibiotic. Besides, the involvement of mutated PBPs other than PBP2a, such as PBP3 and PBP4, has been reported in other investigations [54], [56]. This type of resistance can be overcome by combining ceftaroline with low doses of methicillin. Additional non-*mecA* mechanisms of resistance have been recently demonstrated, where mutated *clpX* endopeptidase, transcription terminator *Rho*, and *pp2c* protein phosphatase have influenced the resistance mechanisms [57].

From another perspective, mutations in the domain V of the 23S *rRNA* gene and the chloramphenicol florfenicol resistance (the presence of *cfr* gene) confer the resistance to linezolid [58], [59]. Moreover, cross-resistance between linezolid and other antibiotics acting on the ribosomal peptidyl transferase center, such as tiamulin, has been reported [60]. As for clindamycin, genetic mutations in the *erm* genes can cause significant alterations of the main binding sites (the 23S ribosomal RNA) by coding the methylase enzyme [61]. Such modifications can either be inducible or constitutively expressed [62]. Notably, the inducible resistance could not.

Regarding daptomycin, *S. aureus* seems to induce changes in the cell membrane and the membrane phospholipid content to make it more positively charged. This would create an electrostatic repulsive action against the positively charged daptomycin-calcium complexes to prevent their binding to the membrane. Such changes would be incurred by gain-of-function mutations in the *mprF* gene, which encodes the multiple peptide resistance factor protein; hence, it increases the expression of positively charged lysyl-PG [63]. Intriguingly, an alternative resistance pathway is mediated by the increased expression of the *dlt* operon and the subsequent enhancement of alanine attachment (positively charged) to teichoic acid in the cell wall [64].

Collectively, the dilemma of antimicrobial resistance is apparent for each introduced antibiotic to the market. These antibiotics lose their clinical values with overuse or if they are dosed incorrectly. Besides, resistance to some antibiotics, such as ceftaroline, may have been established in *S. aureus* strains even before drug introduction. These restrictions could be compounded by other virulence mechanisms that may support the pathogenicity of *S. aureus*.

Virulence factors of S. aureus in SSTIs

The skin represents the first line of defense against invading *S. aureus*, forming a physical barrier that prevents the entry of bacteria into deeper layers and/or internal dissemination. Keratinocytes are the major constituent of such a barrier. In addition to the physical function, keratinocytes can detect the invading microbes via their pattern recognition receptors, which would subsequently initiate the cutaneous innate immune response comprising of a proinflammatory response (interleukin-1α [IL-1α] and tumor-necrosis factor α [TNFα]) along with the production of AMPs (β-defensins) [65]. These changes are characteristic features of early abscesses, which contain multiple viable and dead polymorphonuclear leukocytes (PMNs), fibrin, tissue debris, and live bacteria in the central core [66]. Of note, PMNs play a significant role in abscess formation and resolution. They are heavily recruited to the site of infection in response to host proinflammatory molecules, tissue damage, and bacterial signals [67], [68]. Chemotactic factors produced by keratinocytes, macrophages, PMNs, and T cells would contribute to the influx of neutrophils in SSTIs. However, the resistant strains of *S. aureus* harbor an arsenal of virulence factors that can overcome the physical barrier and the cutaneous immune response; these factors are reviewed below.

Toxins and surface proteins

Toxins have a significant role in SSTIs pathogenesis through different mechanisms (Figure 3).

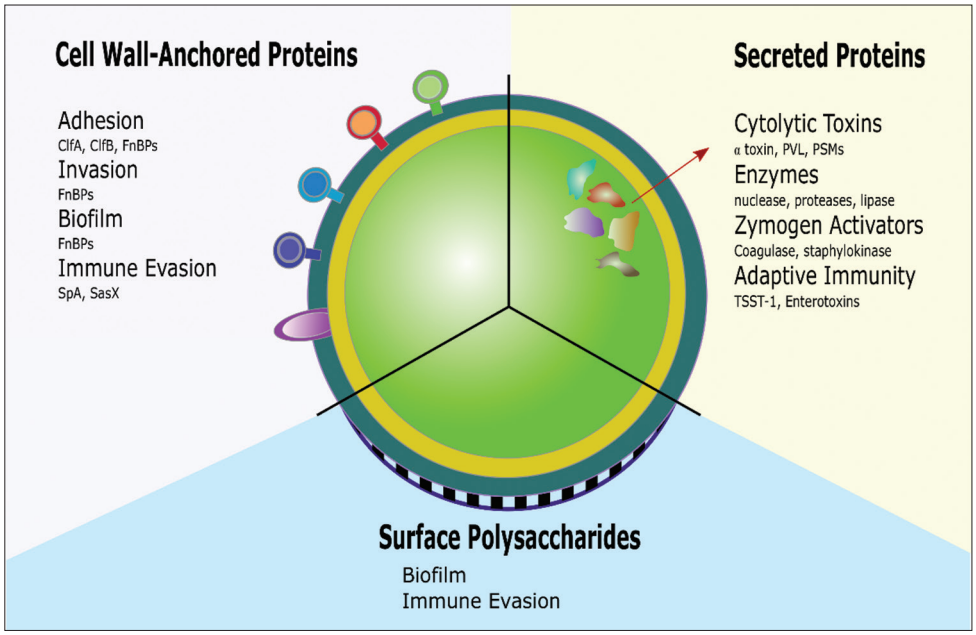

Figure 3: Summary of virulence factors of Staphylococcus aureus located on the cell surface or secreted by the bacterial cell

More specifically, the secreted cytolytic toxins have the most prominent effect on host cells by inducing pore formation and the subsequent cell lysis. These membrane-damaging toxins include bicomponent toxins (panton valentine leukocidin [PVL]), α-toxins, and phenol-soluble modulins (PSMs). PVL was first described in SSTIs in 1932 [69], as it was associated with severe, necrotizing lesions. This bicomponent exotoxin is carried by 90–95% of CA-MRSA strains in Australia [70], [71], indicating a prominent role of PVL in virulence, transmissibility, and fitness of the bacteria. However, less than one-third of HA-MRSA isolates are PVL-positive [72]. PVL is encoded by two genes (*lukS-PV* and *lukF-PV*). It binds to specific complement receptors on the neutrophil surface, causing pore formation and cell lysis. In a case–control study carried out among children presenting to an emergency department, PVL was significantly higher in MRSA isolates in patients with SSTIs than their peers without SSTIs [73]. However, the impact of this virulent factor on the clinical outcomes and the therapeutic efficacy is generally still elusive.

S. aureus α-toxin (α-hemolysin) is another β-barrel pore-forming, water soluble toxin. Neutrophils are not targeted by such a toxin; instead, α-toxin lyses macrophages and lymphocytes, and it can induce morphological changes in the platelets [74]. Actually, the endemic strain in the United States (USA300) is known to produce high levels of α-toxin as a result of expressing significant levels of the accessory gene regulator (*Agr*), which regulates multiple virulence factors [75]. In addition, experimental deletion of *saeRS* and *Agr* genes reduced the expression of α-toxin, and this reduced the number of skin lesions in a murine model [75], [76]. Furthermore, it has been shown that α-toxin is the most significant virulence factor in the PVL-negative CA-MRSA strains isolated in China [77]. Interestingly, therapeutic targeting of α-toxin by neutralizing monoclonal antibodies has significantly prevented dermonecrosis in mouse and rabbit models, and such an effect was optimized by the coadministration of vancomycin or linezolid [78], [79]. This suggests novel approaches for controlling SSTIs caused by MRSA.

Unlike the aforementioned β-barrel pore-forming toxins, PSMs (α and β) are non-cell-specific, receptor independent peptides which have a high ability to lyse neutrophils shortly after phagocytosis by *S. aureus* [80]. This leucocyte-destroying effect would facilitate immune evasion and persistence of skin infections. Presumably, PSMs play the most important role in the pathogenesis of SSTIs as evidenced by the production of higher quantities of PSMs in the clinical isolates of SSTIs as compared to other isolates from patients with infective endocarditis and pneumonia [81]. Intriguingly, these types of proteins can modulate the adaptive immune response in the most virulent MRSA strains via upregulation of the CCR7 receptors located on dendritic cell surfaces, and they enhance IL-10 production and reduce TNF production by $CD4^+$ dendritic cells [82]. Notably, PSM of the alpha type (highly expressed in CA-MRSA) is encoded by the *psm*α operon located in the core genome [83], as well as the *psm-mec* gene located within the MGE element SCC*mec* [84]. This indicates a potential correlation between PSM-mediated virulence and antibiotic resistance in *S. aureus*.

S. aureus also possesses multiple cell-wall anchored (CWA) proteins bound to the PGN which contribute to bacterial virulence. For instance, SasX has been associated with HA-MRSA epidemics in Asia, since it can be involved in immune evasion and abscess formation [85]. Surface protein A (SpA) is another

important factor of immune evasion. Its structural domains are key factors for binding to immunoglobulin (Ig) G, von Willebrand factor, and TNF receptor 1 (TNFR1) [86]. IgG binding provides a latent period during which *S. aureus* can establish itself in the skin, while binding to TNFR1 located on keratinocytes can promote a proinflammatory response. Other virulence proteins have been established, yet their contribution to resistance is still incompletely understood. For example, the clumping factor A (ClfA) contributes to platelet aggregation, and it protects *S. aureus* from phagocytosis by neutrophils via enhancing fibrinogen recruitment to the bacterial cell surface; therefore, it has an implication in abscess formation [87]. Similarly, ClfB has a role in determining the bacterial load at the infection site, which impacts the formation and structure of skin abscesses [88]. Fibronectin-binding proteins (FnBPs) can also control the bacterial burden in the abscess, and they enable the bacterial cell to effectively adhere to and invade keratinocytes [89], [90].

In sum, the secreted toxins by *S. aureus* are the most significant virulent factors that contribute to SSTIs, particularly dermonecrosis. This causes profound inflammation and excessive skin damage. Besides, the cytolytic effect on leucocytes can assist in immune evasion and facilitate the persistence of infection. The increased expression of these factors in MRSA strains indicates that they play a role in resistance; however, the exact resistance-related implications of these toxins are still unclear. Regarding surface proteins, only SasX has been found in resistant *S. aureus* strains, and they seem to be confined to hospital settings.

Quorum sensing

Quorum sensing is an important adaptive process to the external environment in *S. aureus*. It regulates the production of virulent proteins by a sufficient number of bacterial cells, who would have a higher infective potential than smaller populations. This regulatory mechanism is basically dependent on the population density, and it is regarded one of the major controlling mechanisms of pathogenesis. Cell-to-cell communication in quorum sensing is generally regulated by the Agr system [91]. This system is mediated by specific signaling molecules called autoinducing peptides (AIPs). These peptides are encoded by one of the four major transcripts of the Agr system (*agrD*) and are exported through the C-terminal cleavage of the *agrB* gene product. When AIPs reach a critical concentration threshold extracellularly (10 μm), they are detected by a sensor protein encoded by the *AgrC* transcript. Subsequently, the AgrC protein is phosphorylated and the associated AgrA protein binds to the promotor regions for RNAII and RNAIII (P2 and P3, respectively), as well as the PSMα and PSMβ (Figure 4). Consequently, the activation of the Agr system increases the expression of multiple virulence

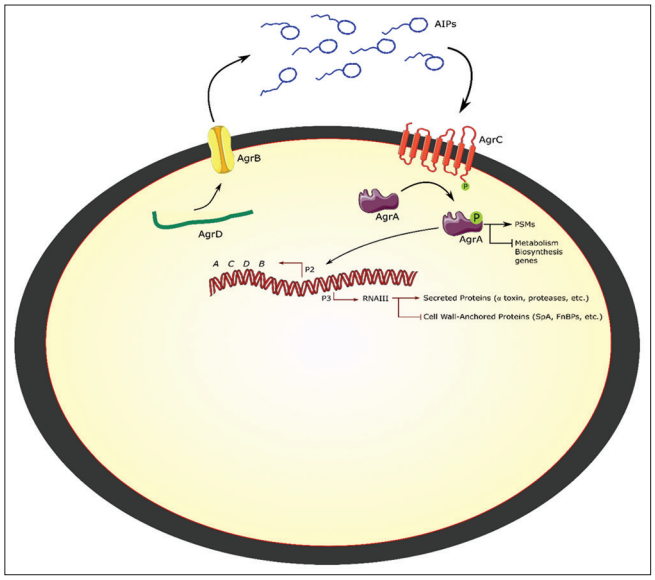

Figure 4: The quorum sensing circuit of Staphylococcus aureus

toxins, including α-toxin, PVL, and PSMs, as well as degrative enzymes, such as proteases [92]. However, it downregulates SpA [93]. Indeed, Agr regulation is critical for controlling the timing of expression of the mentioned virulence factors, where high Agr activity is apparent during the development of acute infection. On the other hand, decreased Agr activity is more prominent in chronic infections and biofilm formation [91].

Biofilm formation

In vivo growth of bacterial cells is more challenging than laboratory-based settings, where nutrients are readily available to the planktonically growing cells. Conversely, *S. aureus* may pursue other survival-supportive pathways in nutrient-deficient conditions. Bacterial cells tend to form multicellular aggregations encased in a self-produced matrix of extracellular polymeric substances (EPSs) [94]. The formation of a biofilm is a complex sequential process of three major phases (Figure 5). First, bacterial cells are attached to a biotic (living) or an abiotic (non-living) surface by hydrophobic, electrostatic forces. Second, these cells grow exponentially into multicellular layers via microbial surface components recognizing adhesive matrix molecules (MSCRAMMs) and accumulation-associated protein until the complete maturation of the biofilm. In this phase, active cells are predominant in the outer layer of the biofilm, while dormant, or possibly non-growing, cells are located in the center. The EPS matrix contains proteins, teichoic acid, exopolysaccharides, and/or micromolecules, such as extracellular DNA (eDNA). Finally, the biofilm ruptures or encased and planktonic cell clusters are dispersed to start a new invasive colony [95].

