Chapter

Antimicrobial Resistance in *Staphylococcus aureus*

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Abstract

Staphylococcus aureus is a Gram-Positive bacteria that are responsible to cause skin infections and also shows toxic shock syndrome. Several antibiotics were given against the *S. aureus* infections but eventually, the prevalence of multidrug resistance of *Staphylococcus aureus* started emerging. Since then Methicillin-resistant *Staphylococcus aureus* strains (MRSA)were very common which causes nosocomial infections. Microorganisms for the need of the survival undergoes mutational changes either in their chromosomal DNA/RNA which confers the resistance. One of the famous examples is the resistance against methicillin in *Staphylococcus aureus*. The evolution of *S. aureus* is successful in developing multiple resistant strains. Plasmids are capable of carrying the resistant genes and also several toxic genes. In a recent study, it has been observed that drug resistance genes are located in the R plasmids and they are also responsible in conferring multi drug resistance and induce less utilization of multiple antimicrobial therapy. MRSA was not only resistant to methicillin, studies proved MRSA strains were resistant to macrolides, tetracyclines, chloramphenicol. Resistance to vancomycin was very evidently observed, and its transfer among the population and rising of resistant strains was becoming a major threat globally. The resistance of all these antimicrobial agents against the pathogenic microorganisms are taking a rise in some patients due to prolong use of the antimicrobial agents by these patients. The multi drug resistance has enhanced the mortality and morbidity rate which referred to the infecting agents as the "Super Bugs". Survival of the microorganisms has increased due to the gradual development of extensive resistance against varied antimicrobial drugs. Possible treatments with combinations are found to be the only hope for infections against *S. aureus*. Few drugs are in development such as Dalbavancin, Oritavancin, Tigecycline. These are the possible treatments upon which the work is going on to reduce the resistance against the invasive MRSA. This chapter highlights the profiles of *Staphylococcus aureus* and the resistance patterns along with transmission and the role of the plasmid in transmitting the resistance.

Keywords: multi-drug resistance, SaPIs, *mec A* gene, clinical MDR, daptomycin, dalbavancin

1. Introduction

Multi-Drug Resistance *of S. aureus* is a massive concern in the clinical world. Immunocompromised, diabetic, and weak immune systems are general medical

problems but patients already suffering from these are more susceptible to the Staphylococcal infections and mainly by *S. aureus* which causes skin infections and soft tissue infections. The severity of the infections caused by *S. aureus* increases when there is overgrowth of the *S. aureus* on the infected part of the body which results in the secretion of toxins and causes a fatal condition known as toxic shock syndrome. Penicillin was used predominantly against infections caused by *S. aureus* but the organism started having resistant strains developed for fighting against Penicillin. Methicillin was the next approach that came up for *S. aureus* but the major failure of methicillin by forming MRSA strains made vancomycin the last hope for *S. aureus* infections. Methicillin is the synthetic antibacterial drug given to *S. aureus* widely. *S. aureus* is resistant to almost all antibiotic drugs that are so far used and among them Methicillin and Vancomycin are the two drugs that have shown resistance in *S. aureus*. In this, we will emphasize the genetic aspects of the resistance that is observed in *S. aureus*. The antibiotic resistance genes are generally present on plasmids, and nonessential for the survival of the organism but it provides the bacterial population with a means to reduce the genetic and physiological load on the majority of cells. Plasmid-borne genes can undergo more radical evolutionary changes without affecting the viability of the cell, as would changes to indispensable chromosomal genes, and established plasmid transfer mechanisms can provide recipient cells with new genetic material which has already been refined by selective pressures elsewhere. Besides plasmids, bacteriophages too have contributed towards development of resistance by transduction. Thus the continuous evolution of *S. aureus* strains was successful to bring forth the vancomycin-resistant strains as well (VRSA). New drug development and treatments are applied to the *S. aureus* mediated infections which have proved to be the immediate possible treatment for this. This chapter will help the readers to acquire a comprehensive knowledge regarding the Multi-Drug Resistance of *S. aureus* along with the resistance mechanism and possible treatments of *Staphylococcal* infections.

