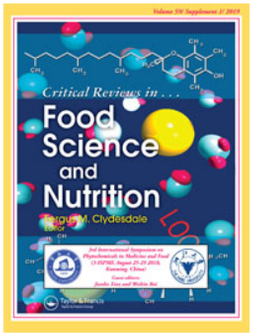

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REVIEW

Novel antibacterial modalities against methicillin resistant *Staphylococcus aureus* derived from plants

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ABSTRACT

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a notorious bacterial pathogen that induces high mortality and morbidity. Due to the emergence of multiple resistance, antibiotic treatments are rapidly becoming ineffective for the related infections. Natural products, especially those derived from plants, have been proven to be effective agents with unique antibacterial properties through different mechanisms. This review interprets the resistance mechanisms of MRSA with the aim to conquer public health threat. Further, recent researches about plant antimicrobials that showed remarkable antibacterial activity against MRSA are recorded, including the crude plant extracts and purified plant-derived bioactive compounds. Novel anti-MRSA modalities of plant antimicrobials such as alteration in efflux pump, inhibition of pyruvate kinase, and disturbance of quorum sensing in MRSA are also summarized which may be promising alternatives to antibacterial drug development in future.

KEYWORDS

MRSA; plant antimicrobials; efflux pump; pyruvate kinase; quorum sensing

Introduction

The global life has reached to the post-antibiotic era and it threatens millions of lives each year according to the World Health Organization (WHO 2014). This disastrous effects ascribe to an indiscriminate and improper use of antimicrobial drugs in veterinary medicine, aquaculture, agriculture and infection treatment in humans. The bacteria resistance can be intrinsic but can also be developed via spontaneous mutation in chromosome or by horizontal gene transfer (Alekshun and Levy 2007; Giedraitiene et al. 2011). The uncontrolled use of antibiotics increases the selective pressure and further accelerates the spread of resistances (Davies and Davies 2010). Multiple drug resistance *Staphylococcus aureus* (MDR *S . aureus*) has been developed to be one of the prominent life-threatening bacterial pathogens. It is not limited to hospitals and is also becoming widespread in natural environments, raising the concern that those antibiotic resistance reservoirs might threaten the health and safety of human (Deleo and Chambers 2009). It is reported that around 90–95% of *S. aureus* strains worldwide are resistant to penicillin, and 70–80% are also methicillin resistant (known as MRSA) in most of the Asian countries, which are reported to be resistant to the last line of antibiotic defense (Hemaiswarya, Kruthiventi, and Doble 2008). The prevalence of multiple drug resistance has retarded the development process of effective synthetic antimicrobial drugs. Therefore, it has necessitated the search for new antimicrobial agents from alternative sources to control the situation.

Plants are the important sources for drugs and alternative medicines to ameliorate symptoms and fight against diseases since ancient times. Nowadays, the use of plant-derived natural agents in medical treatments is drawing more attention due to its potential efficacy and fewer side effects (Subramani, Narayanasamy, and Feussner 2017). Indeed, plants have evolved a wide variety of chemical defenses, referred to as secondary metabolites, to resist the environmental stresses and hostile conditions (Borges et al. 2016; Suzuki et al. 2014). These compounds are divided into phytoanticipins and phytoalexins. The former includes saponins, cyanogenic glycosides and glucosinolates which are stored as inactive forms or produced after pathogen attack. The latter includes terpenoids, glycosteroids, flavonoids and polyphenols which are synthesized and accumulated after exposure to attackers (Furstenberg-Hagg, Zagrobelny, and Bak 2013; Gonzalez-Lamothe et al. 2009). However, most researches of antimicrobial agents from botanical sources were stuck in the bacteriostatic and bactericidal assays (Subramani, Narayanasamy, and Feussner 2017). Understanding the action mechanisms of plant extracts and taking advantage of their rich chemical composition will be beneficial to explore

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Table 1. Recent research on plant extracts and plant-derived compounds against MRSA.

