Mitigation Strategies for Vancomycin-resistant Staphylococcus aureus

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ABSTRACT

In just two hundred years the prevalence of infectious disease started soaring as new modes of travelling were invented. Now people could one place to another quickly carrying several kinds of disease with them. With the advent of diseases, life expectancy was reduced to around 35 years and death was rampant. These dire circumstances led to the discovery of antibiotics that became the main weapon of mankind against infectious diseases post-infection. Humanity was saved from the looming threat of constant epidemics and reduced life expectancy. Soon mankind began to thrive and people started increasing the use of antibiotics to battle all kinds of diseases. With overuse came the problem of misuse of antibiotics. People soon started using antibiotics without proper protocols and dosing regimens. This malpractice soon resulted in the emergence of a capability in bacteria to nullify the effects of antibiotics. The antibiotic resistance meant that the easily curable diseases once again became untreatable maladies. One such bacteria that gained antibiotic resistance was Staphylococcus aureus. S. aureus gained resistance against the methicillin group of antibiotics as they were commonly used against it. After that vancomycin became the drug of choice against methicillin-resistant S. aureus. Soon, people started misusing vancomycin too which quickly led to the development of vancomycin-resistant S. aureus (VRSA). VRSA were usually multi-drug resistant bacteria that effectively rendered, many of the antibiotics being used against them, ineffective. These conditions forced the researchers to look for alternative medication modes and techniques for countering antibiotic resistance.

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1. INTRODUCTION

In the groups of Gram-positive cocci Staphylococcus aureus is an organism often linked with gastroenteritis that occurs due to consumption of contaminated food products like milk (Ayele et al. 2017) and shrimp. The phenomenon of multidrug resistance in food-borne bacteria is a concern of worldwide occurrence (Song et al. 2015). S. aureus and several other bacteria resistant to beta-lactam drugs have been isolated from milk products (Ayele et al. 2017) and shrimp (Arfatahery et al. 2016). Out of many factors acting in the selection of drug-resistant bacterial pathogens, some important ones are misuse of antibiotics and improper disposal of antibiotics in the environment (Cheng et al. 2015; Roca et al. 2015). Thus, the improper use of antimicrobial drugs in marine (di Cesare et al. 2013; Tajbakhsh et al. 2015) and livestock (Yang et al. 2017a) animals ultimately leads to the production of resistant bacterial species. This rise in turn results in increased levels of contamination in foods obtained from animal sources. An alternative tactic to control the rise of antibiotic-resistant bacteria has been suggested. This proposal emphasizes the research on antimicrobial compounds present in plants (Atanasov et al. 2015).

After the identification and reporting of Staphylococcus aureus with Methicillin-Resistant also known as (MRSA) in the 1960s physicians started using vancomycin (VA) as the drug of choice against it. However, soon reports started coming out about the occurrence of Staphylococcus aureus with Vancomycin-Resistant also known as (VRSA) (Alakeel et al. 2022; Saber et al. 2022; Pinheiro et al. 2023).

The isolates of S. aureus from Saudi Arabia were also tested positive for resistance against several drugs of antibiotic nature including vancomycin. This discovery forced the scientists to begin their search for new alternatives against the Vancomycin-resistant Staphylococcus aureus (VRSA). One such alternative option was the use of medicinal plants. Since ancient times, plant extracts have been used for the treatment of diseases caused by bacteria. Now issues like the increase in bacterial resistance against conventional antimicrobial drugs, necessitate the shift of attention to medicinal herbs. A commonly known plant Ziziphus nummularia, also known as sidr in Arabic is potential is a potential candidate for battling antibiotic resistance. It is a branched thorny bush. This plant is native to Saudi Arabian land and it grows in arid, dry regions (Mesmar et al. 2022). Several parts of Ziziphus nummularia can be used for the treatment of a broad range of diseases. Recently it has gained scientific approval for possessing beneficial bioactive substances that act as antimicrobial, antioxidant, antitumor, anti-hypotensive, anti-inflammatory, anti-hypoglycaemic, liver protective and immune system stimulants (Mustafa et al. 2019; Khurshid et al. 2022).