2. Multi-drug resistance

2.1 Overview of multi-drug resistance

Multi-Drug Resistance (MDR) is a global concern that is having a very bad impact on health care. Microbes are getting resistant to antibiotic therapies due to the constant exposure of antimicrobial drugs. In the past decade, microbial infections have raised enormously and this has led to an increased amount of resistance [1]. Multi drug resistance is the phenomenon in which pathogenic organisms are resistant to multiple chemotherapeutic agents [2]. The emergence of MDR rises the mortality and morbidity rates for which they are known as 'Superbugs'. It is said that MDR is a very natural process among microorganisms but the increasing amount of this process is due to several reasons like the use of undefined antimicrobial agents, unhygienic sanitary conditions, poor health care facilities. The omnipresent threat of antibiotic-resistant pathogens entails having very few antimicrobial agents for other infections [2, 3].

2.2 Classification of MDR

Many different definitions for multidrug-resistant (MDR), extensively drug-resistant (XDR) and pandrug-resistant (PDR) bacteria are being used to characterize the different patterns of resistance. Was defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories, XDR was defined

as non-susceptibility to at least one agent in all but two or fewer antimicrobial categories (i.e. bacterial isolates remain susceptible to only one or two categories) and PDR was defined as non-susceptibility to all agents in all antimicrobial categories. MDR is a frequently encountered phenomenon in *S. aureus* which can be broadly classified as primary MDR, secondary MDR and clinical MDR (**Figure 1**) [1, 4–6]. Survival of the microorganisms has gradually developed extensive resistance against varied antimicrobial drugs. Also, there is a failure of many clinical trials which are not always due to the occurrence of resistance but all due to poor bioavailability of drugs, very poor immune system, excessive-high metabolism of drugs.

2.3 Mechanism of multi-drug resistance (MDR)

Before studying the resistance of *S. aureus*, it is very important to take a look upon all the possible biochemical mechanisms of resistance that the microbes show. Microorganisms have the ability to employ several ways to develop multi drug resistance [2]. The resistance of all these antimicrobial agents against the pathogenic microorganisms are taking a rise in some patients due to prolong use of the antimicrobial agents by these patients. Below, is the schematic diagram of all methods of resistance mechanism (**Figure 2**). Microorganisms for the need of the survival undergoes mutational changes either in their chromosomal DNA/RNA which confers the resistance. One of the famous examples is the resistance against methicillin in *Staphylococcus aureus*. The cell wall of the microbes plays a vital role as a barrier and helps in their survival but due to alteration in the chromosomal DNA or genetic mutations the compositions of the cell wall or the plasma membrane changes and this in turn encourage the resistance phenomenon.

Drug Efflux Pumps are one of the major ways for the MDR mechanism. ABC transporters (ATP Binding Cassette) are membrane proteins which are commonly defined as drug efflux pumps that specifically helps in the transport of the drugs in the cell. The P-glycoprotein or multi-resistant protein (MRP) damages