Indeed, biofilms represent a real challenge in the clinical practice. Bacterial cell attachment to the surface of indwelling devices and the subsequent

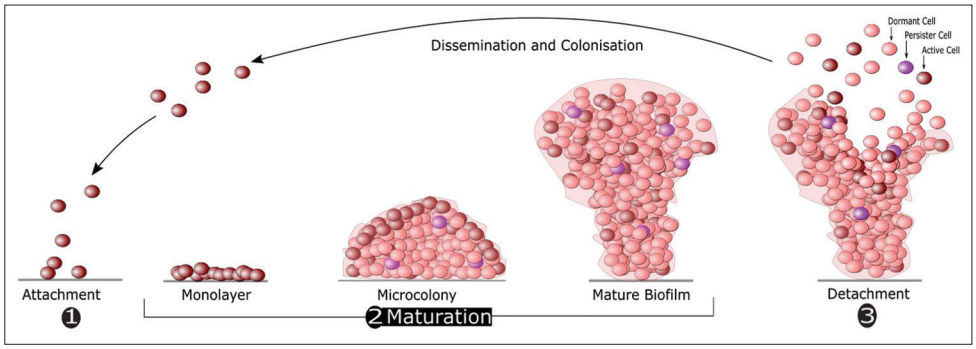

Figure 5: The lifecycle of a Staphylococcus aureus biofilm

biofilm formation are deemed the most common causes of device-related infections [96], [97]. This includes all types of implanted medical devices, such as prosthetic heart valves, central venous catheters, joint replacements, cardiac pacemakers, contact lenses, and intravascular lines. As a result, bacterial cells within a biofilm, particularly dormant cells, are tenacious to the host immune system and resistant to antimicrobial drugs. Such a "recalcitrance" state toward antibiotics is ascribed to tolerance and resistance mechanisms (Figure 6). In particular, the penetrative capacity of antibiotics is reduced with the existence of electrical differences with polymers within a biofilm [98]. Moreover, the presence of dormant cells, known as persisters [98], [99], represent a significant barrier to treatment since antibiotics can essentially act on biosynthetic processes (protein, DNA, and cell wall synthesis) in the growing bacteria. Although these persisters account for only a small proportion of the whole bacterial population (0.1%), they would be able to grow and confer high rates of antibiotic failure and recurrence of infections [100]. Another important mechanism of antimicrobial resistance in a biofilm is the bacterial efflux pump [101]. It removes toxic compounds from the bacterial cells, including antibiotics, and it can mediate multidrug resistance phenotype.

As a consequence, infections associated with biofilm formation have been associated with increased morbidity and mortality. Barsoumian *et al*., [102] found that biofilms produced by MRSA and *Pseudomonas aeruginosa* were associated with more severe infections and higher mortality rates than non-biofilm forming isolates. Surgical removal is inevitably required, causing prolonged hospitalization and

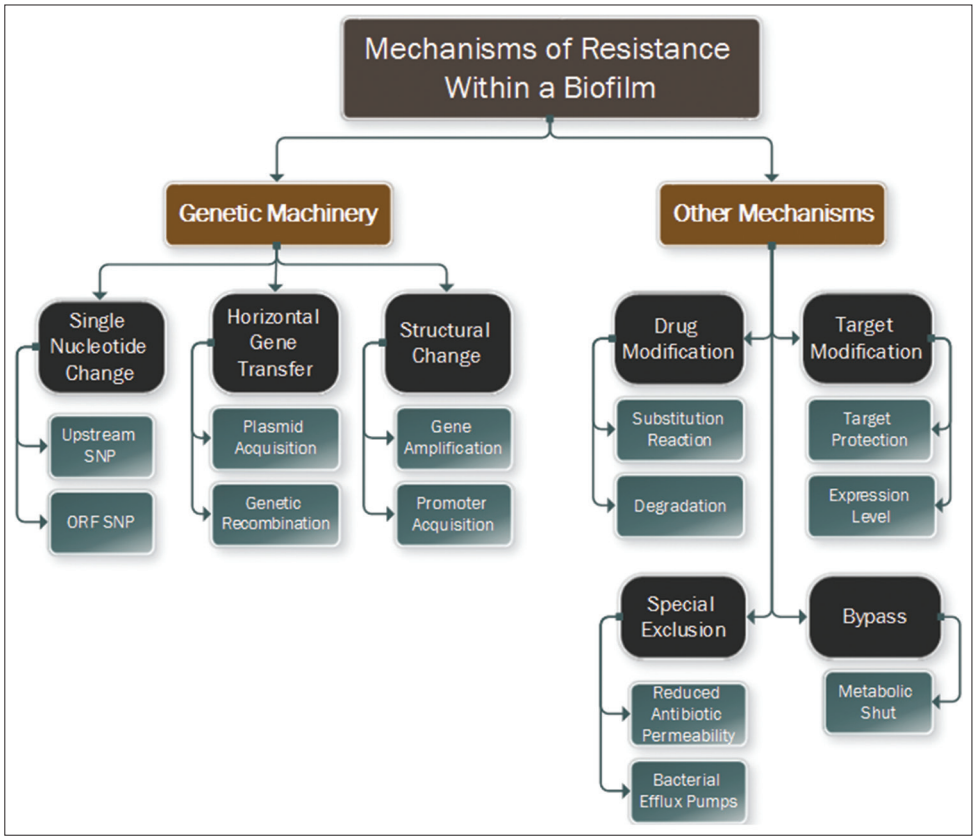

Figure 6: The major mechanisms of antimicrobial resistance in the biofilms of Staphylococcus aureus

significant costs to health-care systems [103], [104]. In skin infections, biofilm formation is a significant phenomenon in almost all types of SSTIs as revealed in a recent investigational study [105]. There is an early evidence indicating that bacterial cells in clinical isolates from patients with impetigo are more potent biofilm formers than those with furuncles [106], whereas other recent studies showed contrasting outcomes [105]. Seemingly, biofilms are formed by the colonizing strains already established on the skin surface, which may partly demonstrate that biofilm formation is crucial for successful colonization [105]. The latter notion is supported by the fact that the increased bacterial density of *S. aureus* on the skin correlates with the capacity of biofilm formation [107]. Nonetheless, the proficient biofilm-forming strains isolated from medical device infections as well as superficial skin infections could not exacerbate patients' symptoms as compared to other strains with a low capacity to form biofilms [108].

Pathogenic Changes Associated with *S. aureus* Infection

Besides the virulence factors of *S. aureus*, there is a variety of pathogenic mechanisms of skin diseases include a complex set of immunological, environmental, and physiological effects. These are responsible for the observed symptomatology and wide variation of severity of SSTIs, including impetigo, erysipelas, cellulitis, furuncles, folliculitis, carbuncles, scalded skin syndrome, and fasciitis [109]. The induced changes occur due to inflammation, the initiation of oxidative stress reactions, and the impairment of healing.

Inflammation

Physiologically, the physical and biochemical barriers of the skin are composed of keratinocytes as well as the associated lipids, sweat, and AMPs [110]. The outer epidermis contains keratinocytes in different stages of maturation, T cells, and Langerhans cells, whereas the inner dermis is formed by collagen, connective tissues, and elastin fibers. These fibers host several types of immune cells, such as dermal dendritic cells, macrophages, mast cells, T and B lymphocytes [111]. Therefore, the skin contains a considerable number of immune cells which can be involved in fighting against *S. aureus* infection. In particular, the first cells that recognize pathogenic microorganisms are the keratinocytes via their pattern recognition receptors, such as the scavenger receptors MARCO and CD36 as well as toll-like receptors (TLRs) and nucleotide-binding oligomerization domain-1 [112], [113]. The induced signaling pathways through these receptors are the main contributors of inflammation. This activates distinct transcriptional factors to form and release cytokines, including interferon γ (IFNγ), IL-1α, IL-1β, IL-17A and F, TNFα, and IL-22. Furthermore, signaling molecules mediate the generation of chemokines and antimicrobial effectors, such as inducible nitric oxide synthase and AMPs [114], [115].

Intriguingly, TLR2 on keratinocytes and resident macrophages recognize *S. aureus* to release neutrophilic chemoattractant and AMPs, such as defensins and LL-37, and this enhances cytokine release and phagocytosis [114]. Therefore, it is thought that TLR2 is a critical element in combatting *S. aureus* infection [116]. The recruitment of monocytes and neutrophils to the site of infection is regulated by dermal and perivascular macrophages [117]. The recruited neutrophils in the skin can phagocytose *S. aureus* cells, undergo degranulation, and form extracellular nets (traps) for bacterial cell killing. This would mediate abscess formation, which would ultimately be encapsulated with a fibrous substance and macrophages (Figure 7). However, toxins produced by virulent strains, such as PVL, PSMs, and α-toxin, can accelerate neutrophil death and subsequently initiate the release of IL-33, IL-1α and other danger associated molecular patterns, leading to variable forms of SSTIs [66].

Oxidative stress

Oxidative stress is another important part of the host immune response. During an oxidative burst (following phagocytosis), macrophages, neutrophils, and monocytes can generate O^-_2, HOCL, and H_2O_2 (components of the reactive oxygen species [ROS]) via the action of NADPH oxidase and myeloperoxidase. This causes bacterial cell death by direct and indirect mechanisms [118], [119]. However, ROS accumulation, either by ROS overproduction or impaired elimination, can lead to oxidative stress. In such an instance, cell damage is induced by protein oxidation, DNA mutation, and lipid peroxidation [120]. In *S. aureus* infections, oxidative stress is promoted in neutrophils, macrophages, and leucocytes, and this is associated with increased free radical production and limited antioxidant effects by such cells [121]. These changes would cause further damage to the injured skin in SSTIs causing increased severity of symptoms and exacerbated inflammation. ROS overproduction can also damage extracellular matrix proteins and alter the functions of fibroblasts and keratinocytes. Besides, it maintains the activation of proinflammatory cytokines and activates metalloproteases [122].

Like ROS, nitric oxide is a reactive oxidant having bactericidal and properties against *S. aureus.* It is virtually produced by all immune cells, and it is regarded an important component of reactive nitrogen species [123]. Nonetheless, excessive nitric oxide levels can induce adverse effects by inducing apoptosis of host

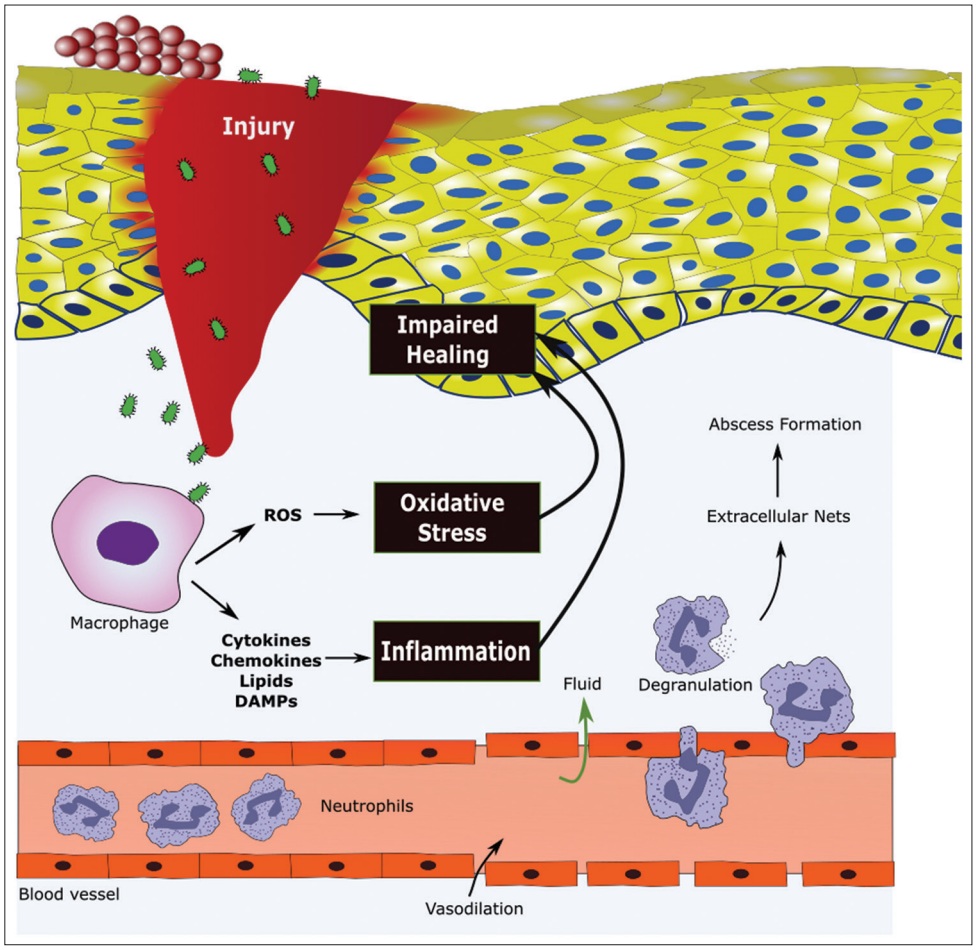

Figure 7: Pathophysiologic response to Staphylococcus aureus skin infection

cells, inhibiting cell proliferation, and preclude antigen presentation and TNFα production by phagocytes. In addition, nitric oxide can be utilized by *S. aureus* to proliferate and to mediate lactic acid fermentation to inhibit the activation of stress regulon [124].

Impaired healing

Normally, wound healing comprises of four stages, including coagulation, inflammation, cell division and epithelial resurfacing [125]. The existence of pathogenic *S. aureus* would interfere with the healing process through protease and toxin secretion, which would promote antibiotic resistance [126]. *S. aureus* toxins reduce the number of fibroblasts, preclude collagen production, and maintain the production of inflammatory mediators [127]. Furthermore, chronic infections characterized by excessive release of oxidants and proteases by immune cells would degrade the extracellular matrix; hence, it cause difficult healing. Recently, it has been shown that biofilm formation in chronic *S. aureus* infection contributes to the inhibition of granulation tissue formation and impaired tissue biomechanics [128]. In addition, these biofilms would deplete oxygen, raise tissue alkalinity, and induce dermal cell apoptosis, which impede wound healing [129].

PART II - STAPHYLOCOCCUS AUREUS IN TWO MAJOR INFLAMMATORY SKIN DISORDERS-AD AND PSORIASIS

S. aureus and skin inflammatory lesions

In the healthy skin, the adaptive immune homeostasis is controlled by commensal skin microbes via interaction with specific populations of effector T cells and antigen-presenting cells (APCs) [130], [131], providing a balance between supporting the commensal microbial survival and protecting against the overgrowth of pathogenic organisms. The relationship between dysbiosis of skin microbes and some inflammatory conditions has recently grabbed the attention of researchers. This was specifically relevant in patients with AD and psoriasis.

