

Global resistance trends and the potential impact of Methicillin Resistant *Staphylococcus aureus* (MRSA) and its solutions

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Developing countries have greater burden of infectious diseases. A number of factors which promote antimicrobial resistance are availability of antimicrobials without prescription, substandard antimicrobial drugs, suboptimal hygiene, immunosuppression, etc. *Staphylococcus aureus* is a pathogen of major concern because of its ability to cause a diverse array of diseases ranging from minor infections to life threatening septicemia and its ability to adapt to adverse environmental conditions. Methicillin resistance among clinical isolates of *S. aureus* is still increasing. The major mechanism is the acquisition of the *mecA* gene that codes for additional penicillin-binding protein2a. Limited treatments for MRSA prompted the research for novel compounds with a broad spectrum of activity and new therapeutic strategies. Medicinal plants are a significant aspect of developing a safer antibacterial principle through isolation, characterization, identification and biological studies.

Key words: Antibiotic resistance; MRSA; *mecA* gene; medicinal plants

1. Introduction

In developing countries, bacterial infections are widespread, especially in informal settlements, due to poor sanitation and unhygienic conditions. Furthermore, diseases such as AIDS, malaria and tuberculosis, result in higher morbidity and mortality than those caused by susceptible pathogens; the global impact of increasing resistance is a major concern [1]. Antibiotic resistance among Gram-positive and Gram-negative pathogens is a worldwide problem both in the hospital and community settings [2]. The appropriate use of antibiotics is one of the humankind's most essential weapons against disease. Intervention need to target inappropriate patterns of use, specifically those that have contributed most significantly to the development of resistance [3]. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents [4]. Drug resistance can be described as a state of decreased sensitivity to drugs that ordinarily cause growth inhibition or cell death. More strains of pathogens have become antibiotic resistant, and some have become resistant to several antibiotics and chemotherapeutic agents, the phenomenon of multidrug resistance [5]. Limited treatment options for infections caused by such multi-resistant microorganisms prompted the search for novel compounds with a broad spectrum of activity and new therapeutic strategies [6].

2. *Staphylococcus aureus*

2.1. History

Staphylococcus aureus was first described at the end of the 19th century in pus from human abscesses. *S. aureus* is a major pathogen that is responsible for not only severe infections of the skin and skin structures but also life-threatening diseases because of its propensity to form biofilms on artificial materials, difficult-to-treat infections of catheters and other devices [7]. *S. aureus* was certainly a significant human pathogen prior to the development of antibiotics. For example, in the last century, *S. aureus* was the major bacterial cause of death in the influenza pandemic of 1918, among those who developed secondary bacterial pneumonia. Following the introduction of antibiotics, *S. aureus* developed resistance to penicillin in the 1940s, and then emerged as an important cause of serious nosocomial infections in the 1950s. With the development and widespread use of chloramphenicol and tetracycline in the 1960s, superinfections due to *S. aureus* occurred including staphylococcal enterocolitis [8]. The incidence of *S. aureus* related infections has increased dramatically since the emergence of methicillin resistant strains and high rates of mortality and morbidity are occurring worldwide [9]. Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major cause of infections in healthcare institutions [10] and more recently in the community [11]. MRSA was first reported in 1961, two years after the introduction of methicillin for the treatment of penicillin-resistant *S. aureus* infections [12]. Despite extensive infection control efforts, methicillin resistance among isolates of *S. aureus* has steadily increased. Data from the National Healthcare-associated Infections Surveillance (NHIS) system of the Centers for Disease Control and Prevention (CDC) show that 50% of healthcare-associated *S. aureus* isolates are now resistant to methicillin [13].

2.2. Characteristics

Staphylococci are nonsporulating, nonmotile Gram-positive cocci that have an average diameter of 1 μm and microscopically appear as grapelike clusters (Fig.1). When grown on blood agar, staphylococci form small (1 to 2 mm), smooth, round colonies that are often pigmented and may be surrounded by a zone of β -hemolysis. Staphylococci are very hardy organisms and can withstand much more physical and chemical stress than pneumococci and streptococci [8].