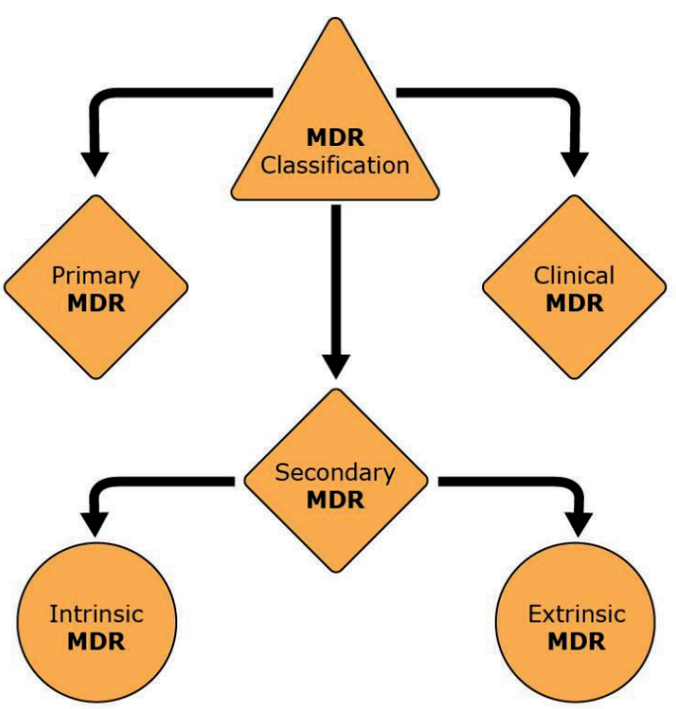

Figure 1.
Classification of MDR.

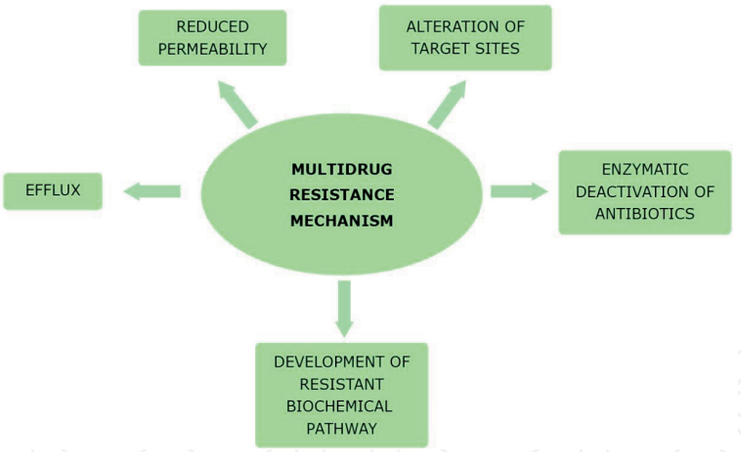

Figure 2.
Multi drug resistance mechanism.

the permeability and influences the ATP-dependent efflux of the drugs which is responsible for decreasing the intracellular concentrations [7–9].

3. Genetic aspect of resistance in *S. aureus*

The genetic determinants of resistance to many antimicrobial agents are believed to have evolved prior to the era of antibiotic chemotherapy. Processes such as phosphorylation, glycosylation, acetylation whose inactivation or chemical transformation is the major cause of the MDR. The schematic diagram shows the possible ways of causing antimicrobial resistance (**Figure 3**) [1, 4, 10–12].

Methicillin-Resistant *Staphylococcus aureus* (MRSA) came into the focus of attention when the Methicillin-Susceptible *Staphylococcus aureus* (MSSA) started adopting a specific gene (methicillin-resistant gene) named as *mecA* which is intervened by a genetic element called Staphylococcus cassette chromosome (SCC) and is transferred into the MSSA via either conjugation or transformation (Horizontal gene transfer). As SCC elements are carrying the gene *mecA* so, the complex is named *SCCmec*. The complex consists of the mecA and several other regulatory genes such as *mecR1*, *mecI*. (**Figure 4**), Demonstrate a schematic diagram of the SSC*mec* element. There is also the presence of a specific complex named Cassette Chromosome Recombinase (CCR) that helps in the integration and excision of the element from the chromosome of *Staphylococcal* species [13–16]. The region, origin of replication (oriC) in the *S. aureus* chromosomal element is accompanied by a special gene named as orfX towards the downstream of the oriC. The gene orfX is popular for encoding a specific enzyme called ribosomal RNA methyltransferase and this gene also has direct repeat sequences that help to protect the Staphylococcus cassette chromosome (SCC). In this way, multiple SCC elements are placed one after another in tandem which results in the formation of the cluster of foreign genes and forms a chromosomal region whose name is oriC environ [13, 17, 18]. Now, there are mainly two types of MRSA. One, the Community-Associated MRSA (CA-MRSA), and the other one is Hospital-Acquired MRSA (HA-MRSA). CA-MRSA has been found to get transmitted among the population from crowded places and the CA-MRSA isolates are highly resistant against methicillin and penicillin as well. Minor skin problems, redness, itchiness, and pain are the symptoms of the body affected by CA-MRSA. HA-MRSA is acquired from the hospital or any health care center. oriC environ has many transposons and insertion sequences (IS) which are capable to induce deletion, recombination, chromosomal