2. Healthcare Challenges against the Issues of *Staphylococcus aureus*

One of the more severe problems concerning infection of *S. aureus* is the outbreak of infections of drug-resistant pathogens and their impact on global human health. The emergence of MRSA had a major effect on the settlements of hospitals that later turned into community-based infections in a particular trend. It mostly happens in people who lack proper medical attention (See et al. 2017; Rowe et al. 2021). In a cascade-effect manner, MRSA has started showing resistance to an extended range of antibiotics belonging to the beta-lactam group (ESBL). This group includes the most commonly used antibiotics like penicillins, carbapenems and cephalosporins (Rasheed and Hussein 2021). 80% of the mortality rate recorded in hospitalized people with MRSA infection is due to the formation of biofilm by invading bacteria (Alonso et al. 2022). The recurrence of MRSA infections can be of any type ranging from cystic fibrosis disorders, soft tissue and skin infections, endocarditis, bacteremia, UTIs, colonization of nares and osteomyelitis (Rowe et al. 2021). MRSA is also a major pathogen of the infection associated with implants (Khatoon et al. 2018). The typical infections caused by *S. aureus* include issues of soft tissues and skin infections. These infections may also include impetigo and purulent cellulitis (Cruz et al. 2021). Occurrence of impetigo is common in

infections caused by *Staphylococcus*. It can be specifically seen at the extremities in the crusty lesions (Alegre et al. 2016). The toxic shock syndrome toxin produced by *Staphylococcus* can lead to the occurrence of toxic shock syndrome (Wang et al. 2007). These infections mainly happen due to the use of absorbable tampons. It involves severe clinical symptoms such as multi-organ coupled septic shock. With the consideration in mind that the important part of *S. aureus* is the resident of the nasal region in humans, Nurjadi et al. (Nurjadi et al. 2015) its connection between pvl (Panton-Valentine leukocidin) can underline, which is mostly discovered in intercontinental travellers, and the genes linked with the harshness of soft tissue and skin infections are lukL/lukS genes. MRSA has been identified as the main cause of health-care setting-associated pneumonia in the statistical analysis performed by Walter et al. (Walter et al. 2018) in the countries of Europe. After *Pseudomonas aeruginosa*, the second major colonizing bacteria of the lungs is *S. aureus,* especially in people suffering from cystic fibrosis. *S. aureus* affects the regulator protein for transmembrane conductance found in the epithelium layer of cystic fibrosis. This effect then causes the mucus to accumulate in the respiratory tract. The mucus engorgement leads to difficulty in breathing and ultimately to the disease and fatality of patients with cystic fibrosis (Stauffer 2017). The main sources for getting infected with nosocomial pneumonia are the endotracheal tubes and overuse of intensive care unit ventilators for the patients which may also lead to infection with biofilm-forming bacteria (Bauer et al. 2002). Medical equipment like pacemakers, defibrillators, and heart valve implants are the sources of cardiovascular infections of *S. aureus* that can usually lead to early-onset endocarditis due to prosthetic valves (Viola and Darouiche 2011).

The most significant cases of increased mortality by endocarditis and sepsis were observed in several types of vascular catheters (Alonso et al. 2022). The clumping factors (Clfs) are fibrinogen-binding proteins such as ClfA and ClfB, along with SdrE, which induce the aggregation of platelets. This aggregation then leads to endocarditis (O´Brien et al. 2002). Past studies have discovered *S. aureus* as the second major etiologic agent of shunt infection (Bhatia et al. 2017; Yakut et al. 2018). Intracranial pressure and meningeal irritation were the notable clinical signs seen during cerebrospinal fluid shunt infections (Kulkarni et al. 2001). An elevated risk of shunt infection was seen in people facing spinal fluid leakage post-surgery of shunt reimplantation. Additionally, *S. aureus* tries to produce a viscous infection of joints and bones called osteomyelitis (Chang et al. 2013). Another recent issue concerning *S. aureus* is the prevalence of its infections during orthopaedic procedures, especially hip or knee arthroplasty (Beam and Osmon 2018).