Plant names	Source	Extraction method	Active phytochemicals/ compounds	Inhibition effect	Reported country	References
Azadirachta indica	Leaf	15% TCA-acetone	Low molecular weight polypeptides (11-14 kDa)	ZOI width: 19.0 mm ZOI height: 15.0 mm	India	Al Saiqali et al. (2018)
Atuna racemosa Xanthostemon verticillatus Syzygium antisepticum Syzygium antisepticum	Leaf Leaf Leaf Stem	Acetone:methanol:water (AMW, 2:2:1)	/	MIC >2, MBC >2 mg/mL MIC: 1, MBC: 2 mg/mL MIC: 0.125, MBC: 0.5 mg/mL MIC: 0.5, MBC: 2 mg/mL	Singapore	Yuan and Yuk (2018)
Moluccella spinose Helichrysum sanguineum Styrax officinalis	Aerial parts	Aqueous extract Organic extract Aqueous extract	Cardiac glycosides, phenols, tannin, steroids, flavonoid	MIC: 6.25 mg/mL MIC: 0.78 mg/mL MIC: 3.125 mg/mL	Palestine	Jaradat et al. (2018)
Acalypha integrifolia Willd.	Leaf	Methanol	Terpenes, tannins, polyphenols, coumarins, flavonoid, sugars	MIC: 0.5 mg/mL	Mauritius	Seebaluck-Sandoram et al. (2018)
Lycium shawii Phyllanthus emblica	Seed	Methanol	Alkaloids, phenolic compounds, tannins and flavonoids	MIC: 10 mg/mL MIC: 8–10 mg/mL	Malaysia	Tayel et al. (2018)
Dracontomelon dao	Stem bark	60% ethanol	Secondary metabolites	ZOI: 11.7 mm MIC: 1.8 mg/mL MBC: 3 mg/mL	Indonesia	Yuniati et al. (2018)
Bauhinia kockiana	Flower	Ethyl acetate	Tannins	ZOI: 11.3-11.7 mm MIC: 62.5-125 μg/mL MBC: 125-500 μg/mL	Malaysia	Chew et al. (2018)
Phyllanthus columnaris	Stem bark	Methanol	Tannins	MIC: 0.78 mg/mL MBC: 1.56 mg/mL	Malaysia	Adnan, Ibrahim, and Yaacob (2017)
Annona squamosa L.	Leaf	Methanol	Corydine, sanjoinine, norlaureline, norcodeine, oxanalobine and aporphine	MIC >25 mg/mL MBC: 12.5 mg/mL	Iraq	Shami et al. (2017)
Ononis pubescens L.	Entire plant	n-Hexane	Cardiac glycosides, carbohydrates, steroids, terpenoids	MIC: 6.25 mg/mL	Palestine	Jaradat et al. (2017)
Astragalus pelecinus	Aerial parts	Aqueous extract	Proteins, phenols, terpenoids, flavonoids, glycosides and alkaloids	MIC: 33 mg/mL	Palestine	Jaradat et al. (2017)
Urtica dioica Lavandula angustifolia	Leaf	70% ethanol	Phenolic acids, flavonoids, flavones and flavonols	ZOI: 14.0–21.3 mm MIC: 0.063–0.500 mg/mL	Portugal	Zenao et al. (2017)
Nigella sativa	Seed	Methanol	/	ZOI: 18-25 mm MIC: 0.39-1.50 mg/mL	Pakistan	Uzair et al. (2017)
Erica manipuliflora Juniperus foetidissima Berberis vulgaris Olea europaea	Bark Leaf Fruit Leaf	Hot aqueous	/	MIC: 0.625 mg/mL MIC: 0.25 mg/mL MIC: 0.101 mg/mL MIC: 0.125 mg/mL	Turkey	Comlekcioglu et al. (2017)
Ferulago trojana	Aerial Rhizome	Methanol	Phenolic, flavonoids	MIC: 48 mg/L MIC: 19 mg/L	Turkey	Selcuk et al. (2017)
Mentha piperita	Aerial parts	Ethyl acetate	/	MIC: 5 mg/mL MIC: 10 mg/mL	Saudi Arabia	Shalayel et al. (2017)
Pongamia pinnata Rubia cordifolia Linn Jasminum officinale Linn Garcinia zeylanica	Bark Stem Leaf Stem	Ethanol	/	ZOI: 4 mm ZOI: 10.75 mm ZOI: 4.6 mm ZOI: 14 mm	Sri Lanka	Gunasekara et al. (2017)
Morus alba	Bark	Ethanol	Cyclocommunol, morusin and kuwanon E	MIC: 4–32 μg/mL MBC: 16–128 μg/mL	China	Zuo et al. (2018)
Solanum schimperianum	Roots	Methanol-water-acetic acid (94:6:1)	Soladunalinidine, Solacallinidine, Solanopubamine	MIC: 31.25 μg	Saudi Arabia	Balabanova et al. (2018)
Commiphora leptophloeos	Bark	Organic aqueous	Hinokinin	MIC: 0.0485–3.125 mg/mL MBC: 0.40–3.125 mg/mL	Brazil	de Souza Pereira et al. (2017)
Zanthoxylum tingoassuiba	Root bark	Dichloromethane methanol	Dihydrocheleryhtrine N-methylcanadine	MIC: 171.7 μM MIC: 153.9 μM	Brazil	Costa et al. (2017)

new antimicrobial drugs or novel strategies against multiple drug resistance bacteria.