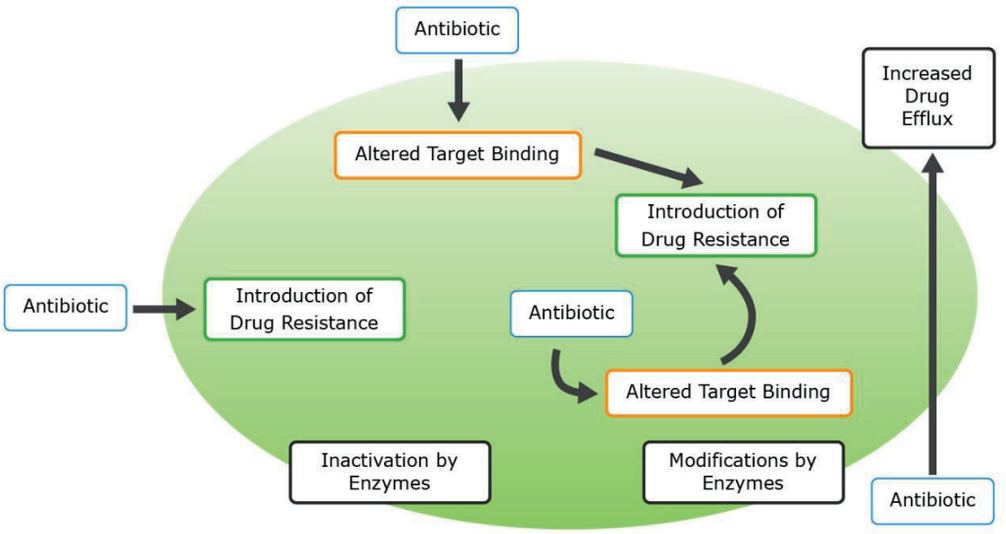

Figure 3.
Schematic diagram of antimicrobial resistance.

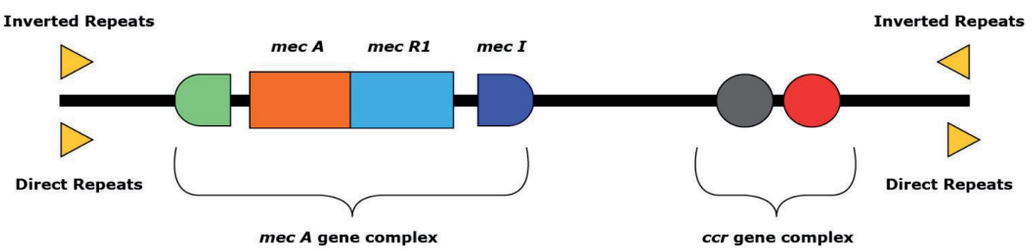

Figure 4.
*A schematic diagram of SCCmec element. The SCC*mec *consists of two components mec. A gene complex and* ccr *gene complex.* mec *gene complex helps to encode the methicillin resistance gene (*mec *A) and other two regulatory genes (*mecR1, mec1*).* ccr *gene complex takes care of the movement of the whole SCC element.*

inversion across oriC and this helps the *S. aureus* to maintain their survival strategy according to the environmental condition [18]. Horizontal gene transfer mediated by phage is one of the prime reason for the evolution of the *S. aureus*. It has been observed in the past studies that the Bacteriophages such as Staphylococcus Phage 80α is a specific helper bacteriophage that is required for the mobilization of SaPIs. This helps to carry the *Staphylococcus aureus* pathogenicity islands (SaPIs). SaPIs are known as mobile genetic elements which are the common residents in the genome of *S. aureus* and are transferred to other cells. These SaPIs are responsible for carrying several toxin genes and also superantigens [19, 20].