UTIs caused by *S. aureus* are rarely observed. However other issues like older age, hospital exposure, urologic surgical procedures, long-term urinary tract catheterization, urinary tract obstruction, and malignancy favour induction of hematuria caused by *S. aureus*. Similarly, it may also cause dysuria, bacteriuria, or bacteremia (Gad et al. 2009). The research by Gjodsbol et al. (Gjødsbøl et al. 2006) denoted that *S. aureus* can be identified in more than 80% of chronic wound infections typified by diabetic foot ulcers, venous ulcers, and pressure sores.

3. Herbal Mitigation of VRSA

In the context of herbal medication, the plants belonging to the genus *Plectranthus* have 3000 well-identified species found in all countries of Africa, Australia, Asia and South America. The plants are well known by local folks as a popular medicine (Figure 1). They are often used by locals for treatment of digestive, respiratory problems, infectious and inflammatory (Waldia et al. 2011; Daglia 2012). There are several species of *Plectranthus* (Kiraithe et al. 2016; Crevelin et al. 2015) including *P. amboinicus* (Swamy et al. 2017), have been under consideration by researchers due to their unique pharmacological characteristics. These studies will help validate the proper use of these medicinal herbs. *P. amboinicus* has its bioactivity by 76 volatiles and 30 non-volatile compounds present in it. These compounds belong to

various types of phytochemicals (Arumugam et al. 2016). Research regarding the pharmacological activities of *P. amboinicus* was conducted from its extracts which are sophisticated volatile compounds. These compounds are naturally synthesized in several portions of the plant by secondary metabolism. These substances have great potential in the biomedicine sector (Swamy et al. 2016). An increased sensitivity of methicillin-resistant *S. aureus* (MRSA) has been observed against the *P. amboinicus* extracts (de Oliveira et al. 2013; Santos et al. 2015).

These researchers studied the crude methanolic extracts of *Ziziphus nummularia* and used them against the VRSA (Vancomycin-Resistant Staphylococcus aureus). The phytochemicals present in these extracts included tannin, phenols, saponin and flavonoids. No steroids and alkaloids were found in the plant extracts although it was separated using TLC. The GC-MS analysis was applied to discover that it contains 2-Octene, (E)- and Eugenol. These substances are the major antimicrobial factor found in the extracts of this plant. A study with similar objectives was presented by Odongo et al. (Odongo et al. 2023). In this study, they checked the antimicrobial potential of extracts from various plants like *Toddalia asiatica, Aloe secundiflora*, *Camellia sinensis* and *Senna didymobotrya* against several types of clinically important pathogens including *S. aureus*. The study made several revelations about the potential antibacterial effect that was achieved through the combination of the extracts from *Aloe secundiflora* and *Clonorchis sinensis* used against *S. aureus*.