In this review, the current knowledge on the development of MDR and MRSA is summarized and the resistant mechanisms are discussed. Further, an effort is made to summarize the recent researches of the crude or purified plant extracts against MRSA. To counteract the resistance problems of MRSA, innovative strategies are suggested based

on literature data. Among them, an overview on the use of phytochemicals as efflux pump and pyruvate kinase inhibitors against MRSA are provided. The use of plant extracts to interfere with bacterial quorum sensing (QS) signaling pathways of MRSA is also reviewed.

The resistance mechanisms of MDR bacteria and MRSA

Multiple drug resistance (MDR) is the bacterial ability to withstand lethal doses of several classes of antimicrobial drugs, even though their structure and action mechanism are diverse (Coates, Halls, and Hu 2011). In order to reduce the antibiotic concentration in the cell, the cell surface might be modified and impermeable barriers can be developed, resulting in the interaction limitation with the drugs or reduction of entry channel number (Fernandez and Hancock 2012). Another mechanism by which bacteria can be resistant is the degradation or modification of the antibiotic, which is mainly through enzymatic hydrolysis. A lot of enzymes including β-lactamase, aminoglycosides, phenicols and macrolides are able to degrade and modify different classes of antibiotics (Bush and Jacoby 2010; Wright 2011). The overexpression of efflux pumps is another mechanism of resistance. Efflux pumps are active transport proteins that provide self-defense mechanism to extrude antimicrobial drugs from the cells, lowering the intracellular drug concentrations to non-cytotoxic levels (Jarmula et al. 2011). Thus the bacteria with the efflux-mediated resistance are able to overexpress efflux pumps to cope with the high drug concentrations (Piddock 2006). In addition, the modifications of the target molecules can also influence the binding affinity of antibiotics (Dzidic, Suskovic, and Kos 2008). It can be achieved by spontaneous chromosomal mutations or acquiring genes that encode modified target with lower affinity (Wright 2011). A classic example is the development of methicillin resistance in *S. aureus*.

Speaking of *S. aureus*, it has developed resistance to a number of antibiotics such as penicillin, amoxicillin, methicillin and oxacillin by the acquisition of horizontal gene transfer of mobile genetic elements (Vuong et al. 2016). The penicillin-resistant *S. aureus* was reported as early as the 1940s due to the acquisition of plasmid-encoded β-lactamase. The β-lactamase-resistant methicillin was then introduced in the 1960s to defend against penicillin-resistant *S. aureus*, which can inhibit transpeptidase mediated peptidoglycan cross-links after binding with cell wall PBPs (Kali 2015).

However, the emergence of methicillin-resistant *S. aureus* arose shortly thereafter (Jevons, Rolinson, and Knox 1961). Methicillin resistance is due to acquisition of *mecA* gene that is carried by staphylococcal cassette chromosome *mec* (SCC*mec*), encoding a homolog of the PBP2 called PBP2a or PBP2' (Hiramatsu et al. 2001). The expression of *mecA* gene is usually inducible and affected by two regulator genes (*mecI* and *mecR1*) and additional genes like *blaI*, *blaR1*, *femB*, *aux* (Chambers 1997). MRSA is probed to have low affinity for β-lactam agents and confers resistant to methicillin, which is due to the active site of PBP2a located in a deep pocket that is not accessed by β-lactams. In fact, the increased use of antibiotics in hospitals has been implicated in development of MDR in hospital-acquired MRSA (HA-MRSA) strains (Otto 2012). Likewise, the overuse of antibiotics in animal feed has caused occurrence of livestock associated MRSA (LA-MRSA) (Kali 2015). Thus, MRSA represents a serious global threat which is not limited in hospitals but also becoming widespread in daily living.