Plasmids are capable of carrying the resistant genes and also several toxic genes. In a recent study, it has been observed that when an *S. aureus* plasmid was sequenced which originated from a different bacterial environment, few trailblazing resistance genes named ampA and vgaC were discovered. The amp resistance gene is resistant to the antimicrobial drug named apramycin and the vgaC resistance gene is resistant against Streptogramin A, respectively. Along with these, many toxin genes are being carried on *S. aureus* plasmids such as exotoxin B (ETB) and enterotoxins (entA, entP, entG, entJ). R plasmids play a major role in mediating resistance among bacteria. Drug resistance genes are located in the R plasmids and they are also responsible in conferring multi drug resistance and induce less utilization of multiple antimicrobial therapy [21–23].

There is also support for the notion that some resistance determinants in staphylococci are derived from genes present in antibiotic-producing organisms. The *S. aureus* ermC methylase encoded on pE194 shares amino acid sequence

homology with the analogous methylase encoded by erythromycin-producing organisms such as *Streptomyces erythraeus* (ermE) [24].

4. Resistance against antibiotics

Due to the high resistance against methicillin and after the failure of the drug, Vancomycin was playing a major role in treating most MRSA infections. Isolates of *S. aureus* were taken from a surgical wound of a Japanese baby and it was observed that the infection was not responding to the drug called Vancomycin. Vancomycin, is an antibiotic made up of glycopeptide and was initially used for the treatment of the MRSA strains as the efficacy of this drug was quite prominent but eventually because of prolong usage of the drug, it was resistant to MRSA infections. The resistance was not via the acquisition of vanA by MRSA infection-causing strain but this was because of unusual thickening of the cell wall which is rich in dipeptides and this results in the decreasing of the drug availability in the body. Despite the issues, in the year 2000, Vancomycin was considered to be one of the prominent drugs against the MRSA strains. The mechanism of the resistance is predicted to be a plasmid-mediated transfer among the species. The genes vraS, msrR, rpoB and graR were found to be mutated which was responsible for the resistance against the Vancomycin [13, 25–27]. Other than Methicillin and Vancomycin, Penicillin and Quinolones were also given to *S. aureus*.

In case of Penicillin, R plasmids encode the enzyme called as *penicillinase,* the plasmid gene that carries the enzyme is *blaz*, and the organisms that were resistant to penicillin were having this gene which inactivated the antibiotic by splitting the β-lactam ring. Slowly, this became a threat and major resistance towards penicillin antibiotic emerged world wide [28–30]. Use of Methicillin started when Penicillin failed to cure the Staphylococcal infections. After major failure of both these antibiotics, Quinolones were used. Quinolones destroy the bacteria by attacking and inhibiting their bacterial topoisomerases which generally ease the super coiling of DNA and also separates DNA strands. Moxifloxacin and Gemifloxacins are useful against the Gram-Positive bacteria but unfortunately *S. aureus* again developed resistance against quinolones [31, 32]. *S. aureus* developed resistance against fluoroquinolones by overexpression of the NorA efflux pumps. Similarly, point mutation is another way by which this organism becomes resistant to quinolones. Point mutation in the subunits of topoisomerase takes place. Such as, point mutation at Gr1A in topoisomerase IV subunit and in GyrA, subunit of Gyrase [28].