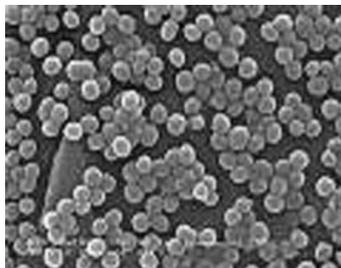


Fig1. Scanning electron micrograph of *S. aureus* [14]

2.3. Cellular structure

The cellular structure of *S. aureus* is complex. Most strains have polysaccharide microcapsules. The cell wall of *S. aureus* is structurally similar to that of Group A streptococci: both have a carbohydrate antigen, a protein component, and a mucopeptide. The carbohydrate antigen is a teichoic acid, which in *S. aureus* is a polymer of N-acetylglucosamine and polyribitol phosphate. Antibodies to teichoic acid can be detected in normal human serum, and elevated antibody titers are present in patients with deep-seated staphylococcal infections [15].

2.4. Virulence factors

The pathogenicity and virulence of *S. aureus* is associated with the capacity of this organism to produce several virulence factors including enterotoxins serotypes A through Q (SEA-SEQ), toxic shock syndrome toxin-1 (TSST-1), cytolytic toxins (α and β hemolysins), exfoliative toxins, Panton-Valentine leukocidin (PVL), protein A, and several enzymes [16]. Important virulence factor in *S. aureus* is PVL, a member of the recently described family of synergohymenotropic toxins. PVL damages the membranes of host defense cells through the synergistic activity of two separately secreted but non-associated proteins, LukS and LukF, causing severe abscesses, necrotizing pneumonia, and increased complications in osteomyelitis [17]. In addition to PVL, other toxins may be produced by *S. aureus*: α -toxin, which causes tissue necrosis and acts on cell membranes; exfoliatin A and B, which cause skin separation in diseases such as bullous impetigo and staphylococcal scalded skin syndrome; enterotoxins A, B, C1, C2, D, and E, which can cause vomiting and diarrhea associated with food poisoning; and toxic shock syndrome toxin 1 (TSST-1), which induces production of interleukin-1 and tumor necrosis factor leading to shock [18].

3. Methicillin Resistant *Staphylococcus aureus* (MRSA)

There are two distinct types of MRSA. Each has a slightly different genetic makeup. To categorize the type of the bacteria and how the condition is spread, MRSA infections are classified as either hospital-acquired or community-acquired MRSA infections (HA-MRSA or CA-MRSA).

3.1. Hospital associated-MRSA (HA-MRSA)

Hospital associated-MRSA is usually resistant not only to β -lactams but also to other types of antibiotics. Because many hospitalized patients are weak and their immune systems are compromised, HA-MRSA infections are often quite serious [19]. HA-MRSA first appeared in the United States in 1968. There are three types of Staphylococcal Chromosomal Cassette (SCC) *mec* in HA-MRSA: types I, II and III [20]. Type I contains no additional resistance determinants, but types II and III contain resistance determinants in addition to *mecA*; these additional genetic elements account for the antimicrobial resistance to many antibiotics in addition to the β -lactam agents. The three SCC*mec* types contained in HA-MRSA have an identical chromosomal integration site and cassette chromosome recombinase genes, which are responsible for horizontal transfer of SCC*mec* [21].