Akinduti et al. (Akinduti et al. 2022) studied the antimicrobial activity of the plant extracts of several plants including *Vernonia amygdalina, Azadirachta indica, Acalypha wilkesiana* and *Moringa oleifera*. These extractions were tested against the isolates of multi-drug-resistant *S. aureus*. The results of these tests revealed the potential impact of these plant extracts as antibacterial agents due to the presence of compounds like saponin, alkaloids and terpenoids in the plant extracts. These discoveries suggested that the extracts of plants might be an alternative to the herbal formation with biologically active substances that can target *S. aureus* even if it is vancomycin-resistant. The antimicrobial activity of plant extracts from *Calpurnia aurea* and their portions were tested against several important pathogenic bacteria including *S. aureus*. The antimicrobial activity was evident in targeting *S. aureus*. It was attributed to all of the fractions found in the plant extract. The other studies regarding the effectiveness of extracts from plants like *Calpurnia aurea* also supported its usability for the treatment of skin infection. These extracts include compounds like saponins and alkaloids that improve their effectiveness (Wasihun et al. 2023). In another research, the leaves of *Artemisia afra* were used to obtain crude extracts. These extracts were then tested for effectiveness against several clinically important pathogens including *S. aureus.* These extracts have shown promising activity with bactericidal potential. Thus suggesting that the plant extract under consideration is a less toxic and economical antibiotic from *Artemisia afra* against *S. aureus* (Haile and Jiru 2022). Edet et al. filtered the raw extracts from *Annona muricata* to verify its effectiveness against multi-drug resistant (MDR) *S. aureus* and the result determined the composition of extracts to be several phytochemicals including glycosides, saponin, flavonoids, alkaloids, hydroxyanthraquinones, polyphenols, anthraquinone, phlobatannins and tannin. The GC-MS study showed the presence of carbonic acid 2-dimethylaminoethylpropyl ester, 1-methyl-4-phenyl-5-thioxo-1,2,4-triazolidin-3-one and trichloromethane, bicyclo[4.1.0]heptan-2-one 6-methyl. Conclusively, all the studies proved that the phytocompounds present in the raw methanolic extracts obtained from *Ziziphus nummularia* had active compounds for anti-VRSA action against pathogenic bacteria (Edet et al. 2022).

4. Use of PAMAN Nanoparticles

Infections of bacterial origin are a serious threat to the security of global human health (Outterson et al. 2016). Another severe case of infectious disease is its occurrence by antibiotic-resistant bacteria that is

estimated to cause 10 million fatalities by the year 2050 (Humphreys and Fleck 2016). Even with an ongoing series of publications regarding innovative and "improved" antibiotics, the clinical presentation of their effects is still not very promising (Burrowes et al. 2011). Strict policies, high costs of development and shorter lifetime effectiveness life-times of new antibiotics, before the first strains of bacteria emerge to develop new antibiotics at a commercial level, have little attraction (Spellberg 2014). Hence formulating new strategies is a matter of urgency to discover options for improved usage of presently available antibiotics. Simultaneously, a change of paradigm is necessary for the aversion of research and development to focus away from the production of antibiotics towards introducing new antibiotics that do not lead to the emergence of antibiotic resistance in pathogenic bacteria. Such a shift in the paradigm of research might be more attractive for companies if it becomes commercially feasible for marketing and later on for clinical uses. A large number of innovative nanotechnology-based antibiotics are already known to mankind (Liu et al. 2019a) Some examples of nanoparticles include metal nanoparticles (Yang et al. 2017b; Zheng et al. 2017; Wang et al. 2019), particularly Ag nanoparticles (AgNPs) (Xiu et al. 2012) that have the capability for disrupting membranes of the cell by releasing Ag ions (Rizzello and Pompa 2014; Wang et al. 2016). The main challenge hindering the practical use of AgNPs is the formation of aggregates in suspension. The aggregation leads to a reduction in the antimicrobial action of AgNPs (Martínez-Castañon et al. 2008). AgNP aggregation can be prevented by using block copolymers (Ji et al. 2020), micelles (Huang et al. 2017) and vesicles (Lu et al. 2013) as a template. The disadvantage of template synthesis is that they are complex and expensive. Both of these issues hinder clinical application. Dendrimers are also a good, alternative template to be used with AgNPs. Such an example is Poly-(amido-amine) (PAMAM) dendrimers which are dendritic molecules with extensive branching and large molecular mass. Its size distribution is narrow with a distinct globular structure (Avila-Salas et al. 2020; Song et al. 2020). It has been used in conjunction with gold nanoparticles as contrast agents in computerized tomography (Liu et al. 2019b) or immune-sensor coatings (Razzino et al. 2020). Hence PAMAM dendrimers are also useful as a suitable template for AgNPs. The relatively larger molecular size of PAMAM dendrimers facilitates the integration of AgNPs and also assists in the conjugation of an additional antibiotic. Conjugation of two antibiotics can result in the reduction of the chances of the pathogenic bacteria developing resistance (Ejim et al. 2011).