Unfortunately, strains of *S. aureus* that are resistant to vancomycin, the common antibiotic choice for MRSA, have already been reported (Hiramatsu 1998). Facing the imminent crisis of antibiotic resistance, significant efforts should be made to screen various sources for the new antimicrobial agents or find alternative interference strategies to disable the resistance.

Plant-derived antimicrobials to fight against MRSA

With the increase in awareness of medicinal value of plants, many studies have investigated the antimicrobial properties of plant extracts against MRSA in current years (Kali 2015; Subramani, Narayanasamy, and Feussner 2017). Indeed, it was indicated that the plant-derived pure compounds showed same therapeutic effects as plant extracts, and some of which have been substituted as medicine ingredients. Table 1 summarizes a series of plant extracts reported against MRSA. The different plant parts including leaves, flowers, fruits, bark, root, stem, rhizome that are extracted by various solvents such as aqueous, dichloromethane, methanol, ethanol, ethyl acetate were classified. The list clearly shows the anti-MRSA activity of plant extracts by determining inhibition zones, minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs). In addition, morphological and ultrastructural changes of MRSA cells after exposed to plant extracts were observed using scanning and transmission electron microscopy, which might result in cell wall alterations, shrunken cytoplasm, burst and distorted cells (Khan et al. 2016; Chew et al. 2018). Most studies also expressed the result of phytochemical screening of these extracts, in terms of the presence of secondary metabolites such as alkaloids, terpenoids, saponins, flavonoids, steroids and glycosides. The mechanisms of antimicrobial action of phytochemicals have been reviewed by Pandey and Kumar (2013). The plant-derived quinones and flavonoids have similar action mechanisms by binding to the adhesions and then interacting with cell wall. However, polyphenols and tannins have showed extra functions such as enzyme inhibition, substrate deprivation, membrane disruption and metal ion complexation. Transcriptome analysis of the a MRSA strain in response to tannins was performed, finding that tannins induced significant modulation in essential ribosome pathways. The reduced translation processes led to inhibition of protein synthesis and bacterial growth (Adnan, Ibrahim, and Yaacob 2017). The terpenoids and essential oils are able to cause membrane disruption, while the alkaloids can intercalate into cell wall and DNA. Two triterpenic acids (ursolic and

oleanolic acids) from *Vitellaria paradoxa* leaf extract showed synergistic effect with β-lactams against MRSA by inhibiting β-lactamases activity. More importantly, they were able to delocalize PBP2 from the septal division site and disturb peptidoglycan synthesis, resulting in the reversion of MRSA phenotype (Catteau et al. 2017).

Despite ample researches have proved the anti-MRSA ability of various plant products, there still lack of adequate information about the action mechanisms of precise phytochemicals against MRSA. In most instances, these phytochemicals are often named generically as alkaloids, terpenoids, flavonoids, etc. A few studies have isolated each phytochemical in pure form to identify the structures and test their anti-MRSA ability, which are also appended in Table 1. Among them, Nzogong et al. (2018) isolated and identified fourteen compounds from the two plant species *Dissotis senegambiensis* and *Amphiblemma monticol.* Compounds 3,4′-di-O-methylellagic acid, ellagic acid and the mixture of ellagic acid +3-O-methy- lellagic acid 4′-O-β-D-xylopyranoside showed prominent antibacterial activity against MRSA. In the study of Ordonez et al. (2011), the sesquiterpene lactones, leucodine and dehydroleucodine, were extracted and purified from the aerial parts of *Gynoxys verrucosa* to against MRSA, and their structures were elucidated by spectroscopic methods and single-crystal X-ray analysis. Dehydroleucodine was found to have much more significant anti-MRSA activity than leucodine, demonstrating that the exocyclic conjugated methylene in the lactone ring was essential for the antimicrobial activity of this sesquiterpene lactone. Chew et al. (2018) reported gallic acid and methyl gallate could exhibit antibacterial activity towards MRSA in *Bauhinia kockian,*. Amphiphilic nature of the two compounds exhibited the anti-MRSA action via three stages: cell membrane attachment; cell membrane fluidity modification; and cell membrane structure disruption. Kuok et al. (2017) introduced *in silico* molecular docking to investigate the binding affinities of 139 compounds of the four herbs (*Magnolia officinalis*, *Verbena officinalis, Momordica charantia*, and *Daphne genkwa*) to MRSA inhibitory targets— PBP2a and PBP4. Results revealed tiliroside, pinoresinol, magnatriol B, and momorcharaside B to be inhibitors of PBP2a or PBP4. In addition, a method integrating biochemometric and molecular networking was devised to comprehensively evaluate the antimicrobial activity of *Angelica keiskei* to against MRSA (Caesar et al. 2018). A subset of chalcone including 4-hydroxyderricin, xanthoangelol and xanthoangelol K was determined to possess anti-MRSA activity. Taken together, crude plant extracts and purified phytochemicals have showed remarkable antibacterial activities against MRSA, and it is of great importance to further clarify the action mechanisms of precise bioactive compounds for the development of antibacterial drugs.