3.2. Community-acquired-MRSA (CA-MRSA)

Community-acquired-MRSA differs in several ways from HA-MRSA. Since the mid 1990s, MRSA strains have emerged in the community setting, causing infections in patients who do not have the risk factors usually associated

with hospital associated MRSA. Such as recent hospitalization, chronic diseases, kidney dialysis, human immunodeficiency virus infection, and intra-venous drug use [22]. Although community-acquired strains (CA-MRSA) cause mostly skin infection but sometimes severe infection resulting in death has also been associated with CA-MRSA [23]. CA-MRSA strains are usually resistant to β -lactams but susceptible to other antimicrobials like trimethoprim-sulfamethoxazole, clindamycin and tetracyclines [24] and carry mostly staphylococcal cassette chromosome *mec* (SCC*mec*) type IV. CA-MRSA strains are also more likely to possess unique combinations of virulence factors and seem to be genetically different from HA-MRSA [25]. CA-MRSA strains carry genes for Panton-Valentine leukocidin, which produce cytotoxins [26].

3.3 Risk factors

Approximately 30% of the population may carry *S. aureus*, usually methicillin-susceptible strains in the nares or on the skin [27]. Skin infection manifestations include boils, abscess, impetigo, folliculitis, cellulitis etc. Many patients suffer from recurrent CA-MRSA skin infections [28, 29]. Colonization of the anterior nares with *S. aureus* has been shown to be a risk factor for invasive infection. These infections include necrotizing pneumonia, necrotizing fasciitis, a septic shock syndrome characterized by multi-organ involvement among children, Waterhouse-Friderichsen syndrome, purpura fulminans, myositis, deep-seated infections of bone and joints, septic thrombophlebitis with extensive pulmonary embolization, and other serious syndromes [30].

3.4 Symptoms

A MRSA infection can cause a wide range of symptoms. (Fig.2) The infection usually first appears as a boil, or a pimple like bump that looks like a spider bite. In reaction to the infection, the immune system sends blood filled with disease-fighting white blood cells to the area. This causes the infected area also to become red, swollen, warm, and painful, all characteristics of inflammation, which is the body's way to combat dangerous microorganisms.



Fig.2 Some infections caused by MRSA

4. Antibiotics

Over the past 6 decades, bacterial populations have responded to the selective pressure of antimicrobial drugs by evolving resistance to all commercially available agents [31]. Decreased discovery rates of new classes of antimicrobial agents have substantiated a notion that, for some bacterial species, we might face clinical infections for which there are no treatment options [32]. Treatment options for both outpatient management of mild soft tissue infection and inpatient management of severe infection are limited because of increasing antimicrobial resistance [33]. Topical drugs such as mupirocin and fusidic acid are still effective against certain strains of MRSA, but resistance is increasing against these drugs as well [34]. Selection of appropriate antibiotics for MRSA infection, dosage, and route of administration, side effects and type of MRSA are shown in Table 1.

Table 1: Antimicrobial agents for the treatment of MRSA infection [33, 35-37]

