Chapter

# Antibiotic Resistant *Staphylococcus aureus*

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## Abstract

Staphylococcus is an adaptable pathogen and leads to rapid development of antibiotic resistance. The major targets for antibiotics are (i) the cell wall, (ii) the ribosome and (iii) nucleic acids. Resistance can either develop intrinsically or extrinsically via horizontal gene transfer, drug site modification, and efflux pumps etc. This review focuses on development of resistance to currently used antibiotics in Staphylococcal infection, novel therapeutic approaches resistance pattern of antibiotics and also the future prospectus for new antibiotics usage.

**Keywords:** Staphylococcus aureus, MRSA, antibiotic resistant

## 1. Introduction

Staphylococcus is normal resident bacterium that lives in nasal cavity, throat, skin and mucous membrane of humans as well as a variety of animals and birds [1]. Approximately 20% of healthy populations are persistent nasal carriers and 30% are intermittent carriers of *S. aureus*. Individuals who are colonized with *S. aureus* are at a great risk of infection and also serve as an important source of transferring *S. aureus* in the community and hospital settings [2].

Based on the Coagulase production, Staphylococci are classified into Coagulase negative staphylococci (CONS) and Coagulase positive staphylococci (COPS). Of these, CONS causing infections are mostly seen in immune-compromised patients [3]. COPS (eg. *S. aureus*) is a pathogen of great concern, because of its intrinsic virulence property, its ability to cause a variety of life-threatening infections (superficial skin infections to deep seated infections), and its capacity to adapt to different environmental conditions [4].

*S. aureus* is a major problem in animals. It causes mastitis or intramammary infections and is a cause of major financial losses in the dairy industry. In Poultry industry, *S. aureus* causes a variety of disease manifestations such as comb necrosis, bacterial chondronecrosis and also leads to leg weakness, lameness and septicemia [5].

In the modern world, antibiotics are used in treatment and prophylaxis of human and animal infection. They are also used in poultry industry to prevent bacterial infection and reduce the financial loss [6]. In some developing and underdeveloped countries, antibiotics are used as growth promoters in animal feed, especially in poultry industry to increase the yield of meat production. Due to irrational use of antibiotics, *S. aureus* has emerged to become increasingly antibiotic resistant. This leads to treatment failure and leaves us with limited choice of antibiotics to be used in future [7]. Resistant bacteria can be transmitted from animals to humans among poultry workers and other agricultural workers, who are in close contact

with these animals. It is documented that, after using the antibiotic, 'Avoparcin' as growth promoter in animal feed, there is emergence of glycopeptide- resistant Enterococcus. These resistant determinants are transferred to other gram positive bacteria such as MRSA via horizontal gene transfer method. These leads to development of resistance to Vancomycin, a drug of choice for the treatment of MRSA. Simarily, 'Tylosin' or Enrofloxacin (a derivative of fluoroquinolones) is used as a supplement in animal feeds. This has resulted in the development of Erythromycin and Ciprofloxacin- resistant Staphylococci [8].

## 2. Mechanisms of antibiotic resistant

### 2.1 Methicillin resistant *Staphylococus aureus* (MRSA)

Alexander Flemming introduced the antibiotic, Penicillin in 1940s for the treatment of bacterial infection. At that time, *S. aureus* infections were well controlled. However, with the widespread use of this antibiotic in the 1950s, Penicillin-resistant *S. aureus* appeared. It produces pencillinase enzyme, which can hydrolyze the beta-lactum ring of Penicillin. In 1959, substitution of the natural aminoadipoyl chain from Penicillin with bulkier moieties, developed a semi-synthetic Penicillin, named Methicillin. However, it was not widely used because of its toxicity. It was replaced by similar, more stable Penicillins like Oxacillin, Flucloxacillin and Dicloxacilllin. These antibiotics show good antibacterial activity and are resistant to beta-lactamase substrate. In 1961 the British scientist, Jevons isolated the penicillin stable resistant *S. aureus*. However, the name Methicillin resistant *S. aureus* (MRSA) continues to be used [9].

Bifunctional Transglycolylase-transpeptidase (Penicillin binding protein 'a' or PBPa) is the inhibitory target of beta-lactam antibiotics in *S. aureus*. The transglycolylase domain is responsible for transferring the disaccharide pentapeptide (L-alanine, D-glutamine, Lysine and 2 D-alanines) from membrane bound lipid to growing chains of polysaccharide. Domain of Transpeptidase (TP) cross-links the glycine bridge and links the D-alanine of 4th position to adjacent chain of peptidoglycan layer to make the cell-wall strong. The active site of Transpeptidase (TP) serine is blocked (i.e., PBP2a) by causing structural analogous changes of D-Ala4 to D-Ala5. This leads to breakdown of beta lactam ring and a penicilloyl-O-serine intermediate is formed [10].