AD

In AD patients, skin barrier functions are significantly impaired, with multiple defects in the innate immune activity and decreased expression of AMPs [132]. Loss-of-function mutations in the FLG gene, which encodes the structural protein filaggrin in keratinocytes and corneocytes, represent a major genetic risk factor for AD development [133]. As a consequence, AD patients are susceptible to increased *S. aureus* colonization and reactive sensitization. It has been shown that *S. aureus* could be isolated from 80 to 100% of atopic skin lesions, often without apparent symptoms of infection [134]. The strain has led a state of temporal dysbiosis in active atopic inflammatory lesions as revealed by microbiome analyses, indicating the contribution of *S. aureus* in AD [135]. Besides, the density of staphylococcal populations has been associated with the severity of eczema [136], and this might correlate with AD flare-up. Indeed, the inherent changes in the morphology and surface composition in dead keratinocytes (corneocytes) of the AD skin might provide a good medium to multiple ligands to which *S. aureus* can be attached [137]. For instance, fibronectin is abundant in AD lesions; thus, it becomes available to the staphylococcal FnBPs [138]. In additional, the cornified proteins cytokeratin and loricrin facilitate the binding to multiple microbial CWA proteins [139].

In addition to the relationship between *S. aureus* colonization and AD inflammation, *S. aureus* possesses a couple of virulence factors that exacerbate the disease, including superantigens, biofilm formation, and virulence proteins (Figure 8). Microbial superantigens are a group of toxins that activate large populations of T cells at small concentrations. These include staphylococcal enterotoxins (SEs), toxic shock syndrome toxin-1 (TSST-1), and the SEs-like proteins [140]. Several SE serotypes have been described, ranging from SEA to SEE and SEG-SEQ. In contrast to conventional antigens, superantigens act without internalization or antigen processing, bind to the variable region of the β chain (Vβ) of T receptors, and bind to subtypes of APCs with the major histocompatibility complex class II (MHC II) different than those bound to conventional antigens [141]. Upon T cell activation, proinflammtory cytokines and chemokines, such as IL-1, IL-2, TNF α, and IFNγ, are massively produced, leading to the development of fever, hypotension, and shock.

In the context of AD, staphylococcal strains that produce large numbers of superantigens are associated with profound T cell activation that could be resistant to the immunosuppressive effect of corticosteroids, a matter which is evident during patient management [142]. T cells exert a major role in skin inflammation, and the lesions could be exacerbated by superantigens produced by *S. aureus*, particularly SEA, SEB, SEC, and TSST-1 (Figure 9). These superantigens bind to MHCII molecules on the surface of APCs and T cell receptors on T cells. Such toxins induce selective accumulation of T cells expressing Vβ and induce Th2 cells to release IL-31, which causes several pathogenic consequences, such as precluding keratinocyte differentiation and reduced filaggrin expression; therefore, patients would experience skin barrier disruption and itching. Furthermore, superantigens act also as allergens and mediate a profound IgE response [143]. As a consequence, histamine is released by mast cells and basophils in sensitized patients (Figure 9).

Besides superantigens, several virulence factors can interact with AD lesions simultaneously. The susceptibility of differentiating keratinocytes to α-toxin increases in AD patients than healthy individuals due to reduced filaggrin expression; hence, the skin barrier is significantly disrupted, allowing the penetration of allergens and irritants [144]. Moreover, the increased expression of SpA and its ligand TNFR1 in AD patients indicates that SpA may exert a potent role in inflammation via inducing a proinflammtory response [145]. Furthermore, PSMs and gamma toxin cause non-degenerative degranulation and lysis of mast cells, respectively, and they can result in excessive skin damage and inflammation [146]. Noteworthy, Jun *et al*., [145] introduced a concept of

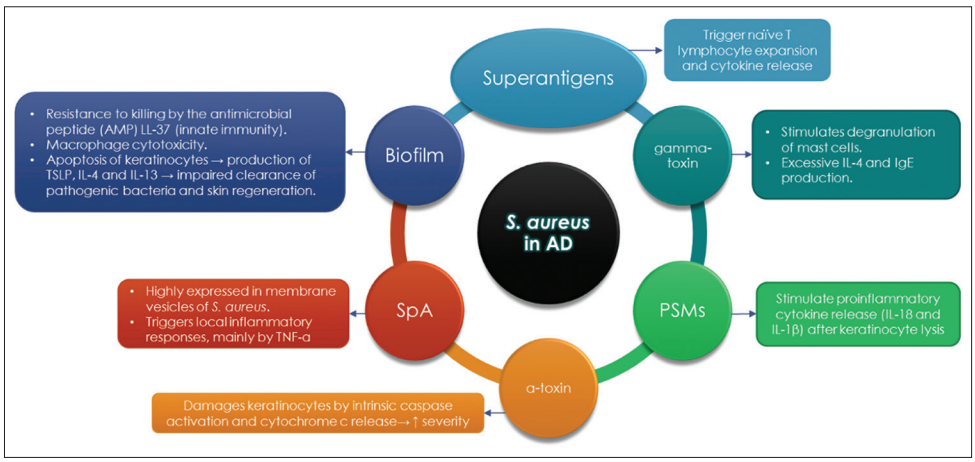

Figure 8: Implications of Staphylococcus aureus in atopic dermatitis

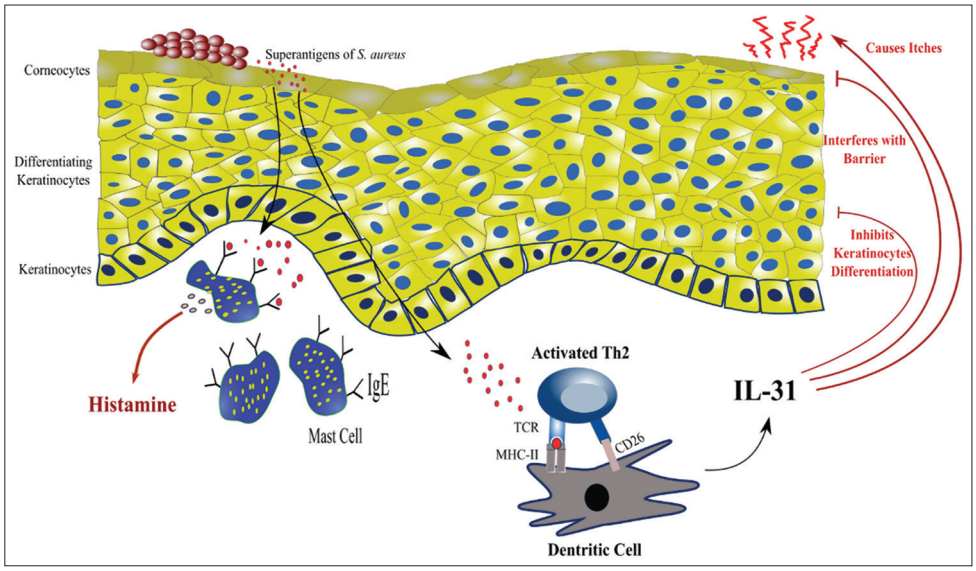

Figure 9: The pathogenic process induced by Staphylococcus aureus in atopic dermatitis patients

membrane vesicles (MVs), which can deliver SpA and potentially superantigens to keratinocytes *in-vitro*. Only intact MVs were able to perform this function, causing worsening of AD symptoms. Finally, biofilm formation is a hallmark property of *S. aureus* strains in AD lesions, while such a phenomenon is less recognized in non-lesional areas [147]. Actually, biofilm producers can lead to increased AD severity, impaired host immune responses, increased rates of recurrent and refractory infections, and increased resistance to antimicrobials as compared to planktonic bacteria [147], [148], [149], [150]. Interestingly, proteases in a *S. aureus* biofilm are responsible for the degradation of cathelicidin LL-37, an important skin AMP; therefore, chronic *S. aureus* colonization is sustained [149].

Notably, reduced *S. aureus* colonization decreases the severity of AD lesions [150], [151]. Nevertheless, although antiseptics and antibiotics can reduce bacterial colonization, relapses can take place within a few weeks due to decolonization. In essence, resistant strains to decolonization by fusidic acid and mupirocin are increasingly reported [152]. As a consequence, the lack of therapeutic efficacy with the emergence of these resistant strains has underscored the importance of avoiding the long-term use of topical or systemic antimicrobial in AD [153].

In sum, skin colonization with *S. aureus* is frequent in AD patients, leading to exacerbation of skin inflammation by several virulence factors. There is a lack of effective topical or systemic antibiotics, even when used over long periods, for bacterial decolonization during AD flare-ups when added to steroid treatment [154]. This might be associated with the emergence of resistant strains or might increase the risk of adverse effects owing to using antibiotics in high concentrations. Recent reports have demonstrated that MRSA strains were isolated from skin lesions in 12.9–26.6% of patients with AD, with the predominance of PVL and SEB toxins compared to susceptible isolates [155], [156], [157], [158]. As such, there is a strong need to develop robust alternatives with a high-efficacy and reasonable safety measures.

Psoriasis

Psoriasis is another chronic inflammatory condition affecting about 0.1–11.4% of the general population [159]. Multiple pathogenic mechanisms have been proposed for disease pathogenesis, including autoimmune reactions, systemic drugs, mild trauma, infections, and stress [160]. The link between skin microbial dysbiosis and disease activity has been investigated, yet most of the studies have focused on *Streptococci*. This is because substantial alteration of the skin microbiota has been dominated by *Streptococcus* spp. in psoriasis lesions; however, streptococcal infection has been involved in initiating a single subtype of the disease (guttate psoriasis) [161], [162]. Nevertheless, the diversity of microbial communities in psoriasis lesions [163], [164] has suggested other microbial signatures which may have additional roles in disease pathogenesis.

The impact of *S. aureus* on disease pathogenesis has been studied elsewhere. The bacteria can exacerbate the disease by acting as a triggering factor, which can initiate a robust immune response mediated by TLRs [165]. More specifically, the triggering molecules include the cytolytic α-toxin, SpA, superantigens (mostly SEA and SEC) [166], lipoteichoic acid, and the staphylococcal PGN. Indeed, the immunomodulatory effects of *S. aureus* in psoriasis are exerted in two major pathways. First, PGN induces the expression of IL-13 and the vascular endothelial growth factor in keratinocytes. IL-13 would further stimulate VEFG expression in a positive feedback loop. The second pathway includes increasing the expression of the human cathelicidin LL-37 by the infiltrating neutrophils and keratinocytes under the

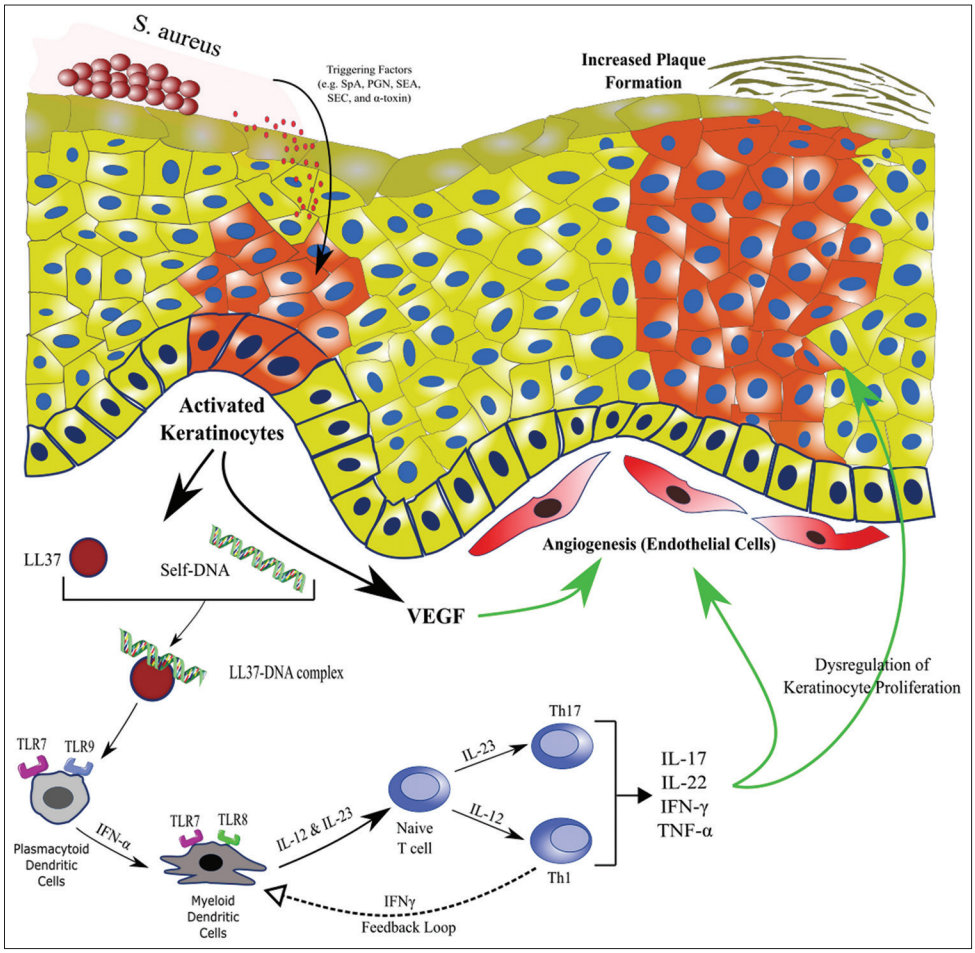

Figure 10: The interaction between keratinocytes and immune cells in psoriasis

influence of PGN and other staphylococcal triggering molecules. Subsequently, LL-37 binds to self-DNA fragments located in the extracellular dermis; hence, such a complex would stimulate the release of IFN-α by plasmacytoid dendritic cells via TLR9 activation. Ultimately, Th1 and Th17 are activated, and IL-22, IL-17, and INF-γ are overexpressed. The released IL mediate further LL-37 production, which repeatedly induces proinflammatory responses as seen in psoriasis (Figure 10).

The aforementioned postulated mechanisms may explain the inflammatory pathways induced by *S. aureus* virulence factors in psoriatic lesions. This might be influenced by the degree of bacterial colonization and infection. A systematic review and meta-analysis of 21 comparative studies has shown that patients with psoriasis had a 4.5-fold increased risk of *S. aureus* colonization compared to healthy controls [167]. Besides, the proportion of psoriasis patients with MRSA were significantly higher than MRSA-colonized healthy controls (8.6% vs. 2.6%, respectively) [167], However, the impact of other cocolonizing organisms may be evident. Chang *et al*., [163] have demonstrated a loss of microbial community stability, with a significantly increased colonization of *S. aureus* and other pathogenic strains at the expense of other immunoregulatory bacteria, such as *Propionibacterium acnes* and *Staphylococcus epidermidis*. In the same context, Fyhrquist *et al*., [164] have recently shown that while *S. aureus* was the dominating microbial species in AD lesions, skin psoriatic lesions were colonized by multiple genera, such as *Streptococcus*, *Staphylococcus*, and *Corynebacterium*. Actually, patients with psoriasis are 36% less likely to encounter *S. aureus* colonization on their skin lesions compared to AD patients [167].