Novel strategies: plant-derived efflux pumps inhibitors (EPI)

Microbial efflux pumps have been recognized as major substances in mediating multidrug resistance, and it has also been shown that production of efflux pumps is up-regulated in MRSA. To date, many multidrug resistance efflux pumps have been described for *S. aureus* (Jang 2016). NorA, NorB, NorC, MdeA, SdrM, LmrS, MepA and SepA are chromosomally encoded, while QacA, QacB, QacG, QacH, QacJ and Smr are plasmid encoded systems (Costa et al. 2013). These pumps can be served as promising antibacterial targets. One of the most studied efflux pumps in *S. aureus* is NorA. It belongs to the MFS group which consists of 400–600 amino acids with 12 or 14 transmembrane spanners (Pao, Paulsen, and Saier 1998). Some review papers have been published to summarize the NorA-induced efflux inhibitors from a NorA overexpressed *S. aureus* (Prasch and Bucar 2015). Given that efflux pumps are tightly related to antimicrobial drug resistance, the development of efflux pump inhibitors (EPIs) will serve as a promising method to circumvent the drug resistance mechanism (Schindler, Jacinto, and Kaatz 2013). The potency of specific antibiotic might be restored by using of EPIs to lower the efflux pumps activity in resistant *S. aureus* strains and synergistically increase the susceptibility of the bacteria.

Compounds extracted from natural products are of interest because of their ability to inhibit efflux pumps in MRSA. The first clinical drug shown to reverse an MDR phenotype in *S. aureus* is a plant alkaloid called reserpine. Gibbons and Udo (2000) indicated 17 μM of reserpine afforded a fourfold tetracycline MIC reduction in MRSA which encodes for the TetK efflux protein, indicating that TetK is reserpine susceptible. Similarly, Smith et al. (2007) reported ferruginol, a diterpene isolated from *Chamaecyparis lawsoniana*, was also able to cause fourfold tetracycline MIC reductions in MRSA strain possessing the TetK pump. Mohtar et al. (2009) evaluated the efflux inhibitory activity of 13 alkaloid compounds as potential EPIs against MRSA. Results showed quinine isolated from Cinchona tree's bark, piperine isolated from *Piperaceae* family and harmaline isolated from *Perganum harmala* could cause ethidium bromide (EtBr) MIC reduction ranging from two- to eight fold. Speaking of EtBr, it was often used as a substrate to test the efflux inhibition activities of EPIs against MRSA by a fluorometric efflux assay, and the list of plant-derived EPIs includes 4',5'-O-dicaffeoylquinic acid isolated from wormwood (*Artemisia absinthium*) (Fiamegos et al. 2011), triterpenoids from *Momordica balsamina* (Ramalhete et al. 2011), carnosic acid from *Rosmarinus officinalis* (Oluwatuyi, Kaatz, and Gibbons 2004), coumarins from *Mesua ferrea* (Roy et al. 2013) etc. Silybin is a flavonolignan component of the extract from the milk thistle seed, which can reduce the expression of the quinolone resistance protein NorA and quaternary ammonium resistance proteins A/B (Qac A/B) efflux genes in MRSA. In addition, silybin eliminated the plasmids of MRSA, which appeared to serve an important role in bacterial resistance to antibiotics by carrying the resistant genes. The inhibition of *nor A* and *qac A/B* genes expression and elimination of plasmids by silybin enhanced the sensitivity of MRSA to antibiotics, indicating that silybin EPI was able to reverse the MDR phenotype in MRSA (Wang et al. 2018). Similarly, down-regulation of MFS and MATE family efflux