PBP2a is encoded by mec A gene. This mecA gene is a mobile genetic element integrated into the chromosomal element (SCCmec) of Methicillin sensitive *S. aureus*. The mecA gene is transfered to other *S. aureus* via horizontal gene transfer mechanisms. Resistance confered by mec A gene is broad spectrum and shows resistance to all beta-lactum antibiotics except Ceftaroline and Ceftobiprole [11].

SCCmec contains two essential components such as mec gene complex and ccr gene complex. The mec gene complex contains mecA and is associated with regulatory and insertion sequences. It has been classified into 6 different classes (A, B, C1, C2, D and E) along with ccr complex (Cassette chromosome recombinase) genes. It encodes for the enzyme, 'recombinase' that helps in integration and excision of SCCmec into the chromosome. There are 3 different types of recombinase enzymes, namely ccrA, ccrB, and ccrC. Recombinase enzymes are further classified into eight different types based on the existing recombinase and allotypes in the different characteristics.

SCCmecs are classified into 8 types and subtypes according to 'International Working Group on the Staphylococcal Cassette Chromosome elements' [12].

### 2.2 Vancomycin resistant *S. aureus*

Vancomycin is a glycopeptide antibiotic which has been used as the first line drug in the treatment of MRSA infections. It was introduced for human use in late 1958 [13] and resistance to Vancomycin was reported in *Enterococci* by 1980s. Thereafter slowly *S. aureus* showed reduced susceptibility to Teicoplanin (structurally similar to Vancomycin) in European countries [14]. The first VRSA (Vancomycin Resistant *S. aureus*) was identified in 2002, in Michigan, USA. In the same year, total 52 isolates carrying Van gene were identified in USA, India, Iran, Pakistan, Brazil and Portugal [15]. *S. aureus* having reduced susceptibility to Vancomycin is classified into 3 groups based on the MIC value by CLSI as follows [16]:

1. Vancomycin Susceptible *S. aureus* (VSSA) with MIC ≤2 μg/ml

2. Vancomycin Resistant *S. aureus* (VRSA) with MIC ≥16 μg/ml

3. Vancomycin Intermediate *S. aureus* (VISA) with MIC 4-8 μg/ml

#### *2.2.1 VISA*

The first vancomycin intermediate *S. aureus* was reported in 1997 from Japan with MIC value of 8 μg/ml [17]. VISA strains are generally preceded from heterogeneous Vancomycin resistant *S. aureus* (hVISA). hVISA is the precursor of VISA and is composed of cell subpopulations with various degrees of Vancomycin resistance. Vancomycin-intermediate *S. aureus* (VISA) are those isolates with a MIC between 4 and 8 mg/l, whereas heterogeneous VISA (hVISA) strains appear to be sensitive to Vancomycin with susceptible range of 1–2 mg/l, but containing subpopulation of Vancomycin-intermediate daughter cells (MIC ≥4 μg/ml). Vancomycin-resistant *S. aureus* (VRSA) are defined as those having MICs of at least 16 mg/l [16]. This means that, in the same culture plate, some strains are sensitive and some strains show Intermediate resistance to Vancomycin which may lead to treatment failure [18]. The underlying mechanism is still not completely known. However, scientists have put some efforts to identify the genetic determininants of VISA via different molecular identification methods such as comparative genomics, proteomics, transcriptomics etc. This lead to identification of genes responsible for VISA such as WalKR, GraSR, and VraSR [19]. The following are the fundamental characteristics of VISA phenotypes [14]:

1. Increased cell wall thickness

2. Reduced cross- linking of peptidoglycan

3. Decreased autolytic activity of bacteria

4. Changes in surface protein profile

5. Dysfunction of *agr* system (The accessory gene regulator *(agr)* of *S. aureus* is a global regulator which secretes virulence factors and surface proteins) and changes the growth profile of bacteria.

GraRs gene regulates the transcription of cell wall biosynthesis and specifically up-regulates the genes responsible for capsule biosynthesis operon. It also

up-regulates the *dlt* operon and the *mprF/fmtC* genes, which are linked to teichoic acid alanylation and alters the cell wall charge. Moreover, the GraRS mutation can modify the expression of *rot* (repressor of toxins) and *agr* (accessory gene regulator). This leads to downstream effect of global regulators [20].