In a nutshell, *S. aureus* colonization in psoriasis lesions may play a role in psoriasis pathogenesis. Such effect is mediated via a superantigen-driven TLR9 pathway with selective T cell recruitment. Distinct types of skin cells, such as dendritic cells and keratinocytes, contribute to the process of plaque development. However, its impact on the inflammatory processes is less apparent than that in AD patients given the diversity of colonizing organisms in psoriasis patients. While several aspects of psoriasis pathogenesis are still enigmatic, the identification of early triggering factors, among which *S. aureus* products are involved, could provide novel therapeutic targets for the prevention and treatment of psoriasis.

PART III - NEW TREATMENT STRATEGIES TO SURMOUNT ANTIBIOTIC RESISTANT SKIN INFECTIONS USING HERBAL COMPOUNDS

Novel Treatment Strategies for Anti-microbial Resistant Skin Infections: Herbal Treatments

From the foregoing discussion, the frequent isolation of *S. aureus* and MRSA strains in skin infections and some inflammatory conditions may corroborate their role in disease pathogenesis. For skin infections, several antibiotics have been used in the clinical practice. In dermatology, mupirocin (pseudomonic acid) has been extensively applied topically to treat patients with SSTIs and to eliminate nasal carriage of MRSA [168]. However, mupirocin resistance has been shortly detected 2 years after its first introduction in 1985 [169]. Furthermore, the increased use of such antimicrobial for the management of chronic infections has been associated with increased rates of resistance. While early studies showed that mupirocin resistance in clinical isolates ranged between 7.7% and 19% in the late 1990s [170], more recent reports demonstrated incidences that reached as high as 31.3% [171].

This raises the need for identifying and developing new methods of treatment. Plant-based medicines have been used since 5000 years ago for the treatment of multiple conditions, including infectious diseases [172]. From the historical perspective, much is known about the origin and development of many medicinal plants in the traditional medicine of the ancient Greeks, Ayurveda, and traditional Chinese medicine [173], [174]. In North America, the use of medicinal plants has begun by the Native Americans, and subsequently conveyed to the European settlers [175]. In Australia, the Aboriginal pharmacopeia has essentially been incorporated with a diverse set of herbal remedies that served the Aboriginal people as well as the colonists who arrived in the late 1700s [176]. Thus, the historical basis of medicinal plant applications in disease management, including infectious diseases, is robust and continues to this day.

Recently, the interest in herbal remedies has greatly increased. A specific search in the PubMed database between 1970 and 2020 shows that there are more than 19500 publications concerning the antimicrobial activity of medicinal plants and their extracts. Of them, about 950 scientific articles investigated herbal implications in skin infections caused by *S. aureus* and/or MRSA. Plant-derived compounds have exhibited favorable outcomes in combating the emergence of antibiotic resistance; hence, they may potentiate the action of antibiotics and restore the activities of antibacterial agents against which *S. aureus* has developed a resistance [177]. The antimicrobial properties of herbal agents emerge from multiple factors. First, herbal bioactive compounds have a complex composition, such that a single active constituent may improve the action of another one. This would extend the biological activities of a given compound in a significant manner rather than using that constituent solely. The potentiation of antimicrobial effects is ascribed to increased membrane permeability, disrupted efflux pumps, or precluded enzymatic degradation [178]. Second, the existence of multiple active constituents may exert significant additional actions on several targets. These include anti-inflammatory, antioxidant, and/or healing-promoting effects. Acting on multiple physiological mechanisms would facilitate treatment and improve the outcomes. Finally, herbal compounds within a single extract may exert synergistic actions, accounting for multiple benefits. The pharmacokinetic and physiochemical effects of herbal compounds are improved, including the chemical solubility, reabsorption, and bioavailability. Besides, the resistance mechanisms of bacteria may be partly counteracted. In additional, the adverse effects of a distinct compound may be alleviated by another compound in the same extract. As a consequence of these benefits, it has been shown that mixtures of herbal compounds can possess significant antimicrobial effects than the isolated compounds [179]. The most beneficial plant-based compounds in the treatment of *S. aureus* dermatological infections are listed below according to their chemical structures.

Terpenes

Terpenes and terpenoids constitute a large group of bioactive herbal compounds with a substantial chemical diversity. More specifically, more than 40,000 structural forms of terpenes have been identified, of which a few classes possess pharmaceutical properties [180]. In general, the basic building blocks of terpenes are the 5-carbon isoprene (C_5H_8) units. The addition/removal of functional groups in terpenes creates a group of derivatives named terpenoids, which have variable antimicrobial activities according to the structural changes [181].

Furthermore, the most influential factors of antimicrobial activity of phenolic terpenoids are the hydroxyl group and the delocalized electrons.

Carvacrol

Carvacrol is a monoterpene found in the essential oils of *Thymus vulgaris*, *Origanum vulgare*, *Trachyspermum ammi*, *Citrus bergamia*, and *Lepidium africanum* [182]. It has recently attracted the attention of researchers owing to its wide-spectrum antimicrobial activities. Besides, it has strong antioxidative properties

due to the presence of a hydrophilic phenolic OH group and a substituted hydrophobic aromatic ring [183].

In the literature, the MIC of carvacrol was divergent. It ranges from 78 to 500 μg/mL for *S. aureus* [184], [185]. *S. aureus* cells exposed to minimum bactericidal concentrations (MBCs) of carvacrol for 24 h had depressed PGN structures with deformed and wrinkled cell membranes, indicating the leakage of intracellular content [186]. It is likely that carvacrol has the ability to affect bacterial cell membrane integrity by disrupting the proton gradient via exchanging the hydroxyl proton for potassium ion. The hydroxyl group may also cause significant changes in the composition of phospholipids and fatty acids in the cell membrane, causing changes in the membrane fluidity and permeability [187]. Carvacrol and other components of the essential oil may additionally disperse distinct enzymes which mediate fatty acid synthesis, such as multicomponent membrane desaturase [188]. This can be directly carried out by increasing saturated C16 and C18 fatty acids and decreasing unsaturated C18 fatty acids. Indirect effects can involve the interference with enzymes involved in fatty acid synthesis (multicomponent membrane desaturase) as well as cis-trans isomerase which regulates the adaptive mechanisms to environmental stresses (Figure 11) [187]. These effects are exerted simultaneously with the aid of essential oil components rather than a single phytochemical compound, and this would ultimately increase the amount of fatty acids, decrease membrane fluidity, and increase membrane rigidity [189].

From another perspective, Mouwakeh *et al*., [190] have shown that intracellular accumulation of ethidium bromide was significantly increased in the presence of carvacrol in methicillin-susceptible strains and MRSA, and this subsequently associated with the inhibition of mepA efflux pump activities. Efflux mechanisms are known methods of antimicrobial resistance [191]. Interestingly, membrane integrity of both susceptible and resistant bacterial cells decreased by 50% by the half MIC, and preformed biofilms by these strains have been reduced effectively by 11–35% after carvacrol treatment [190].

When tested against oxacillin and vancomycin-resistant *S. aureus* strains, carvacrol significantly reduced biofilm formation compared to negative controls, and it also had low MICs against those strains (250 μg/mL) [192]. Evidence indicating the proven activity of the hydroxyl group is supported by the lack of significant inhibitory effects exerted by another phenolic compound lacking such a structural group (p-cymene) [190]. Other postulated mechanisms of action include the induction of ROS and modification of fatty acids in the bacterial cell membrane [193], [194].

However, despite these robust experimental outcomes, the applicability of carvacrol in skin infections remains limited due to its permeability, which results in poor skin retention and the need to frequent applications. Recently, Mir *et al*. [195] developed a drug delivery system based on biodegradable polymeric nanoparticles (NPs) which could be incorporated into *ex vivo* skin lesions infected by lipase-producing *S. aureus* strains. A hydrogel matrix loaded with NPs improved the release of carvacrol and enhanced its skin retention after 24 h. This sustained effect was also effective against MRSA at the sites of infection. However, future comprehensive studies that investigate the biocompatibility, pharmacodynamics, and pharmacokinetics of carvacrol *in-vivo* are needed to prove its efficacy and safety as a viable alternative of conventional antibiotics.

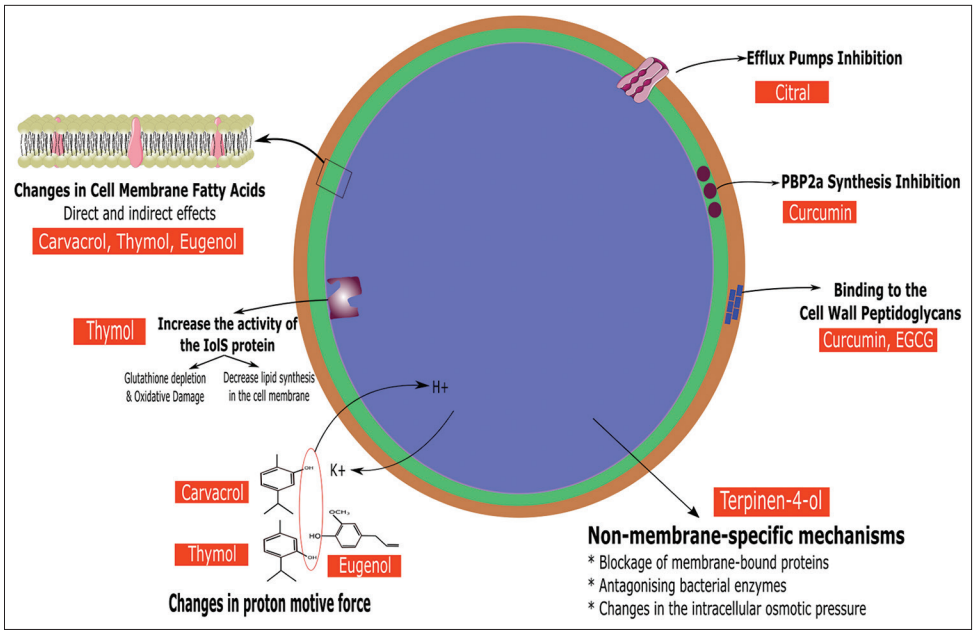

Figure 11: Summary of the effects of herbal active compounds on MRSA

Thymol

Thymol is a natural phenolic monoterpene present in many types of essential oils extracted from *T. vulgaris*, *O. vulgare*, *T. ammi* and other multiple types of plants [196], [197]. It is a carvacrol isomer with antioxidant, antimicrobial, anti-inflammatory, and antispasmodic properties [198]. The antioxidant effects of thymol have supported its use as nutritious substances in functional foods [199]. In addition, there is an evidence showing anti-cancer effects of thymol [200]. Thymol has also been utilized in the dentistry practice in a combination with chlorhexidine to prevent the development of caries [201].

Focusing on the antimicrobial activity, the first investigational study of thymol activity against *S. aureus* was in 2004 [184]. Results showed significant inhibitory actions against both susceptible and resistant strains, and there was no significant difference between these strains. In a more recent study, García-Salinas *et al*., [186] showed that thymol exhibited strong bactericidal activities. In particular, scanning electron microscopy (SEM) revealed a significant damage of the PGN layer following the exposure to MBCs of thymol for 24 h. Notably, a thymol-carvacrol combination had an additive bactericidal effect, whereas the combined effects of thymol and the active compound cinnamaldehyde was not apparent. The additive actions of thymol and carvacrol may be related to the similarity in their chemical structures [186]. Similarly, Kifer *et al*. [202] found that the MIC of thymol against *S. aureus* planktonic cells ranged between 0.250 and 0.375 mg/mL, while the minimum biofilm-eliminating concentration of a mupirocin-thymol combination was two-fold higher than the MIC. Another combination comprising of thymol, EDTA, and vancomycin acted synergistically to reduce the colony count of MRSA strains [203]. As such, it seems that thymol combinations with other antimicrobials are more effective in reducing the burden of infection; however, more extensive studies are needed to assure the clinical efficacy and safety of these combinations.

As with other phenolic terpenes, thymol has the ability to move across the watery extracellular medium (due to its relative hydrophilicity) and induce significant alterations in membrane permeability via leakage of protons, potassium ions, and ATP [204]. This leads to leakage of intracellular components [186]. The bactericidal effects of thymol could be attributed to its capacity to bind to IolS and increase its activity as revealed in bioinformatics analyses [205]. IolS is a protein which is able to reduce aldo-keto reductase (AKR) substrates, such as carbonyl substrates, in the presence of NADPH. AKRs play important roles in the metabolism of steroids, which are pivotal for bacterial cell membrane fluidity. Thymol has a significant implication in enhancing the AKR activity of IolS; therefore, NADPH is depleted, leading to the depletion of glutathione (GSH), increased susceptibility to hydroxyl free radicals, oxidative damage, and eventually bacterial cell death [205]. NADPH depletion can also decrease the rate of lipid synthesis and adds to the cell membrane compromise.

Notably, thymol activity against *S. aureus* biofilms has been investigated elsewhere. Kifer *et al*. [202] indicated that the biofilm inhibitory potency of a thymol-mupirocin combination was significantly higher than that of mupirocin combinations with other monoterpenes, including menthol and 1,8-cineole [202]. It is therefore possible that thymol has enhanced the activity of mupirocin through disturbing membrane permeability; thus, a synergistic action was evident. Similar observations regarding antibiofilm activities were recently reported, where bacterial growth within preformed biofilms was significantly reduced after thymol treatment compared to non-treatment or treatment with cinnamaldehyde [186]. The mechanism by which thymol can inhibit biofilm formation is still unknown. It has been postulated that the phenolic phytocompound can interfere with the production of MSCRAMMs and the release of eDNA within biofilms [206].

It is noteworthy that the subcytotoxic doses of thymol were 0.090, 0.060 and 0.060 for fibroblasts, keratinocytes, and macrophages, respectively [186]. These values were lower than the MIC and MBC concentrations, but they were higher than those reported for the skin antiseptic chlorhexidine. Indeed, these important experimental outcomes corroborate the safety of thymol and support a rationale to its integration in skin preparations as an alternative to chlorhexidine for the treatment of infected wounds.

In addition, thymol application in skin diseases could extend to AD lesions. While intact MVs are required to effectively deliver effector molecules, such as SpA and superantigens, to keratinocytes in AD lesions and promote inflammation (discussed in section 4.4.1), topical application of thymol suppressed AD exacerbation in a mouse model by the disruption of MVs [207]. More specifically, thymol treatment disrupted the membranes of EVs, and thus it inhibited the expression of pro-inflammatory cytokines and chemokines induced in response to MVs, suppressed the inflammatory responses mediated by Th1, Th2, and Th17, and decreased the levels of IgG2a and total IgE. Therefore, in addition to the direct antimicrobial properties, thymol can alleviate the exacerbation of *S. aureus* infection via targeting EV-induced inflammatory responses in AD [208].