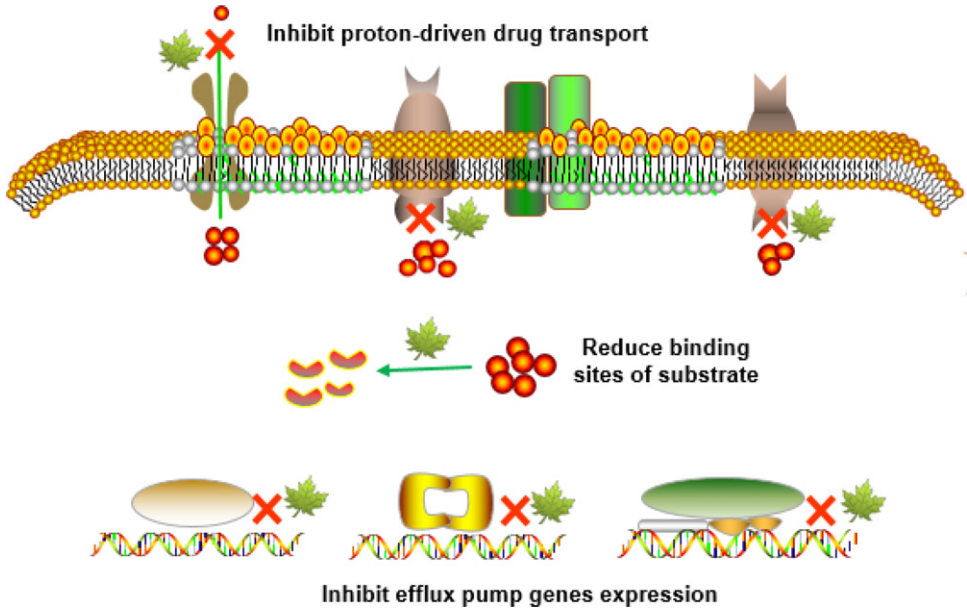

Figure 1. Schematic representation of possible action mechanisms of EPIs derived from plants against MRSA (Christena et al. 2015; Kakarla et al. 2017; Wang et al. 2018).

genes such as *norA*, *norB*, *norC*, *mdeA* and *mepA* was observed when treated by clerodane diterpene from *Polyalthia longifolia* (Gupta et al. 2016). Christena et al. (2015) reported that pinostrobin, a flavonoid nutraceutical, displayed drastic 128 fold reduction in ciprofloxacin MIC in MRSA. Notably, studies with NorA mutants of *S.aureus* revealed efflux inhibition of pinostrobin is not mediated by NorA. Indeed, pinostrobin was considered to enhance the membrane permeability, which possibly augmented its EPI effect and in turn accounted for the effectivity of pinostrobin in curtailing drug resistance (Christena et al. 2015). In addition, cumin spice (*Cuminum cyminum*) was demonstrated to have the ability to inhibit LmrS drug transport (Kakarla et al. 2017). LmrS is a proton-driven multidrug efflux pump in MRSA and confers resistance to many antimicrobial agents (Andersen et al. 2015). The studies indicated that cumin extract specifically acted on the LmrS and inhibited the ethidium transport across the membrane at low concentrations. At relatively higher concentrations, it was suggested that these agents dissipated the proton motive force generated by respiration during metabolism (Kakarla et al. 2017).

In conclusion, efflux pump inhibition for MRSA might be achieved by several modes of action of EPIs which are illustrated in Figure 1: a) reduce the binding sites of substrate by competitive or noncompetitive manner, resulting in the blockage of efflux pump; b) inhibit the expression of efflux pump genes and eliminate the plasmids encoded efflux proteins; c) disturb the proton gradient across the membrane and disrupt the pump's required energy source.

Novel strategies: plant-derived pyruvate kinase inhibitors (PKI)