Antibiotics	Dosage	Route of administration	Main side effects	Type of MRSA
Trimethoprim–sulfamethoxazole	2 double-strength tablets (160/800 mg) every 12 h (Adult)	Orally only	Nausea, vomiting, rash, myelosuppression (esp. thrombocytopenia), Stevens-Johnson syndrome, central nervous system (CNS) disturbances, hepatotoxicity	CA-MRSA
Doxycycline	100 mg every 12 h (Adult)	Orally only	Nausea, photosensitivity, deposition in teeth/bones	CA-MRSA
Minocycline	100 mg every 12 h (Adult)	Orally only	Nausea, photosensitivity, deposition in teeth/bones, vestibular toxicity	CA-MRSA
Clarithromycin	500 mg twice daily (Adult)	Orally only	Nausea, vomiting, diarrhoea, urticaria, rash	CA-MRSA
Erythromycin	500 mg four times daily (Adult)	Orally only	Mild gastrointestinal upset with nausea, vomiting, diarrhoea, urticaria, rash	CA-MRSA
Rifampin	600 mg every day (Adult)	Orally only	Discoloration of body fluids, liver function abnormalities, GI disturbances and nervous system symptoms, such as nausea, vomiting, headache, dizziness, and fatigue.	CA-MRSA
Sodium fusidate	250 mg twice daily; 500 mg three times daily (Adult)	Orally only	Nausea, vomiting, jaundice	CA-MRSA
Tetracycline	500 mg four times daily (Adult)	Orally only	Photosensitivity, rash, nausea, vomiting, diarrhoea, dysphagia, hepatotoxicity	CA-MRSA
Clindamycin	300-600 mg every 6-8 h (Adult)	Orally only	<i>Clostridium difficile</i> diarrhea	CA-MRSA
Linezolid	600 mg every 12 h (Adult)	Intravenous or orally	Myelosuppression (esp. thrombocytopenia) Contraindicated in concomitant SSRI use	CA-MRSA and HA-MRSA
Vancomycin	1 g every 12 h	Intravenous (orally for <i>C. difficile</i> enterocolitis)	Red-man syndrome	HA-MRSA
Daptomycin	4-6 mg/kg daily	Intravenous only	Muscle toxicity	HA-MRSA
Tigecycline	100 mg once, then 50 mg every 12 h	Intravenous only	Nausea, vomiting, photosensitivity, deposition in teeth/bones	HA-MRSA
Dalbavancin	1 g on day 1, then 500 mg on day 8	Intravenous only	Mild GI intolerance	HA-MRSA
Ceftobiprole	500 mg every 12 h	Intravenous only	Few serious adverse events in phase 3 trials	HA-MRSA

4.1. Mechanisms of Antibiotic Resistance

Resistance can be caused by a variety of mechanisms: (i) the presence of an enzyme that inactivates the antimicrobial agent; (ii) the presence of an alternative enzyme for the enzyme that is inhibited by the antimicrobial agent; (iii) a mutation in the antimicrobial agent's target, which reduces the binding of the antimicrobial agent; (iv) post-transcriptional or post-translational modification of the antimicrobial agent's target, which reduces binding of the antimicrobial agent; (v) reduced uptake of the antimicrobial agent; (vi) active efflux of the antimicrobial agent; and (vii) over production of the target of the antimicrobial agent [38].

4.2. Mechanisms of methicillin resistance

Methicillin is a β -lactam antimicrobial which binds penicillin-binding proteins (PBP) in the cell envelope and prevents cross-linking of the peptidoglycan (PG) chains in the cell wall. *S. aureus* renders methicillin ineffective by the production of an alternative PBP (PBP2a), which has reduced affinity for β -lactams [39]. Molecular targets for MRSA detection resistance to β -lactam antibiotics is due to acquisition of the exogenous gene, *mecA* that is incorporated into a large segment of DNA called Staphylococcal Chromosomal Cassette (SCC) *mec* that was first described by Katayama and co-workers in 2000 and which encodes for the penicillin-binding protein 2a (PBP2a) [40].

4.3. Mechanisms of Vancomycin Resistance

Vancomycin has long been the last-resort antibiotic against MRSA. Vancomycin inhibits peptidoglycan polymerization by binding to the D-Ala-D-Ala termini of peptidoglycan precursors disaccharide pentapeptide and impairing the subsequent reactions of transglycosylation and transpeptidation, thus compromising cell wall integrity [41].