Terpinen-4-ol

Terpinen-4-ol is the principal component of tea tree oil, which is obtained by steam distillation of the Australian native plant *Melaleuca alternifolia*. The first evidence regarding the antimicrobial effects of tea tree oil has been published three decades ago, when Carson *et al*. [209] found that the MIC against MRSA was

0.25 mg/mL. Since then, such an essential oil has proven effective as an antiviral and antifungal agent and it had bactericidal activities against several types of bacteria, including MRSA, methicillin-sensitive *S. aureus*, and coagulase-negative *S. aureus* [210], [211], [212], [213].

Nevertheless, the main component of the essential oil has different results. The MIC and MBC of terpinen-4-ol were significantly better than tea tree oil against both coagulase-negative and MRSA clinical isolates taken from skin samples of patients who had undergone spinal surgery [213]. Moreover, the $\log_{10}$ reduction in viable count of MRSA was significantly greater following an experimental exposure of the isolates to 5% terpinen-4-ol compared to 5% tea tree oil [213]. Likewise, Noumi *et al*. [214] have pointed out that the MIC against MRSA strains isolated from skin lesions and the blood ranged between 0.048 and 1.52 mg/mL for terpinen-4-ol and 6.25–50 mg/mL for the whole volatile oil, and the difference was statistically significant.

It is therefore plausible that the presence of a non-oxygenated component in tea tree oil, such as γ-terpinene, might have accounted for the reduced aqueous solubility of the compound, thereby reducing the effective concentrations of active molecules in the bacterial cell surface. On the other hand, the use of terpinen-4-ol exclusively has exerted potent antimicrobial properties given the combined hydrophilic (to diffuse through the surrounding media) as well as hydrophobic properties (to deliver the active compound to the cell surface) [213]. In contrast, a recent investigation of the available commercial preparations of tea tree oil used for cutaneous infections [215], it has been shown that the antimicrobial batches with the highest concentrations of terpinen-4-ol were the least effective in combatting MRSA and *P. aeruginosa* compared to those with low concentrations of the active compound. The synergistic effect of different compounds in the mixture may be notable; however, this warrants further investigations.

In general, it seems that antimicrobial preparations of tea tree oil formulated based on ISO 4730 specifications are effective in reducing the MRSA burden. This includes ≥30% terpinen-4-ol and <15% 1,8-cineole. To get an insight into the main mechanism of action involved in the bactericidal effect of terpinen-4-ol, Carson *et al*. [216] revealed a significant increase in the optical density of bacterial suspensions treated by tea tree oil or terpinen-4-ol exclusively for 60 min (but not 30 min) compared to control suspensions. Besides, the authors viewed multilamellar, mesosome-like structures in the cytoplasm of many treated cells, which were not seen in untreated cells. These findings indicate that the active compound compromised the cellular morphology of bacterial cells and induced a delayed cell lysis. Such a delayed effect might be explained by non-membrane-specific mechanisms, such as blockage of membrane-bound proteins or antagonizing bacterial enzymes [216]. The non-specific mechanism can be further corroborated by the lack of a significant difference in the antimicrobial activity between terpinene-4-ol and its L-isomer [213], [217]. Alternatively, terpinen-4-ol might cause changes in the intracellular osmotic pressure, which weakens the cell wall and causes rupture of the cell membrane [216]. Concomitantly, sublethal doses of terpinen-4-ol might alter membrane permeability and impact its capacity to regulate the osmotic pressure and its ability to exclude toxic materials. This might partly exclude a direct effect on the cytoplasmic membrane [216], [218].

Of note, the appearance of mesosomes and the loss of cytoplasmic content may support the inability of terpinen-4-ol to lyse *S. aureus* cells, and these findings are comparable to other antimicrobial agents, such as vancomycin and phenethyl alcohol [219], [220]. Recently, Ramadan *et al*. [221] have shown prominent bactericidal effects of a tea tree preparation (44% terpinen-4-ol) incorporated into silver NPs. The authors found that *S. aureus* cells contained the NPs in their cell walls and cell membranes, and these structures became detached with a severe damage of the whole bacterial cells as viewed by transmissible electron microscopy (TEM). Therefore, the antimicrobial effect of terpinen-4-ol may be attributed to a series of cellular events that would ultimately alter the chemiosmotic control of bacterial cells.

Regarding antibiofilm activities, it has been shown that different concentrations of terpinen-4-ol, reaching as low as MIC/16, were effective to inhibit the adhesion of biofilm-forming *S. aureus* strains to polystyrene and glass surfaces [214]. In addition, these concentrations inhibited biofilm formation and were able to eliminate 73.8–91.2% of the formed biofilms. This might be related the mentioned capacity of bacterial killing. Furthermore, terpinen-4-ol may possess inherent abilities to disrupt the extracellular matrix of bacterial biofilms similar to the whole tea tree oil as revealed by SEM studies [214], [222]. However, the methodological differences in the assessment of biofilm viability should be considered while interpreting the favorable effects of tea tree oil or its components [222], [223].

In line with these promising effects, it is necessary to employ the non-specific antimicrobial actions to develop robust anti-MRSA formulations. Nonetheless, it is imperative to investigate the possibility of developing a resistance to terpinen-4-ol. Interestingly, clinical resistance has not been reported and the experimental attempts to generate resistant bacteria were unsuccessful so far [224], [225]. Conversely, it has been shown that the Gram-negative *P. aeruginosa* possesses special efflux pumps that would be able expel the active compounds of tea tree oil, including terpinen-4-ol, 1,8-cineole, and α-terpineol [226], [227]. On the other hand, it is imperative to investigate the role of terpinen-4-ol or its major essential oil, tea tree oil, on the development of resistance to other antimicrobial agents. Hammer and colleagues [228] showed that culturing of *S. aureus* with subinhibitory doses of

terpinen-4-ol or tea tree oil induced no significant difference in the frequency of resistance to vancomycin, mupirocin, or ciprofloxacin. In addition, there were no significant differences in the MICs of these antimicrobial agents, indicating no changes in the susceptibility profiles of the bacteria [228]. Similarly, habituation to tea tree oil yielded a slight (nonsignificant) increase in MICs of vancomycin, mupirocin, linezolid, and fusidic acid, when these antibiotics were tested against MRSA, coagulase-negative *S. aureus*, or methicillin-susceptible *S. aureus* [229]. Therefore, tea tree oil or terpinen-4-ol can be effectively used as a topical antiseptic to control skin infections by *S. aureus*, including resistant strains.

Notably, the application of terpinen-4-ol in skin infections can be further supported by its anti-inflammatory effects. It has demonstrated that terpinen-4-ol reduced the lipopolysaccharide (LPS)-induced pro-inflammatory responses elicited by peripheral blood monocytes and macrophages [230], [231]. More specifically, it reduces TNFα and IL-1, IL-8, and prostaglandin E2 [230]. These inhibitory effects are primarily mediated by modulating the activation of NF-κB or the ERK MAPK pathways [231]. Terpinen-4-ol was also able to reduce superoxide production by monocytes, but not neutrophils, in response to LPS [232]. Furthermore, terpinen-4-ol can alleviate histamine-induced wheal and flare reaction [233]. Given that the parent essential oil has evident tissue remodeling activities, such as antiproliferative effects on dermal fibroblasts as well as enhancing the expression of epidermal growth factor receptor, tissue inhibitor of metalloproteinase, and matrix metalloproteinase 1 [234], it is likely that may exhibit similar properties, or at least act synergistically with other oil components, to alleviate inflammation. It is therefore possible to use terpinen-4-ol as a topical anti-inflammatory agent.

Regarding the clinical aspects, early studies showed that tea tree oil can induce weak to moderate sensitizing reactions, but the sensitizing potency is augmented by oxidation [235], [236]. The oxidized products of terpinen-4-ol and α-terpinene are strong sensitizers as reported previously [237], [238]. However, the application of 5% and 10% terpinen-4-ol on the skin of health volunteers and dermatitis patients, respectively caused no irritation or sensitization [239].

In sum, terpinen-4-ol has prominent antimicrobial activities although the exact mechanism of action on *S. aureus* cells is still unclear. It can be used as a topical antiseptic, preferably in combination with other antimicrobial agents, to eliminate MRSA colonization and *S. aureus* skin infections. It also possesses tissue remodeling effects and anti-inflammatory activities which target the pro-inflammatory cytokines as well as superoxide production. Further studies are required to assess the therapeutic potential of terpinen-4-ol in skin inflammatory conditions mediated by *S. aureus*, such as AD and psoriasis.

Citral

Citral is a monoterpenoid aldehyde (3,7-dimethyl-2,6-octadienal) found in the essential oil of multiple plants, including *Backhousia citriodora* F. Muell, *Litsea cubeba*, *Ocimum basilicum*, and *Cymbopogon citratus* [240], [241]. There are two geometric stereoisometric forms: citral A (the E-isomer) and citral B (the Z-isomer) [242]. Owing to its characteristic aroma, citral is used as a flavor enhancer and as a scent in perfumes. Citral has also antitumor, antiparasite, and antimicrobial effects [243], [244], [245].

The antibacterial activity of citral has been demonstrated elsewhere [246], [247]. Systemic administration of citral *in vivo* has led to significant reductions of oxidative factors (hydroxyl radicals and malondialdehyde) and cytokines (TNF-α, IL-1β, IL-6), and this was associated with an increased survival of MRSA-infected mice [248]. Experimentally, the MIC against MRSA 2071 ranged from 75 to 150 μg/mL, which was lower than that of other antibiotics (>500 μg/mL) [249]. Citral, either individually or being integrated into lemon grass essential oil, has been associated with significant inhibitory actions against MRSA and VRSA isolated from wound pus samples [250]. Therefore, it seems that citral has antibacterial and anti-inflammatory actions.

Notably, the main mechanism of action of citral does not supposedly target bacterial cell wall. This is because the difference in the bacteriolysis assays showed no significant difference in the optical density between citral-treated and non-treated *S. aureus* cells, indicating the lack of extracellular nucleic acids, which is inherently observed with bacterial cell rupture [249]. Alternatively, citral can significantly inhibit efflux pumps as revealed by reduced extrusion of ethidium bromide from bacterial cells [249]. Moreover, citral may interact with bacterial DNA; hence it forms chimera to inhibit the biological activity of DNA as shown by ultraviolet spectroscopic analysis [251].

Of note, citral can synergistically interact with other antibiotics. For instance, Gupta *et al*. [249] revealed 4-to 32-fold reduction in the MIC values of norfloxacin when it was combined with citral against six MRSA clinical isolates. Other synergistic actions with erythromycin, streptomycin, and penicillin were apparent, yet such a synergism involved lower numbers of clinical isolates.

Importantly, citral has been found to activate transient receptor potential (TRP) ion channels present in sensory neurons. Besides its antimicrobial activities, citral can induce a sustained inhibition of TRPV1-3 and TRPM8 [252]. This might indicate the usefulness of citral against allodynia, itch, and other dermatological and superficial sensory types of pain. However, citral has been reportedly associated with some adverse reactions, such as allergic contact dermatitis and sensitization [253], [254]. Future large-based studies

would possibly unravel additional aspects regarding the efficacy and safety of topical citral preparations.

Eugenol

Eugenol is the main constituent of clove oil, comprising 45–90% of its essential oil. Eugenol is also found in cinnamon (*Cinnamomum verum*), beans, soybeans, and bay laurel [255], [256]. It is a phenolic compound (4-allyl-2-methoxyphenol) commonly used a preservative and a flavoring agent in food industry and the cosmetic field [257]. The phytocompound has been a focus of research owing to its growing roles in preventing chronic illnesses, such as cancer, inflammatory diseases, and other conditions [258], [259]. It has pharmacological effects on almost all body systems through its comprehensive anti-inflammatory, antioxidant, local anesthetic, analgesic, and cardioprotective properties [260].

As for MRSA-related conditions, eugenol has demonstrated promising results. Generally speaking, the MICs of eugenol range between 42 and 665 μg/mL against multiple MRSA strains [261], [262], [263]. Besides, at sub-inhibitory concentrations, the compound was effective against *S. aureus* biofilms. In particular, eugenol-supplemented MRSA samples isolated from food handlers exhibited a marked reduction of the biofilm mass in a dose-dependent manner compared to untreated samples; this was further corroborated by light microscopy assays [262]. In addition, bacterial cell aggregation and cell-to-cell connection were prevented as visualized by SEM [263]. Interestingly, molecular docking experiments showed that eugenol can interact with sarA, which is a key regulator of biofilm formation in *S. aureus* [262], [264]. It can reduce the expression of biofilm-related genes, such as *icaD*, *SEA*, and *sarA* genes [263].

It has been also shown that eugenol can eliminate established biofilms at the MIC; this effect could be mediated via reducing the number of viable *S. aureus* cells, promoting cell lysis, or the disruption of cell-to-cell connection. As with other hydrophobic phenolic compounds, eugenol would result in disruption of the cell membrane, loss of normal shape of the bacteria, and reduced cell-to-cell detachment. As a lipophilic phytochemical, eugenol can interact with bacterial cell wall and cytoplasmic membrane, influence the hierarchy of polysaccharides, phospholipids, and fatty acids, disrupt the permeability of cell membrane, and ultimately induce cell lysis [182]. It is worthy to note that a combination of eugenol and carvacrol (at concentrations of 0.02% and 0.01%, respectively) could act synergistically against MSSA and MRSA, and such a combination would reduce the established biofilms by 99% [263].

Eugenol has also been tested against mupirocin-susceptible and low-level-resistant *S. aureus* strains, revealing an MIC of 240 μg/mL. Furthermore, a combination of eugenol and mupirocin exhibited an additive antimicrobial effect against mupirocin-resistant strains, while antagonistic and inconsistent effects were apparent against the susceptible strains [265]. These outcomes indicate the importance of eugenol in eradicating the colonizing strains which might show resistance to mupirocin.

Phenolic compounds

Curcumin

Curcumin is a natural polyphenolic compound present in the rhizome of turmeric (*Curcuma longa*) as well as in other *Curcuma* species [266]. In addition to its widespread use in the culinary world, turmeric has long been known in Asian medicine for its therapeutic properties as an antioxidant, anticancer, anti-inflammatory, and antimutagenic compound [266], [267], [268]. Similarly, curcumin (1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione) has proven beneficial in pain control and the management of metabolic syndrome, inflammatory conditions, and degenerative eye diseases [269], [270].