A frequently used antibiotic for controlling clinical infections is Vancomycin (Ozcan et al. 2006; Dalton et al. 2020). Vancomycin has been used in the past as a part of single-conjugated systems for antibiotic applications (Choi et al. 2013). Conjugation of vancomycin with PAMAM dendrimers has shown five orders of better-targeting magnitudes against cell surfaces of bacteria. These are much better than the simple vancomycin solution (Choi et al. 2013). However, Choi et al. (Choi et al. 2013) only discussed the targeted attacking and killing efficacy of vancomycin in PAMAM dendrimers with single-conjugation without conjugating a second antibiotic or evaluating the possibility of antimicrobial resistance being developed in the bacteria under attack. Recently, a new type of PAMAM-based dendrimer for antibiotics, with dual-conjugation has been developed. It is hetero-functionalized as it kills the vancomycin-resistant *S. aureus* strain in vivo and in vitro while preventing tissue damage that may occur upon usage of AgNPs (Gu et al. 2019) or vancomycin (Abdullah et al. 2016) in high concentrations. In vivo, the killing of bacteria was confirmed by an experiment on a murine-infected wound model through the application of a single low dose of topical Van-PAMAMAgNP dendrimers (2 mg/kg vancomycin, which is a lower dose as compared with other animals under consideration as the literature suggests that topical application necessitates up to 40 mg/day/kg dose for seven days continuously) (Ozcan et al. 2006). The most significant role of these innovative hetero-functionalized Van-PAMAM-AgNP dendrimers having dual-conjugated is their ability to prevent the development of antibiotic resistance in vancomycin-sensitive strains of *S. aureus*. These two notable characteristics of Van-PAMAM-AgNP dendrimers were developed using unmodified AgNP. On the

other hand, Van-PAMAM-AgNP dendrimers were discovered to be blood and tissue-compatible up to an Ag equivalent concentration of 8 µg/mL in vivo and in vitro. In contrast to unmodified ones the modified AgNPs often display a reduction in biocompatibility (Li et al. 2021). The combination of vancomycin with AgNPs seems crucial for low-dose bactericidal action against vancomycin-resistant staphylococci. Both single-conjugated PAMAM-AgNP dendrimers and Van-PAMAM cannot efficiently kill strains of *S. aureus* at low doses, irrespective of their vancomycin resistance. AgNP can induce damage to the cell walls of bacteria by releasing Ag ions (Kaur et al. 2019), thereby allowing entry of vancomycin into the intracellular spaces (Kaur et al. 2019).

Fig. 1: Herbal Treatment options for Vancomycin-resistant *Staphylococcus aureus*.

5. Conclusion

Staphylococcus aureus is a globally prevalent bacteria found in nearly all kinds of media making it a prevalent organism suitable for study models. Sometimes it is also found in isolates from infection sites like in case of ectopic skin infections in Saudi Arabia. The infections caused by *S. aureus* were easily treated in the past using regular antibiotics like the methicillin group. Soon, people started misusing this antibiotic leading to the development of resistance in bacteria. These became the methicillin-resistant *S. aureus*

(MRSA). After the discovery of methicillin resistance scientists started using Vancomycin as the drug of choice against MRSA. Soon, people also started vancomycin haphazardly that soon led to antibiotic resistance in bacteria against it. The discovery of vancomycin-resistant *Staphylococcus aureus* (VRSA) was the last nail in the coffin of humanity battling against pathogenic *S. aureus*. This discovery was an alarming situation for mankind as this meant there were no more antibiotic options for humans to use against pathogenic *S. aureus*. Soon, researchers started shifting their attention towards the alternative remedies of generally all infectious diseases particularly *S. aureus* infection, besides the use of traditional; antibiotics. This shift in the research and development paradigm soon resulted in the emergence of new herbal and nanoparticle options for battling infectious diseases in the modern era. The herbal approach implements the use of plant extracts as antibiotics or antibiotic carriers. Similarly, the nanoparticles may be used as lone antibiotics or antibiotic carriers.

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