5. Medicinal plants as antibacterial agent

The increase in prevalence of multiple drug resistance has slowed down the development of new synthetic antimicrobial drugs, and has necessitated the search for new antimicrobials from alternative sources. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs used as therapeutic agents. One way to prevent antibiotic resistance is by using new compounds which are not based on the existing synthetic antimicrobial agents [42]. Nature has served as a rich repository of medicinal plants for thousands of years and an impressive number of modern drugs have been isolated from natural sources, notably of plant origin [43]. Herbal medicine, based on their traditional uses in the form of powders, liquids or mixtures, has been the basis of treatment for various ailments in India since ancient times. The use of herbs as complementary and alternative medicine has increased dramatically in the last 20-25 years [44]. Screening active compounds from plants has led to the discovery of new medicinal drugs which have efficient protection and treatment roles against various diseases, including cancer [45] and Alzheimer's disease [46]. Development of newer anti-infective and therapeutic regimes that revert or overcome drug resistance is the need of the hour [47]. Sixty percent of the world population and 80% of the population in developing countries rely on traditional medicine for curing many diseases [48, 49]. Furthermore, most studies on the antimicrobial activity of plant extracts have been restricted to analysis of their bacteriostatic and bactericidal properties. Investigations into the antipathogenic potential of natural products may open new avenues for drug development in the control of antibiotic resistant pathogens [50]. The natural products play a major role from ancient civilizations to the current 20th century and more than half of the drugs in the market are natural products or their derivatives. Pharmaceutical industries are giving importance to the compounds derived from traditional sources. Only a small percent of plants have been investigated for their bioactivity. Photochemicals from medicinal plants showing antimicrobial activities have the potential of filling this need, because their structures are different from those of the more studied microbial sources, and therefore their mode of action may too very likely differ. The screening of plant extracts and plant products for antimicrobial activity has shown that plants represent a potential source of new anti-infective agents [51-54]. The continuing resistance of MRSA to many antibiotics resulted in the search of new anti-MRSA sources of plant origin, and in recent years many plant extracts showed anti-MRSA activity (Table 2).

Table 2: List of some plant extracts/isolated compounds for antibacterial activity against MRSA with Minimum Inhibitory Concentration (MIC) and/or zone of inhibition

Plant	Extract / Compounds	MIC (µg/ml)	Zone of inhibition (mm)	References
<i>Croton tonkinensis</i>	Diterpenoids	32	23	[55]
<i>Swietenia mahagoni</i>	Limonoids	-	23	[56]
<i>Callistemon rigidus</i>	Fractions from methanol extract	1.25	29	[57]
<i>Dictyota acutiloba</i>	Fractions from chloroform and acetone extract	0.69	15	[58]
<i>Andrographis paniculata</i>	Chloroform	1000	15	[59]
<i>Sclerocarya birrea</i>	Hexane (oil)	-	10	[60]
<i>Retama raetam</i>	Butanol extract	0.512	-	[61]
<i>Terminalia avicennioides</i>	Ethanol extract	18.2	17.6	[62]
<i>Phyllanthus discoideus</i>	Ethanol extract	20.5	16	
<i>Erythrina variegata</i>	Isoflavonoids	6.25	-	[63]
<i>Rosa damascena</i>	80% Ethanol	-	34	[64]
<i>Olea europaea</i>	Olive leaf extract	12.5	-	[65]
<i>Atuna racemosa</i>	Ethanol extract	16-32	-	[66]
<i>Punica granatum</i>	Water extract	12.5	-	[67]
<i>Planchonia careya</i>	Triterpene	800	-	[68]
<i>Baccharis grisebachii</i> ,	Dichloromethane extract	125	-	[69]
<i>Oxalis erythrorhiza</i>				
<i>Fabiana bryoides</i>	Ethanol extract	20	-	[70]
<i>Erodium malacoides</i>	Ethanol extract	128	-	[50]
<i>Calophyllum</i> species	Calozeoyloxanthone	4-8	-	[71]

6. Conclusion

The problem of microbial resistance is growing and the outlook for the use of antimicrobial drugs in the future is still uncertain. The emergence of MRSA organisms with reduced susceptibility to many antibiotics is a serious and ongoing concern. MRSA will continue to evolve, hence the absolute necessity to control it before it really does get out of hand. Therefore, action must be taken to reduce this problem. The ultimate goal is to offer appropriate and efficient antimicrobial drugs to the patient. Natural products offer a potentially rewarding route for the identification of novel antimicrobial agents. Further understanding of the structural and functional properties of plants may help in the standardization of drug formulations to be used against antibiotic-resistant *S. aureus*.

7. References

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