Notably, the medicinal properties of curcumin have largely been focused on the anticancer activities [271]. Nonetheless, the antibacterial activity of the phytochemical was documented as early as in 1949 [272], when curcumin exhibited promising inhibitory effects against *Salmonella paratyphi*, *S. aureus*, and *Mycobacterium tuberculosis*. Studies indicating inhibition of susceptible and resistant *S. aureus* strains are increasingly reported. For example, Gunes *et al*. [273] have determined MICs against MSSA and MRSA at 219 and 217 μg/mL. Similarly, other broth microdilution assays revealed MICs ranging between 187.5 and 500 μg/mL for *S. aureus* [274], [275].

To further assess the main mechanism of action, Mun *et al*. [276] conducted viability assays, western blotting, and morphological TEM studies on four clinical MRSA strains exposed to curcumin. The authors found that the antibacterial activities of curcumin were significantly enhanced by increasing the membrane permeability via triton X-100 and Tris; the inhibitory effects were further increased by increased curcumin concentrations. Furthermore, the active botanical compound bound to PGN in a dose-dependent manner. TEM images showed damage of bacterial cell membrane after the exposure of *S. aureus* cell to half MIC for 8 h as indicated by cytoplasmic disruption and separation [276]. Intriguingly, western blot analyses showed a significant reduction of PBP2a levels from MRSA by the addition of 250 μg/mL curcumin to 32 μg/mL oxacillin. It seems that curcumin interferes with RNA, and thus inhibits PBP2a protein synthesis.

The synergistic activity between curcumin and oxacillin has inspired the researchers to further investigate the interaction with other antimicrobial agents. Curcumin has reduced the MICs of ampicillin,

norfloxacin, and ciprofloxacin, and time-kill curves revealed marked reduction of the bacterial count following 24 h of treatment [277]. Teow and Ali [278] have also shown that 25 μg/mL curcumin acted synergistically with amikacin and gentamicin. Based on a disc diffusion assay using a sub-inhibitory concentration of curcumin (50 μg/disc), the diameter of inhibition zones increased significantly by 52.6%, 26.6%, and 24.9% for cefixime, tetracycline, and vancomycin respectively [279]. In additional, the curcumin derivative diacetylcurcumin reduced MRSA biofilm survival significantly compared to vancomycin via damaging the general architecture and interfering with the synthesis of amorphous cell clusters [280].

Other therapeutic benefits of curcumin seem to provide considerable support to dermatological infections. For example, in mice models, daily topical application of 1% curcumin gel decreased the severity of psoriasis-like inflammatory lesions; this was exerted by interfering with the proinflammatory cytokines, such as IL-17A and IL-22, and by the inhibition of potassium channels of T cells [281], [282]. In the clinical settings, oral turmeric has proven effective in reducing erythema, induration, and scaling in patients with scalp psoriasis compared to a placebo, and it potentiated the anti-psoriatic activity of topical steroids by reducing IL-22 and TNF-α levels [283], [284]. In another phase II RCT, oral curcumin was also effective in plaque psoriasis, without apparent adverse outcomes [285].

From another perspective, curcumin can reduce oxidation and thereby promote wound healing. A transdermally applied preparation caused a significant reduction of hydrogen-peroxide-induced damage to fibroblasts and keratinocytes [286], [287]. In additional, curcumin promoted the activity of anti-oxidant enzymes, such as GSH peroxidase, catalase, and superoxide dismutase [288]. Fibroblast proliferation and deposition is another important mechanism by which wound healing is accelerated by curcumin as shown in animal models [289]. During the early proliferation phase, curcumin can induce apoptotic effects; thereby it eliminates unwanted inflammatory cells from the wounds. Finally, it improves wound contraction via enhancing the production of TGF-β [289], [290], [291].

Despite these beneficial findings, one of the major limitations with curcumin use is its poor bioavailability [292]. This is attributable to its poor absorption and rapid metabolism and excretion. Therefore, several mechanisms have been tested to improve the bioavailability of the compound, predominantly by interfering with its metabolism. For instance, the bioavailability of curcumin can be increased by 2000% with the coadministration of piperine, the main active component of black pepper, since such a combination can influence intestinal drug absorption and the drug metabolizing enzymes [293]. For topical use, the bioavailability has been augmented via the application of specific curcumin-loaded formulations, such as oleic acid-based polymeric bandages [289], transdermal patches [286], liposomes [294], and chitosan NPs [295]. It is therefore plausible to investigate the interaction between curcumin and other chemical substances which are commonly used in topical preparations in order to get an insight into the development of therapeutically effective formulations used for particular skin infections/conditions.

Epigallocatechin gallate (EGCG)

EGCG is a powerful antioxidant botanical compound and a major constituent of catechins extracted from green tea (*Camellia sinensis*). Catechins are polyphenolic compounds belonging to the family of flavonoids. The antimicrobial activity of green tea extract has been demonstrated in the literature. For example, the MIC of green tea extract for MRSA was 0.4 mg/mL, and the activity of the extract against the laboratory *S. aureus* strain ATCC 25923 was comparable to that of oxacillin [296]. Thus, aerosolized green tea has been suggested for respiratory MRSA infections as recommended in case reports and RCTs [297], [298], [299]. Intriguingly, green tea has exhibited inhibitory activities against β-lactamase, and it showed a synergistic effect with β-lactam antimicrobial agents against MRSA isolates [300]. These effects are largely attributable to the polyphenolic components of green tea extract, such as EGCG, epicatechin gallate (ECG), epicatechin, and epigallocatechin.

Focusing on EGCG, it has been suggested that the anti-adhesive properties of the compound may account for its antimicrobial actions [301]. In particular, EGCG at its MIC values can interact with the bacterial cell wall and interfere with the adhesion of skin pathogens to epithelial cells without inducing significant changes to the mammalian cells [302]. This is because polyphenols exploit macromolecules, such as carbohydrates and proteins, and thus they interact with specific adhesion structures located on the bacterial cell wall or on fimbriae [303], [304].

Importantly, the most prominent antimicrobial actions are mainly related to the interference with β-lactamase and the reversal of MRSA resistance in experimental studies [305]. In essence, modulation of resistance is mediated via the gallate moiety of EGCG and other polyphenols in green tea extract. Indeed, it has been reported that EGCG can reverse the resistance of MRSA; such a property was not evident in (-)-epicatechin-3-cyclohexylcarboxylate and (-)-epicatechin-3-cyclohexylcarboxylate [300], [305].

As a consequence, the MIC of antibiotics against resistant strains could be lowered by EGCG. For instance, in addition to the anti-MRSA activities of EGCG at an MIC of 100 mg/L, a combination of EGCG and ampicillin/sulbactam at subinhibitory doses was synergistically effective in a dose-dependent manner against MRSA isolated from 28 clinical isolates [306].

More specifically, the MIC_{50} of the ampicillin/sulbactam combination decreased from 32 to 8 and 4 mg/L in the presence of 6.25 and 25 mg/L EGCG, respectively [306].

While it was initially thought that MRSA reversal is mediated by interfering with the synthesis of PBP2a [300], a different mechanism has been proposed by Zhao *et al*. [307] The group found that EGCG (25 μg/mL) reversed MRSA resistance to oxacillin, ampicillin, methicillin, benzylpenicillin, and cephalexin, and it induced a supersusceptibility to β-lactam antibiotics in susceptible strains [307]. Therefore, the synergistic effect was equally effective against β-lactamase producers and non-producers. This way, it has been suggested that EGCG is nonspecific to MRSA, and it cannot directly modulate the synthesis or the production of PBP2a [307].

Such a nonspecific effect was supported by several findings. First, the growth of both resistant and susceptible strains was similarly inhibited in a dose-dependent manner. Second, the induced supersusceptibility of susceptible strains to antibiotics, such as oxacillin, may emphasize the lack of direct relation between EGCG and the *mecA* gene as well as PBP2a. Third, there was an additional synergism against MRSA between EGCG and DL-cycloserine, which can inhibit D-alanyl-D-alanine synthetase, leading to the abrogation of PGN synthesis. This mechanism is unlikely related to PBP2a expression. Finally, PBP2a mRNA expression and PBP2a production were only suppressed by low MICs of ECGC as indicated by latex agglutination assays [307].

Collectively, it seems that EGCG acts directly and synergistically on PGNs on the bacterial cell wall, thereby reducing the tolerance of MRSA to osmotic changes. Novy *et al*. [308] demonstrated similar PGN-targeted resistance-modulating effects in a combination with oxytetracycline. Synergism was also evident in combinations comprising of EGCG with carbapenems [309]. In addition, penicillinase production from penicillin resistant strains was previously inhibited in 21 MRSA strains [310]. Sudano Roccaro and coworkers [311] first described a resistance-modulating mechanism, in which the presence of EGCG at concentrations below its MIC (50 μg/mL) decreased the MICs of tetracycline from to against MRSA and *S. epidermidis* resistant strains; the effect was mainly related to the inhibition of Tet(K) and Tet(B) efflux pumps. This disruption of high-energy efflux pumps would favor the dynamic equilibrium toward antibiotic influx and eventually increase the accumulation of tetracycline inside the bacterial cells.

Therefore, EGCG may be clinically effective in eliminating cutaneous and digestive tract MRSA infections in combination with β-lactams and tetracyclines. However, the administration of sublethal doses of EGCG may have clinical implications. Bikels-Goshen *et al*. [312] showed that the exposure of four strains of *S. aureus* to 20 μg/mL of EGCG had no effects on bacterial growth rates, but rather increased the resistance of the bacteria to cell wall-targeting antibiotics, including oxacillin, ampicillin, and vancomycin. Moreover, EGCG induced the expression of stress shock proteins, which would ultimately leads to adaptation and increased tolerance to heat treatment [313]. Besides, there are significant differences in the mean cell wall thickness, indicating marked morphological changes in EGCG-exposed bacterial cells as visualized by TEM [312].

In contrast, Blanco *et al*. [314] found that subinhibitory doses of EGCG prevented biofilm formation in *ica*-positive staphylococcal strains, which have the abilities to construct multilayered biofilms. In such experimental study, slime-producing *S. aureus* formed pale grey colonies on Congo red agar plates (rather than black colonies), indicating the loss of the matrix-producing ability. Interestingly, SEM analysis showed that the exposure to EGCG (1/4 MIC) prevented polysaccharide secretion, and thus it disrupted glycocalyx formation [314]. It is important to note that the PGN inhibitory effects of EGCG could also influence the initial docking phase of biofilm formation by disrupting the interaction between the surface to be colonized and the bacterial cell wall [315]. Recently, it has been shown that EGCG can exert anti-amyloidogenic activities, which affect the assembly of α-PSM fibrils. The interference with these biofilm-associated fibers would have a promising potential to weaken or disrupt the amyloid matrix in biofilms produced by *S. aureus* [316].

Therefore, the favorable resistance modulatory activities as well as the anti-biofilm effects of EGCG may provide a strong rationale for further investigations concerning the treatment of chronic wound infection. These activities would possibly add to the established wound healing effects of the compound [317], [318], [319]. Topical preparations comprising EGCG would be more stable and would have better bioavailability because they would not be subject to degradation and oxidation by the intestinal microbiota [320], [321].

Organosulfur

Allicin

Allicin is a natural organosulphur compound present in crushed garlic (*Allium sativum* L.). Garlic has been known for a range of health benefits, including the treatment of headache, arthralgia, leprosy, tuberculosis, digestive diseases, and epilepsy, as well as cardiovascular protection [322], [323]. Cellular rupture during garlic crushing converts alliin (under the effect of allinase enzyme) to a number of enzymatic products named allyl thiosulfinates. Of them, allicin (diallyl thiosulfinate) has been considered the major active compound. The antimicrobial actions of allicin

were demonstrated early in 1944 [324]. Besides, as with other allyl thiosulfinates, allicin has antioxidant, lipid-lowering, anticancer, and anti-atherosclerotic effects [325].

The pure form of allicin is highly volatile, has strong odor, and is poorly mixed with water [326]. The allicin molecule breaks down within 16 h at 23°C [327]. Therefore, in order to stabilize allicin molecules, Cutler and Wilson [328] have developed two topical formulations based on cold aqueous extraction of allicin in a liquid extract and a cream formulation. The authors found that the majority of clinical mupirocin-resistant *S. aureus* strains had MBCs for 128 μg/mL allicin, and the MIC required to kill all strains was 256 μg/mL.

Interestingly, thiol-specific reactivity has been proposed as a potent mechanism of action against microbes. That is, allicin can act by *S*-thioallylations of the low molecular weight thiol GSH in chemical reactions involving thiol-disulphide exchange [329], [330]. Considering the chemical structure and the electrophilicity of allicin, this phytochemical is virtually highly reactive with sulfhydryl-baring, thiophilic molecules, such as GSH and cysteine [331]. This way, the *S*-allyl component of allicin could be exchanged with the thiol constituent of a bacterial coenzyme, enzyme, or metabolite [332]. In agreement with these findings, it has been shown that electrophilic pyridyl disulfides, which have similar chemical reactivity to allicin [333], could exert bacteriostatic actions against MRSA via the interaction with thiophilic enzymes/metabolites [334].

Recently, Loi *et al*. [335] provided supportive evidence, where allicin-induced oxidative and disulphide stresses were demonstrated in resistant *S. aureus* strains. Indeed, the oxidative shift in the redox potential of GSH as well as the widespread *S*-thioallylations are robust mechanistic antimicrobial pathways. However, thiol homeostasis could be regenerated via the HypR-controlled disulfide reductase MerA and the protective thiol bacillithiol, leading to direct allicin detoxification [335]. As such, future investigations may reveal the possible implications of these adaptive mechanisms to develop anti-allicin resistance.

In addition to the previously mentioned antibacterial effects, Leng *et al*. [336] demonstrated that allicin had anti-hemolytic actions in *S. aureus* culture supernatants, indicating effective reductions of α-toxin. Intriguingly, these anti-virulence properties are particularly exhibited by reducing the expression of the *AgrA* by 6.3-fold [336]. Actually, the application of allicin could be optimized for the treatment of toxic syndromes mediated by virulent *S. aureus*. This might be potentiated by the use of protein-synthesis-targeting antibiotics, such as linezolid and clindamycin at sub-inhibitory concentrations [337]. In contrast, the administration of β-lactam antimicrobial agents at sub-inhibitory doses can stimulate α-toxin expression via enhancing exoprotein synthesis [338].