Pyruvate kinase (PK) is an enzyme involved in catalyzing the final step of glycolysis, converting phosphoenolpyruvate (PEP) to pyruvate and ATP from ADP (Suzuki et al. 2008). Recently, it has been identified as a highly interconnected essential hub protein in the MRSA with structurally discrete features unlike mammalian orthologs, which can be made as a novel antimicrobial target (Cherkasov et al. 2011). This was based on that hub proteins are critical for bacterial survival and are expected to be less prone to genetic mutations. Therefore, the potential for the development of resistant bacteria might be reduced by targeting the PK. The disruption of PK can lead to interruption of carbohydrate metabolism and energy depletion, and thus ultimately resulting in bacterial cell death (Zoraghi et al. 2014). In view of this, recent developments have led to the search of compounds derived from plants that potently and selectively inhibit MRSA PK. Diosmin (3′,5,7-trihydroxy-4′-methoxyflavone-7-rutinoside) and diosmetin (3′,5,7-trihydroxy-4′-methoxyflavone) are natural flavonoids found in a variety of citrus fruits (Chan et al. 2013). It was shown that diosmetin was able to significantly suppress the MRSA PK activities in a dose dependent manner with inhibitory activity of 48% observed at 10 µM diosmetin. Two human isoforms, M1 and M2, were tested to determine diosmetin could inhibit MRSA PK selectively without affecting the human ortholog. In contrast, diosmin did not show inhibitory activity against MRSA PK enzymatic activity. The difference might be due to the presence of rutinose in the diosmin chemical structure, which might hamper its antibacterial activities in vitro. Chan et al., (2011) studied the effect of baicalein, the active constituent derived from *Scutellaria baicalensis* Georgi. on inhibiting the enzymatic activity of MRSA-specific PK. The results demonstrated that baicalein alone significantly inhibited 60% of MRSA PK enzymatic activity at a concentration of 10 µg/mL. It was suggested that the inhibition of MRSA PK by baicalein could lead to ATP deficiency, which might further contribute to the antimicrobial activity of baicalein against MRSA. Indeed, there has been scarce study about

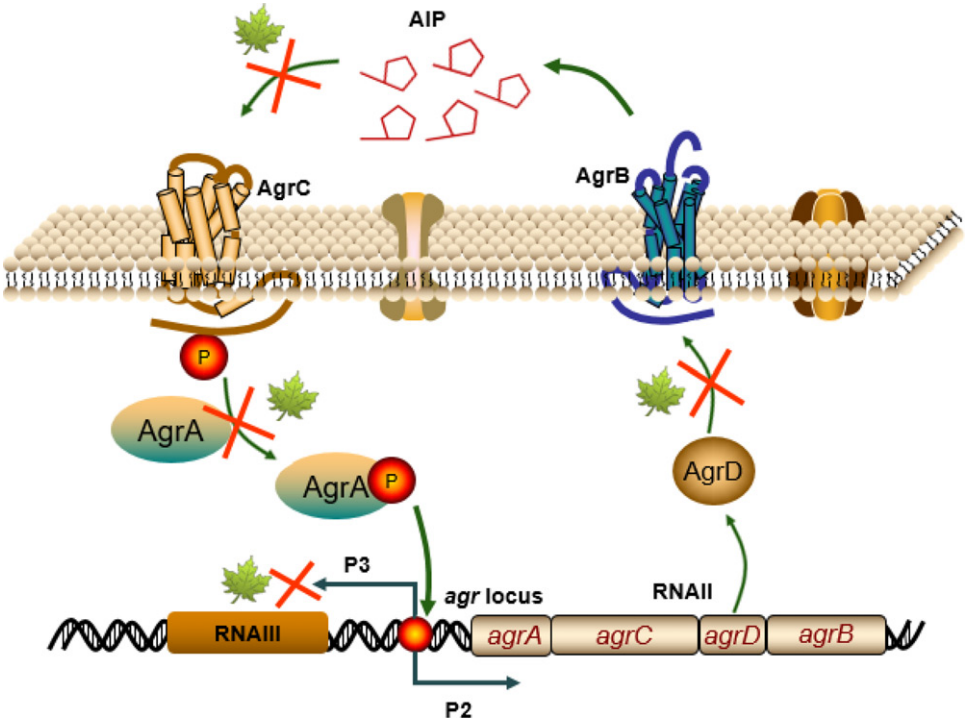

Figure 2. Schematic representation of possible action mechanisms of QSIs derived from plants against the *S. aureus* accessory gene regulatory (agr) system (Cech et al. 2012; Quave, Plano, and Bennett 2011; Quave et al. 2015).

the plant-derived PK inhibitors yet, but sources from natural products like marine sponges or marine invertebrates are explored more deeply (Kumar et al. 2012; Kumar et al. 2014; Zoraghi et al. 2011). They even reported the discrete structural features of the MRSA PK tetramer using x-ray crystallography, and elucidated the essential structural requirements for PK inhibitors in "small" interfaces that provided for tetramer rigidity and efficient catalytic activity.