4.1 Transmission pattern of resistance

Transmission of MRSA infections can take place from person to person who is contaminated with such infections. Proper hygienic condition is required to maintain infection from getting spread. Although the mode of transmission of infections mainly relies upon direct contact but contact with contaminated fomites can also transmit the infection. Several other factors of the host such as immunocompromised patients, defects in neutrophils, or destruction of the skin barriers can also give rise to the infections. *Staphylococcus aureus* has shown evolutionary changes in it and this phenomenon completely relies on the plasmid gene transfer mechanisms. The conventional mechanisms such as horizontal gene transfer popularly conjugation and transformation are followed by the strains to spread the resistance among the population or community but there is a very unique mechanism of *Staphylococcus* named SaPI-helper phage [33, 34]. Through all the studies it is quite evident that plasmids are the fundamental element that is helping in mediating the virulence and the resistance genes among the population of the *S. aureus* [35–37].

5. Treatment and future aspects

Drugs that are discussed to be used for MRSA infections are Daptomycin and Linezolid. Daptomycin is a synthetic drug that is the class of antibiotics that destroy the cell membrane ability by a calcium-dependent binding phenomenon which leads to bactericidal activity in a concentration-dependent way. So, one of the widely used antibiotics and which shows good efficacy even more than methicillin and vancomycin. Therefore, for any MRSA bacteremia, Daptomycin is considered to be very effective [38–40]. There were many topical drugs used against the MRSA strains. These anti MRSA drugs were quite effective. Mupirocin, is one of the anti MRSA topical drug which is applied on the skin for curing skin infections caused by *S. aureus* [41]. The mechanism of Mupirocin is, it binds to the isoleucyl t-RNA synthetase which inhibits the protein synthesis of the organisms resulting in the destruction of the organism [42]. Fusidic acid is another topical drug used against staphylococcal infections and it was reported effective as well. Fusidic acid binds to the elongation factor G of bacteria and interferes with the translocation process resulting in the inhibition of the protein synthesis [28].

Similarly, Linezolid which belongs to the oxazolidinones class predominantly inhibits the protein synthesis in the 50S ribosome of the cell. Linezolid shows a good amount of efficacy against several toxin-producing strains such as toxic shock syndrome toxin, Panton-Valentine leukocidin, α-hemolysin [38]. But the resistance against Linezolid was also observed. So, the combinatorial theory was taken into account. Combinatorial theory helps to mix multiple compounds to balance the inadequate conditions of other compounds and increase efficacy of drugs. The combinatorial theory started with Vancomycin and it shows synergistic interaction with β-lactams widely. Studies cleared that the capacity of clearing the MRSA infection-causing strains was not high in amount when the patients were only subjected to Vancomycin but in combination with β-lactams the clearance efficiency was much higher in amount. Combination with Vancomycin shows a specific effect named as Sea-Saw Effect where if the susceptibility of the vancomycin is decreased which results in decrease of transcription of the *mec A* gene and this increases the susceptibility of the β- lactams [43–46]. Combination with the Daptomycin has also been applied to check the outcome. This combination was to some extent very much successful as it enhanced the destruction of both Daptomycin-susceptible as well as Daptomycin-non-susceptible strains of MRSA. This combination showed high efficacy against the clearance of the bacteremia from the patient's body [47, 48].

Few drugs are in development such as Dalbavancin, Oritavancin, Tigecycline. Tigecycline inhibits protein synthesis and it shows broad-spectrum antibiotic activity. These are the possible treatments upon which the work is going on to reduce the resistance against the invasive MRSA. The prospect of the medication for *S. aureus* infections also lies in traditional medicines. The traditional herbal medicines are believed to have anti-MRSA activity. The bioactive phytoconstituents present in the plants such as Mansonone *F* from *Ulmus davidiana*, β-asarone from *Acorus calamus* rhizome, Prenylated flavonoids from *Desmodium caudatum* root, galloylated flavonol rhamnosides from *Calliandra tergemina* leaves, eupomatenoid-5 from *Piper regnellii* leaves are important for the MRSA treatment as they constitute anti-MRSA activity [49]. Some of the new relevant information regarding the treatment of AMR in *S. aureus* has come forward. Quinopristin-dalfopristin and Linezolid are another set of antimicrobial agents which have come up with activity against the resistance in *S. aureus*. Both these agents are protein inhibiting agents and Quinopristin-dalfopristin mainly exhibits bactericidal effects and Linezolid is bacteriostatic. Current studies against the treatment of this disease deal with the