From another point of view, innovative approaches may have significant roles to augment the antimicrobial potential of allicin. Sharifi-Rad *et al*. [339] investigated a combination comprising of allicin and silver NPs against the MRSA ATCC14458 strain. Based on single and combined analyses, silver NPs plus allicin had a significant synergistic effect (MIC 0.4 mg/mL) compared to either allicin or NPs individually (MICs of 2.2 or 5.6 mg/mL, respectively) [339]. In addition, a topical ointment with the combination applied to experimentally infected wounds in mice showed significant inhibitory effects as indicated by a significant reduction of the colony forming units with ointment use compared to either exclusive compounds or a control medium [339]. Similarly, allicin provoked the antimicrobial activity of chlorhexidine against MRSA when the combination was applied to hernia repair materials [340]. In addition, allicin potentiated the activity of vancomycin in a prosthetic joint infection experimental model [341]. This might raise the possibility of biofilm-counteracting effects of allicin. In another experiment, Majumdar *et al*. [342] assessed the efficacy of a smart drug delivery system based on releasing the active antimicrobial substance with increasing concentrations in response to the amount of MRSA in the target. The authors showed that an allicin-containing extract induced significant bacterial cell death, which acted in a pH-dependent controlled manner (increased bacterial metabolites reduced the pH) [342]. Such outcomes indicate the need to investigate the role of allicin in RCTs to corroborate the efficacy and safety of topical skin formulations in MRSA skin infections. These would be preceded by pharmacokinetic studies aiming at improving the bioavailability of the compound through novel delivery systems.

Single and Combined Herbal Formulations in Dermatology

The topical application of specific formulations from medicinal plants has grabbed the attention of clinicians during the past two decades to overcome the rising trend of MRSA resistance. *In vitro* studies investigating the impact of active compounds from medicinal plants on MRSA strains obtained from clinical isolates showed promising outcomes (Table 2). However, human-based studies are still insufficient to unravel the clinical benefits of these compounds.

In 2001, Sherry *et al*. [343] tested the effects of a topical formulation of eucalyptus leaf oil extract (PT) in two patients with MRSA-infected wounds. In one patient, PT cream (1.0 g daily) without antibiotics facilitated wound healing completely within 2 weeks, and the patient showed no signs of inflammation. The second patient received PT liquid (0.5 g daily), showing marked reduction in inflammation 5 days after initiation,

Table 2: The effects of herbal compounds on MRSA clinical isolates

Active compounds	Source plant	Main components	Method	Outcomes	References
Lemon myrtle oil	*Backhousia citriodora*	Geranial (51.4%), Neral (40.9%), Citral (4.3%)	ADM	0.20% v/v	[389]
Extracts	*Cortex moutan, Cortex phellodendri, Flos lonicerae, Rhizoma atractylodis, Herba menthae*	NA	MIC	1 mg/mL	[390]
Supercritical carbon dioxide extract	*Usnea barbata*	Usnic acid (4% w/w)	MIC	1 mg/mL	[391]
Essential oil	*Eucalyptus globulus*	Eucalyptol (47%)	MIC	8.56-85.60μg/mL	[392]
Essential oil	*Juniperus communis*	NA	MIC	>2% v/v	[393]
Essential oil	*Juniperus officinalis*	α-Pinene (39.8%)	MIC	20mg/mL	[394]
Essential oil	*Kunzea ericoides*	α-Pinene (61.6%)	MAC	0.2% v/v	[395]
Lavender oil	*Lavandula angustifolia*	NA	MIC	0.5% v/v	[393]
Lavender oil	*Lavandula angustifolia*	Linalyl acetate (37%), linalool (31%), terpinen-4-ol (15%)	MIC	1 mg/mL	[396]
Lavender oil	*Lavandula stoechas*	α-Fenchone (39.2%)	MIC	31.3μg/mL	[397]
Essential oil	*Matricaria recutita*	Chamazulene (31.5%) and α-bisabolol (15.7%)	ADM	26.50mg/mL	[398]
Tea tree oil	*Melaleuca alternifolia*	Terpinen-4-ol (35.2%), γ-terpinene (22.5%), α-Terpinene (11.4%)	MAC	0.35% v/v	[395]
Tea tree oil	*Melaleuca alternifolia*	Terpinen-4-ol (40%), δ-terpinen (13%)	MIC	512–2048mg/L	[399]
Tea tree oil	*Melaleuca alternifolia*	Terpinen-4-ol (>35%)	MIC	0.30–0.63% v/v	[400]
Tea tree oil	*Melaleuca alternifolia*	NA	MIC	0.25% v/v	[393]
Tea tree oil	*Melaleuca alternifolia*	Terpinen-4-ol (>35%)	MIC	0.25%	[401]
Tea tree oil	*Melaleuca alternifolia*	Terpinen-4-ol (42.8%) and γ-terpinene (18.2%)	ADM	0.3% v/v	[389]
Essential oil	*Melaleuca cajuputi*	1,8-Cineol (67.6%)	MIC	2.5mg/mL	[394]
Essential oil	*Melaleuca cajuputi*	1,8-Cineole (55.5%)	MAC	0.3% v/v	[395]
Essential oil	*Mentha piperita*	Menthol (47.3%), menthone (22.2%), 1,8-Cineol (12.1%)	MIC	0.6mg/mL	[394]
Essential oil	*Origanum vulgare*	Thymol (24.7%), p-Cymene (14.6%), carvacrol (14%)	ADM	0.13% v/v	[184]
Essential oil	*Rosmarinus officinalis*	1,8-Cineole (26.6%), camphene (11.4%), α-pinene (20.1%),	MIC	0.03% v/v	[402]
Essential oil	*Thymus vulgaris*	NA	MIC	0.5% v/v	[393]
Essential oil	*Thymus vulgaris*	Thymol (48.1%), γ-terpinene (15.40%), p-cymene (15.60%)	ADM	18.50μg/mL	[392]
Essential oil	*Cinnamomum camphora (Linn.) Presl.*	Linalool (26.6%), eucalyptol (16.8%), α-terpineol (8.7%)	MIC	0.8 mg/mL	[403]
Essential oil	*Stachys viticina Boiss.*	Endo-borneol (29.1%), eucalyptol (21.3%), and epizonarene (7.9%)	MIC	0.039 mg/mL	[404]
Root essential oil	*Chrysopogon zizanioides (L.) Roberty*	NA	MIC	62.5 μg/ml	[405]

ADM: Agar dilution method, MAC: Macrodilution method, MIC: Microdilution method, NA: Nonavailable.

with complete wound closure within 3 weeks. Both patients showed no clinical symptoms of recurrence after 12 weeks. This indicates the efficacy of eucalyptus oil.

Further clinical studies were mostly concerned with tea-tree oil for MRSA decolonization. Caelli *et al*. [344] have conducted an RCT to assess the impact of a tea tree oil intervention formulation comprising of a 4% nasal ointment and 5% body wash versus a standard therapy including the application of a triclosan body wash and 2% mupirocin nasal ointment. The authors found a slight, but nonsignificant, improvement in the number of MRSA-cleared patients in the tea tree oil arm; however, the small number of patients allocated to each number (n = 15) might have accounted for the lack of significant effects.

In a larger RCT, the efficacy of a tea tree oil body wash (5%) was compared to that of a standard Johnson's Baby Softwash to clear MRSA colonization in patients admitted to intensive care units [345]. The results revealed insignificant differences between the study groups in terms of new MRSA colonization, percentage of patients colonized, and clinical deterioration indicated by the maximum increase in the scores of the sequential organ failure assessment scale. Similarly, compared to placebo (saline gauze dressing), topical application of a preparation of tea tree oil for 4 weeks has accelerated MRSA wound healing within 28 days without adverse events, and the resistant strains were completely eradicated in 87.5% of patients allocated to the active intervention group [346]. Notably, the used preparation comprised of 10% tea tree oil (of which ≥30% terpinene-4-ol) and 90% paraffin wax. Besides, tea tree 10% cream was equally as effective and safe in clearing MRSA colonization as a standard cream preparation of mupirocin, chlorhexidine, and sulfadiazine [347]. Actually, the comparative findings to mupirocin are relatively encouraging since the resistance to such an antimicrobial agent is rapidly evolving and there is a need to find alternative preparations for decolonization and management of MRSA skin infections [348]. Despite being encouraging, evidence regarding the combined efficacy of tea tree oil components needs to be further addressed by more robust RCTs.

Integrative Approaches

The application of integrative medicine practices has long been studied in several ways. This includes the incorporation of complementary or alternative approaches into a wider aspect of treatment plans in order to improve health, promote healing, or assist in disease treatment [349]. In dermatology, the involvement of diagnostic and therapeutic modalities as a supplement or a substitute for traditional dermatologic practice would produce promising choices by combining these recent and old knowledge bases. This could be attained by herbal compounds obtained from plant preparations.

Decolonization for the prevention of MRSA

In the era of MRSA emergence, it is necessary to combat skin infections by multiple strategies. Decolonization of vulnerable patients against MRSA is an important prophylactic strategy that entails the use of suitable antiseptics, including chlorhexidine and octenidine. However, as with antibiotics, the extensive use of antiseptics has resulted in the appearance of

clinical isolates with increased MICs, indicating the development of bacterial resistance [350]. Therefore, some potentiated therapies comprising of herbal treatments combined with antimicrobial agents may be efficient.

For example, Hendry *et al*. [351] tested the efficacy of adding eucalyptus oil to chlorhexidine digluconate (CHG) against MRSA grown in suspension and biofilm. Fractional inhibitory concentration index (FICI) assays showed synergistic activities of the combination in both the suspension and biofilms. Although the main constituent in eucalyptus oil was 1,8-cineole, the main inhibitory effects of the crude oil was generally superior to the main constituent alone [351]. This indicates the contribution of other components, such as linalool and methyl chavicol, which have affected the integrity of the cytoplasmic membrane in a synergistic manner. Indeed, such a study revealed interesting outcomes, given the poor penetrative abilities of alcoholic and aqueous preparations of CHG alone. This is because the addition of eucalyptus oil can significantly enhance the delivery of CHG into the dermis and epidermis and thus could provide promising antimicrobial activities [352].

Recently, Kwiatkowski *et al*. [353] have investigated the impact of the essential oil extracted from lavender (from *Lavandula angustifolia* Mill) combined with the antiseptic octenidine dihydrochloride (OCD), against which efflux pump proteins in *S. aureus* have led to a rapid emergence of antimicrobial resistance [350]. Lavender essential oil (LEO) containing high amounts of linalool (34.1%) and linalyl acetate (33.3%) provided a synergistic effect with OCD against *S. aureus* ATCC 43300 and other clinical isolates as revealed by time-kill curve assays. Besides, subsequent analyses using Fourier Transform Infrared Spectroscopy showed significant modifications in the bacterial cell wall of MRSA cultured in LEO/OCD containing media. Although the existing terpene alcohol (linalool) and ester (linalyl acetate) had previously exhibited weaker antimicrobial activities against MRSA compared to other phenolic compounds, such as thymol and carvacrol [354], the combined effects of LEO/OCD decreased the mean MIC of LEO from 14.86 mg/mL to 1.29 mg/mL [353].

Recently, El-Kalek and Mohamed [355] have tested the efficiency of four essential oils and six methanol extracts against MRSA specimens isolated from the skin, ears, urine, and eyes. While the highest antibacterial activities were exerted by lemongrass oil (LEGO), *T. vulgaris* extract, and cardamom oil, the FICI values of LEGO and amoxicillin combinations ranged between 0.82 and 0.86, indicating robust synergistic effects against MRSA M2, M16, and M18. Furthermore, TEM images showed swelling of the bacterial cell wall and disruption of the cytoplasmic membrane. The authors suggested that the lipophilicity of LEGO components (from *C. citratus*), particularly the monoterpene alcohol, might have played a significant role in penetrating the lipid layer of the cell membrane; therefore, they caused intracellular leakage [355]. The effects of LEGO have been further corroborated when Warnke *et al*. [356] showed significant differences in the zones of inhibition between LEGO (20–29 mm) and chlorhexidine (1–10 mm). The inhibitory zones were also better, but insignificantly different, than those produced by tea tree oil and eucalyptus oil.

Integrative therapies with antibiotics

Over the past few decades, the use of antibiotics has been considered less effective in the treatment of MRSA infections. The progressive trend of resistance against vancomycin and other anti-MRSA antibiotics has been associated with limited outcomes

Table 3: Summary of phytochemicals acting synergistically with antibiotics against MRSA

Source plant	Pure compounds	Antibiotic	Mechanism of action	References
Garcinia mangostana L.	Alpha-Mangostin	ampicillin and minocycline	Unavailable	[406]
Scutellaria amoena C.H. Wright	Baicalin	Beta-Lactam Antibiotics	Inhibition of β-lactamase	[378]
Stephania tetrandra S. Moore	Bisbenzylisoquinoline Alkaloids	Cefazolin	Multidrug efflux pump inhibition	[407]
Arctostaphylos uvaursi	Corilagin	Oxacillin	Inhibition of PBP2a expression	[408]
Arctostaphylos uvaursi	Corilagin	Penicillin	Inhibition of PBP2a expression	[409]
Origanum vulgare	Essential oil	Tetracycline	Efflux pump inhibition	[365]
Lippia origanoides	Essential oil	Amikacin and neomycin	Efflux pump inhibition	[367]
Mezoneuron benthamianum and Securinega virosa	Ethanol and chloroform extracts	Norfloxacin	Efflux pump inhibition	[358]
Daphne genkwa	Extract	oxacillin	Binding to PBP2a	[376]
Alpinia officinarum	Galangin	gentamicin	Inhibition of β-lactamase	[380]
Lupinus argenteus	Isoflavones	Norfloxacin	NorA efflux pump inhibition	[361]
Cytisus striatus	Isoflavonoids	Erythromycin	NorA efflux pump inhibition	[410]
Pinus nigra	Isopimaric Acid	reserpine	NorA efflux pump inhibition	[411]
Cymbopogon citratus	Lemon grass essential oil	Amoxicillin	Disruption of the bacterial cell membrane	[355]
Canarium odontophyllum	Methanol extract	Oxacillin	inhibit cell wall synthesis	[370]
Punica granatum	Methanolic extract	Ampicillin, oxacillin, tetracycline, chloramphenicol, and gentamicin	NorA efflux pump inhibition	[412]
Ipomoea violacea	Oligosaccharides	Norfloxacin	NorA efflux pump inhibition	[362]
Jatropha elliptica	Penta substituted pyridine	Ciprofloxacin	NorA efflux pump inhibition	[361]
Camellia sinensis	Phenols and flavonoids	tetracycline and ampicillin	Inhibition of β-lactamase	[379]
Sophora species	Sophoraflavanone G	Vancomycin, Gentamicin, and Methicillin	Augments the inhibitory actions on cell wall synthesis	[413]
Rosa canina L.	Tellimagrandin I	Penicillin	Inhibition of PBP2a expression	[409]
Acalypha wilkesiana	The 9EA-FC-B fraction	Ampicillin	Inhibition of PBP2a expression in the planktonic form and biofilm	[374]
Duabanga Grandiflora	The F-10 fraction	Ampicillin	Inhibition of PBP2a expression	[375]

PBP2a: Penicillin-binding protein 2a.

and increased mortality. It is therefore plausible to find integrative substances which could restore the effectiveness of these antimicrobial agents by reversing their resistance mechanisms. The application of herbal preparations is reviewed below according to the target resistance mechanisms against which these compounds have possibly acted. A summary of these phytochemicals is also demonstrated in Table 3.