Novel strategies: plant-derived quorum sensing inhibitors (QSI)

The bacterial cell-cell communication in *S. aureus* employs autoinducing peptides (AIPs) as signal molecules, and their behavior depend on cell density (Quave and Horswill 2014). This mechanism is referred as quorum sensing, mainly regulated by *agr* (accessory gene regulatory system) in *S. aureus* (Gordon, Williams, and Chan 2013; Le and Otto 2015). As shown in Figure 2, the *agr* locus is composed of two adjacent but divergent transcripts called RNAII and RNAIII under control of P2 and P3 promoters, respectively (Thoendel and Horswill 2010). The RNAII transcript is an operon of *agr*BDCA. The AgrD is the peptide precursor of AIP, is processed and secreted through AgrB endopeptidase at the cytoplasmic membrane. At the threshold concentration, AgrC, a membrane-bound histidine kinase, is activated by binding AIP. The phosphorylation of the AgrA response regulator induces activation of the P2 and P3 promoters and transcription of RNAII and RNAIII. The high RNAIII levels lead to the production of numerous virulence factors, such as hemolysis, proteases, lipases (Arya and Princy 2013).

Applying inhibitors to block this quorum sensing to attenuate virulence and pathogenesis is the core of this approach (Rudkin et al. 2012; Scutera, Zucca, and Savoia 2014). This strategy has advantages over conventional antibiotics by disarming the pathogen via non-bactericidal pathways (Quave and Horswill 2014). Given that most virulence factors are not essential for bacterial viability, the quenching of virulence may not select or enrich for bacterial resistance, and preserving the beneficial resident flora in the meanwhile (Clatworthy, Pierson, and Hung 2007). The strategies were summarized to inhibit staphylococcal virulence expression by disturbing *agr* quorum sensing system, including competitive inhibition of AgrC-AIP binding and suppression of RNAIII expression (Chan, Coyle, and Williams 2004). The concept of screening plant-derived products against *S. aureus* based on anti-quorum sensing mechanism is relatively new; nonetheless, some promising findings have been published. Quave, Plano, and Bennett (2011) reported some degree of quorum sensing inhibiting is evident in 90% of the 168 Italian plant extracts screened, including those extracts with no growth inhibitory activity. It indicated extracts from three medicinal plants (*Ballota nigra*, *Castanea sativa*, and *Sambucus ebulus*) exhibited a dose-dependent response in the production of δ-hemolysin. a small peptide encoded in the RNAIII transcript. The results supported the idea that the inhibition of virulence factor production could be accomplished without growth inhibition, thus potentially avoiding selective pressures for drug-resistance. In another work, based on the observation *agr* P3 promoter reduction and reduced levels of δ-toxin production, Quave et al. (2015) suggested *Castanea sativa* (European Chestnut) leaf extracts rich in ursene and oleanene might directly target the core machinery of the *agr* system, including inhibition of AIP docking with AgrC, prevention of AIP production through AgrB, or reduction of AgrA activation. Cech et al.

(2012) also reported goldenseal *Hydrastis canadensis* L. leaf extracts (but not the isolated alkaloids berberine, hydrastine, and canadine) at sub-inhibitory concentrations showed quorum quenching activity. It was believed that the effect was not to kill the bacteria but to quench the agr quorum sensing system by attenuating signal transduction through the AgrCA two-component system. Such attenuation would eventually cause the reduced production in toxin by *H. canadensis* exposed MRSA, proving that quorum quenching effect was able to control the disease via quorum sensing system by triggering the pathogenic phenotype.

Future perspectives

The above literature reveals that a broad range of plant extracts possess anti-MRSA ability, however, there are no plant-derived antibiotics applied in clinical therapy yet. More research should focus on the isolation of precise bioactive components from plants, identify their structures and investigate their modes of action. The effort to develop new drugs is threatened by the fact that the isolation and identification process is time-consuming, therefore, new techniques such as high-throughput screening and computer-based methodology can be introduced. Among the promising strategies, the use of phytochemicals as efflux pumps inhibitors, pyruvate kinase inhibitors and quorum sensing inhibitors have attracted significant attention. In the future, the combinatorial therapies based on several strategies targeting different bacterial sites can be performed to fight against multidrug-resistant microorganisms. In addition, the majority of research are still restricted to the in-vitro laboratory experiments, in vivo-animal model studies and human clinical trials would be needed to further confirm the antibacterial actions and optimize a high therapeutic efficacy dosage at an acceptable toxicity level.

Conflict of interest

The authors declare no competing interests.

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