various combinations of antimicrobial agents [50]. Several compounds are known to inhibit the synthesis of fatty acid in bacteria and with this, two antibacterial agents have shown greater efficiency against *S. aureus*. Triclosan and Isoniazid are the two antimicrobial agents which target the FabI in the *S. aureus.* Fab I is one of the essential enzymes utilized in fatty acid elongation and it plays a major role in *S. aureus.* High throughput screening of the FabI inhibitors has led to come up with a new molecule AFN-1252 also called Affinium Pharmaceuticals was identified and proved to be efficient against the MRSA strains [51]. Multiple combinations were analyzed and several limitations emerged from those. Vancomycin and Rifampicin were in great demand for diagnosing MRSA infections but later on, Rifampicin proved not to be a better option for treating the disease as this drug is the primary drug given against one of the concerned diseases named Tuberculosis. This combination has exhibited higher possibilities of rising the resistance against Rifampicin and this was the major reason for the failure of the Vancomycin-Rifampicin combination against *S. aureus*. Similarly, Trimethoprim/Sulfamethoxazole and Rifampicin was a major failure because of the poor efficacy along with multiple side effects. Also, it was found to be resistant to infections. Among all this, Vancomycin is the only drug that is still either used in combinations or as monotherapy for treating MRSA infections but some of the new antibiotics such as Ceftaroline, Tedizolid, Plazomicin are proved to be successful among other antibiotics and are under research and development for further studies of treating the MRSA infections [52]. Other explored combinations with vancomycin have shown adverse nephrotoxicity. So, it is said that intensive research is required for novel approaches against the treatment of resistance to *S. aureus*. Above all the discussed conventional therapies for the treatment, either majority of them were proved to be ineffective or have shown severe side effects in the patients.

According to the future perspective, there is an immense need for an alternative strategy for treating the resistance against *S. aureus*. Treatment methods such as using nanoparticles are one of the efficient ways of delivering the drug directly to the patients. Under the nanoparticle treatment strategy, there is a unique feature of using ligands that are target specific for certain receptors in bacteria. AuNPs were surface modified by Vancomycin helps in reducing the bacterial growth and also the iron oxide nanoparticles are modified with the porphyrin platinum and Vancomycin which results in thermal degradation of the resistance strain of *S. aureus*. Another very interesting aspect is the usage of SiRNA therapy which enhances the MRSA inhibition. Vancomycin nanocomplexes are proved to have effective anti-MRSA effects which are very new to the study of alternative strategies [53].

The major limitation or failure that rises is intrinsic mechanisms of bacterial resistance and the target-specific antibiotics or drugs have disappointed to come up with any useful product. Another unique novel approach has come forth which combines the genomic information on the drug target and undergo chemical modifications along with efficacy testing [50].

6. Conclusion

Staphylococcus aureus is a major cause of bacterial infection in humans, which has been able to acquire resistance to a variety of antibiotics. MSRA is an emerging issue globally because apart from causing nosocomial infection also emerged as one of the key causative agents of community-acquired infections. Antibiotic resistance in *S. aureus* involves various mechanisms which are drug efflux, expression, or mutation of target proteins, leading to its rapid evolution which requires innovative approaches to develop novel treatment methodologies. A very limited amount of

treatments are available for MRSA and this has become the reason for increasing the mortality rates. Appropriate use of the antimicrobial agents as the MDR is a very natural phenomenon and handling this type of phenomenon needs extra care to minimize the growth rate of resistant MRSA isolates further in the future. The development of new drugs is also in progress so that the resistance can be reduced. Anti MRSA topical drugs are extensively in use for treating skin infections. The new approaches have been initiated by the use of Fusidic acid, Linozolid against *Staphylococcal* infections.

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Conflict of interests

There is no Conflict of Interest in working with this chapter.

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