Inhibition of efflux pumps

Antibiotics and antiseptics could be exported from the bacterial cells via specific efflux pumps, such as NorA and NoB which account for the resistance against norfloxacin, ciprofloxacin, and the antiseptic chlorhexidine in MRSA [350], [357]. Medicinal plants can modify these resistance mechanisms and thus revert the action of antibiotics.

Ethanol extracts of *Mezoneuron benthamianum* and chloroform extracts of *Securinega virosa* potentiated the activity of norfloxacin by a factor of 4 [358]. Considering the established resistance mechanisms of MRSA against norfloxacin, these extracts could potentially have acted via inhibition of the efflux pumps. In addition, plants belonging to the *Berberis* spp. produce the antimicrobial alkaloid berberine as well as an inhibitor of *S. aureus* NorA pump named 5'-methoxyhydnocarpin (5'-MHC). The latter can significantly reduce the MIC of berberine and both compounds act as plant chemical defense mechanisms against pathogenic organisms [359].

Similarly, isoflavones from *Lupinus argenteus* [360], penta-substituted pyridine from *Jatropha elliptica* [361], oligosaccharides from *Ipomoea violacea* [362], and essential oils from *Pelargonium graveolens* and *Zanthoxylum articulatum* [363], [364] have all synergistic actions with norfloxacin against MRSA via the same mechanism. Likewise, efflux pump inhibition has been mediated via several other herbal remedies, such as the essential oils of *O. vulgare* and *Salvia fruticose* with tetracycline as well as the essential oils of *Lippia origanoides* with amikacin and neomycin [365], [366], [367].

Other integrative therapies have been demonstrated in the literature although they have not been tested in dermatological preparations. For instance, saponins obtained from *Panax ginseng* (Korean red ginseng) showed weak antibacterial activities against three MRSA strains (MIC 100 μg/mL), yet these compounds have synergistically acted with kanamycin and exerted additive effects with cefotaxime [368]. In another experiment, ursolic acid and oleanolic acid isolated from *Alstonia scholaris*, a tropical tree native to Southeast Asia, have induced efficacious synergistic effects with tetracycline and ampicillin at 1/2 MICs of the herbal compounds [369]. These pentacyclic triterpenoids might have exerted their potentiating effects via a mechanism different than that of the used β-lactam antimicrobials because of the structural dissimilarities between them. Similarly, the methanol extract from *Canarium odontophyllum* Miq. (native to the tropical rainforests in Southeast Asia) was effectively combined with oxacillin, causing an eightfold reduction of the antibiotic inhibitory concentration [370]. Besides, the same extract has provided an additive effect with vancomycin. Additivity, defined as a relative improvement in the antibacterial activity when the concentration of either active compound has been increased, can be clinically relevant although concerns about the toxicity of high concentrations may still be apparent. Interestingly, synergism between substances occurs when they act via different mechanisms of action, while additivity takes place when the compounds exert the same mechanism of action [371], [372]. This suggests that the methanolic extract acts by inhibiting cell wall synthesis, which is the same mechanism of vancomycin action.

Effects on modified target sites

Modifications of the target site are one of the most common mechanisms by MRSA. This would reduce the affinity of bacterial cells for antimicrobial agents. Resistance to β-lactams is mostly conferred by complete replacement of the target site (PBPs) by the acquisition of PBP2a. Plant active compounds have been implicated in modulating the resistance via this mechanism. For example, a bioactive fraction (F-10) from *Duabanga Grandiflora* has shown effective inhibitory outcomes against MRSA ATCC 43300 in combination with ampicillin, which were significantly different than those exhibited by the antibiotic or the herbal compound alone [373]. This yielded FICI indices ranging between 0.18 and 0.31 using 1/4 to 1/32 MIC of the F-10 fraction. Further analyses showed attenuated PBP2a expression with using the F-10 fraction alone and a total inhibition of protein expression when a combination of subinhibitory concentrations of F-10 and ampicillin had been used. It is possible that the F-10 fraction has interfered with the regulatory genes of *mecA* transcription, namely *mecI*, *mecR1*, and *mecR2*, which has eventually led to blockage of PBP2a synthesis [373]. Some essential phytochemical found in the F-10 fraction, such as flavonoids, tannins, glycosides, and sterols, might have accounted for the resistance modulatory action; thus, such a fraction may be further tested in future preparations against MRSA.

The same mechanism of action could be targeted by a combination of ampicillin and the ethyl acetate extract of *Acalypha wilkesiana.* More specifically, the 9EA-FC-B fraction from such an extract has reduced the MIC of ampicillin by 32-fold against MRSA, and a significant synergistic action was shown compared to either compounds alone [374]. This resulted in the inhibition of PGN synthesis through blocking PBP2a production in MRSA. Interestingly,

the same fraction has proven effective in precluding biofilm formation by MRSA via inhibiting the initial cell attachment and reducing the produced PBP2a in the biofilm matrix [375].

In another recent study, Kuok *et al*. [376] tested the anti-MRSA activities of four medicinal plants used in the Chinese traditional medicine: *Daphne genkwa*, *Verbena officinalis*, *Magnolia officinalis*, and *Momordica charantia*. The authors showed significant synergistic actions between the *D. genkwa* extract and oxacillin (FICI value of 0.38). Additional *in silico* molecular docking investigations revealed robust binding affinities and interactions between the flavonoid tiliroside in *D. genkwa* and specific residues in PBP2a. This interaction may account for the observed synergy and underscore the importance of further investigations.

The influence of herbal compounds on modified target sites can also involve those modifications implied by enzymatic alterations. Macrolide resistance is a clear example of this type, where enzymatic methylation of the ribosome 23S-rRNA leads to changes in the macrolide binding site. The enzyme adenine-N^6-methyltransferase, which is encoded by the *erm* gene family, could be the main target of two plant extracts (from *Alnus incana* L. fruits and *Geranium pratense* L. rhizomes), leading to modulating the resistance to erythromycin [377]. Presumably, active compounds present in these plant extracts have either reduced the expression of *erm* genes or blocked specific active locations on the resistance-mediating enzyme.

Effects on the drug-modifying enzymes

The production of enzymes that degrade or modify antimicrobial drugs is another example of bacterial resistance. This could be mediated by β-lactamases, which act by the hydrolysis of β-lactam antibiotics, such as penicillin and cephalosporins. Extracts and herbal compounds isolated from *C. sinensis* (green tea) have been found to interfere with the activity of β-lactamase [300]. The flavonoid baicalin (extracted from *Scutellaria amoena*) has also exhibited significant inhibitory activities via interfering with β-lactamase [378]. As such, some herbal bioactive compounds might be synergistically used with other antibiotics.

For example, Aqil *et al*. have tested the efficacy of ten medicinal bioactive compounds extracted from Indian plants on clinical isolates of β-lactamase-producing MRSA [379]. Of these, an extract from *C. sinensis* had potent antimicrobial activities (MICs of 1.8–7.5 mg/mL), and it showed synergism with ampicillin and tetracycline. Other β-lactamase-targeting herbs have also yielded synergistic effects with tetracycline, including *Lawsonia inermis*, *Terminalia chebula*, *Punica granatum*, and *Terminalia belerica*. In an antimicrobial susceptibility assay, Lee *et al*. [380] found that the MICs of galangin, a flavonoid obtained from the Korean herb *Alpinia officinarum*, ranged between 62.5 and 125 μg/mL, whereas those of gentamicin were 1.9–2000 μg/mL. The combined effects of time and antibacterial concentrations were synergistic against MRSA clinical isolates (FICI 0.25). However, the authors failed to conclude the major mechanism of action by which the synergism has occurred. The fact that galangin had prevented the action of β-lactamase produced by *Stenotrophomonas maltophilia* [381] and the interaction which had been previously reported between gentamicin and β-lactam antibiotics [382] may all explain a possible effect of gangalin on β-lactamase. Such activities need to be addressed in future experimental studies.

Combination Therapies

Some combinations of herbal compounds were effective in reducing the burden of resistance of MRSA. Tawfiq *et al*. [383] have shown that a combination comprising of the stem bark methanol extracts of *Faidherbia albida* and *Psidium guajava* was synergistically effective against clinically resistant isolates from boils. Those extracts contained considerable proportions of flavonoids, alkaloids, and tannins, which have acted against multiple targets. That is, the impact of tannins was evident since they have had MIC of 0.78 mg/mL against MRSA clinical strains, and they downregulate 44 genes encoding 30S and 50S MRSA proteins [384]. On the other hand, alkaloids act against a wide range of molecular targets, such as disruption of the outer membrane and influencing cell division [385].

Based on the most effective herbal preparations, Yarnell and Abascal [386] have suggested comprehensive formulations for cutaneous and systemic MRSA infections. For mild or early skin infections, the authors presented a special formula comprising of 25% tea tree oil (*M. alternifolia*), 25% *Santalum spicatum* essential oil, 25% *O. vulgare* oil, 20% *Rosmarinus officinalis* oil, and 5% absorption enhancer. This could be applied 2–3 times daily in a base of honey or in a cream formula containing *A. sativum* (30 mL) as a base cream. Additional tinctures may be added to the cream containing resistance-modulating phytochemicals (such as *C. sinensis*). Patients with more severe MRSA topical infections can apply these preparations more frequently along with taking fresh cloves of garlic orally several times per day.

Safety Aspects of Herbal Remedies

Given that most of the conducted studies of herbal remedies have involved microbiological

investigations without clinical considerations, little is known about the possible adverse events that may emerge with their use, either topically or systemically. The adverse events of phytochemicals applied to the skin in preclinical studies are reviewed in [239].

However, data from clinical evidence showed acceptable safety profiles. Caelli *et al*. [344] reported that five patients (out of 15) who had received an IF of tea tree oil therapy experienced mild burning and swelling of nasal mucosa. In another RCT comparing the efficacy of tea tree oil versus standard treatment using chlorhexidine, mupirocin, and sulfadiazine to clear MRSA carriage, the application of the plant oil was well-tolerated, producing no adverse events [347]. However, Blackwood *et al*. [345] revealed that 1.03% of patients assigned to a decolonization regimen of tea tree oil (5%) body wash experienced body rash and they withdrew from the study compared to no reported adverse events in patients who had received Johnson's baby softwash.

Concluding Remarks and Implications

Health-care professionals strive to develop novel therapies relying on effective antimicrobial agents to combat the progressively emerging MRSA infection. In the dermatological practice, the use of efficacious preparations is compounded by the abilities of antimicrobial compounds to penetrate the skin layers. Several herbal compounds have been widely investigated in the literature, providing promising outcomes. They have induced exclusive antibacterial effects or enhanced skin antisepsis in conjunction with traditional antiseptic/antibiotic agents. The antibacterial effects of active phytochemicals were exerted via different mechanisms, including the disruption of fatty acid synthesis in the cell membrane, the interference with the proton gradient, and binding to the PGNs in the cell wall. Effective combinations of these active metabolites in essential oils or plant extracts have been documented, where the antibacterial activities were apparent on multiple targets. Interestingly, plant-derived compounds have proven effective in modifying the antimicrobial resistance of MRSA through targeting NorA efflux pumps, PBP2a expression, or bacterial enzymes, allowing traditionally ineffective antibiotics to be reutilized. Finally, there are notable *in-vitro* and *in-vivo* effects of herbal compounds on MRSA biofilm formation, preformed biofilms, wound healing, and inflammation. These would not only indicate the usefulness of plant-based therapies on the virulence mechanisms of MRSA infections, but also on the co-associated chronic inflammatory conditions, such as AD and psoriasis.

The use of such herbal remedies would have several benefits. First, intuitively, the antibacterial metabolites of plants represent an integrative part of the chemical defense strategy against the diverse microbial population in the surrounding environment; therefore, these compounds are expectedly efficacious against MRSA as well as other pathogenic microorganisms. Second, there is a plethora of chemical compounds that can be applied topically or used systematically, providing a wide variety of chemicals that could be therapeutically effective. Third, the obtained preparations would create a cheaper alternative of the current antimicrobial preparations, which had been clinically limited by the development of bacterial resistance. Fourth, the reported synergism between various herbal metabolites within essential oils or extracts would have a promising clinical relevance; such that the inhibitory concentrations of active chemicals could be reduced and hence the safety aspects of could be reserved. Fifth, the applicability of herbal metabolites to other areas of medicine, such as cancer, might open novel ways to their multi-targeted approaches.

However, the application of these herbal compounds may have several limitations. There is a lack of robust RCTs which compare their efficacy and safety against established preparations. In 2014, a Cochrane systematic review showed no conducted RCTs concerning the role of Chinese herbal medicines in the treatment of SSTIs [387]. This might be supported by the lack of widely used plant-derived preparations for topical skin infections, particularly in patients infected with resistant bacterial strains. Although many of the botanical compounds reviewed in the current work have been used as supplements as immune system enhancers and nutritional supplements, none of these natural compounds have been approved by the FDA.

The applied methodology in antimicrobial screening may pose another limitation. The reviewed studies have mostly relied on phenotypic screening methods, such as broth microdilution and agar dilution. The inherent limitations of these methods when multiple active compounds are used could have led to false negative results; such that, the true antimicrobial compounds could have not been identified [388]. Furthermore, regional differences in extraction methods, raw plant composition, and instrumental variations may all lead to wide variations in the obtained outcomes. From another point of view, the solubility and bioavailability of natural botanical compounds may reduce their potential expansion as antibacterial agents in dermatology. In essence, the delivery of active phytochemicals to the skin need to be optimized by technological advances, such as NPs and microemulsions, and these approaches require further validation for clinical use.

Therefore, future investigations are needed to assess the benefits of established anti-MRSA botanical compounds loaded into nanocarriers on randomized patient groups with various types of SSTIs, considering skin tolerance and the clinical efficacy of these

compounds. This would in turn help develop guidelines for SSTI treatment based on reliable evidence and will assist in reducing the associated burden of topical infections and their potential invasive abilities to other organ systems.

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