*2.2.2 VRSA*

Vancomycin resistance is mediated by Van cluster which are found in bacteria such as S*. aureus, E.fecalis, E.faceium, Clostridium difficile*, Acintomycetes (Amycolotopsis orientalis*, Actinoplanes teichomyceticus, and* Streptomyces toyocaensis) as well as anaerobic bacteria from the human bowel flora such as *Ruminococcus* species and *Paenibacillus popilliae* [21].

Based on the Van gene, homologues Vancomycin resistance is classified into several gene (Van) clusters which encode for the enzymes which synthesize D-Alanyl-D-lactate and D-alanyl-D-serine. Eleven van gene clusters have been discovered till now, namely, *VanA, VanB, VanD, Van F, VanI, VanM, VanC, VanE, VanG, VanL, and VanN* [22].

1. *vanA*, *vanB*, *vanD*, *van F*, *vanI*, and *vanM* encode for synthesis of d-Alanyl-Lac ligase and are responsible for high-level Vancomycin resistance with MIC range > *256 mg/ml*

2. *vanC, vane, vanG, vanL, and vanN* clusters encode for synthesis of D-ala-ser-ligases and are responsible for low level Vancomycin resistance with MIC range 8-6 mg/ml [23].

VRSA resistance mechanism is mediated by van A operon, which is carried on the mobile genetic element (Transposon) Tn1546. VanA cluster is encoded by 5 proteins such as VanS, VanR, VanH, VanA and VanX, having the following functions;

1. *vanS and vanR* together form two-component system and upregulate the vanA gene clusters in the presence of Vancomycin

2. *VanH, VanA, and VanX* are responsible in modifying D-ala-ala precursors of cell wall to D-ala-D-lac, which confer resistance to Vancomycin

3. *vanH* produces dehydrogenase enzyme which reduces pyruvate to D-lac.

4. *vanX* produces D,D dipeptidase that hydrolyses the native precursors and prevents the synthesis and cros- linking of cell wall peptidoglycan [24].

Enterococcus spp. is the major reservoir of Vancomycin resistance and it is transferred to other bacterial species by the horizontal gene transfer method of bacterial conjugation. The Inc18 incompatibility conjugative plasmid naturally occurs in Enterococcus but not in Staphylococci spp. The Inc18 contains pSK41-like multi-resistant conjugative plasmids. These plasmids are transferred from *E. faecalis* to *S. aureus* [25].

*2.2.3 Treatment challenges*

Deletion of Van cluster components has lead to recovery of Vancomycin sensitivity. This is a promising target for new drug development [26]. For example, hydroxyethylamines, posphinate and phosphonate transition-state analogues have

been used for the inhibiton of VanA [27, 28]. Phosphinate based covalent inhibitors, and sulfur-containing compounds have been demonstrated in VanX inhibitors [29]. These inhibitors can be used in combination with Vancomycin to increase uptake of the antibiotic inside the bacterial cell [21].

### 2.3 Mechanisms of tetracycline resistance

Three different tetracycline resistance mechanisms have been described:

1. Ribosomal protection, which is the most common resistance mechanism,
2. Active efflux of the antibiotic and
3. Enzymatic inactivation of the drug.

All these mechanisms are based on the acquisition of one or several tetracycline resistant determinants, which are widely distributed among bacterial genera [30]. Additionally, mutations in the rRNA, multidrug transporter systems or permeability barriers may be involved in developing resistance to several antibiotics including Tetracyclines [31].

Efflux of the drug occurs through some export proteins from the major facilitator super family (MFS). These export proteins are membrane-associated proteins which are coded for by *tet* efflux genes and export Tetracycline from the cell. Export of Tetracycline reduces the intracellular drug concentration and thus protects the ribosomes within the cell.

Ribosome protection proteins that protect the ribosomes from the action of Tetracyclines [32] are cytoplasmic proteins. They are similar to elongation factors EF-Tu and EF-G that bind to the ribosome and cause changes in ribosomal conformation. This prevents Tetracycline from binding to the ribosome, without altering or stopping protein synthesis. This occurs by a ribosome-dependent GTPase activity, which confers resistance mainly to Doxycycline, Minocycline and a wider spectrum of resistance to tetracyclines than is seen with bacteria that carry tetracycline efflux proteins.

#### *2.3.1 Tetracycline resistance genes*

There are at least 38 different characterized tetracycline resistance (*tet*) genes and three Oxytetracycline resistance genes (*otr*) to date [33]. These genes include 23 genes which code for efflux proteins, 11 genes for ribosomal protection proteins, three genes for an inactivating enzyme and one gene with unknown resistance mechanism. Most environmental *tet* genes encode for transport proteins, which pump the antibiotic out of the bacterial cell and keep the intracellular concentrations low to make the ribosomes function normally [34]. The most common genes found in *S. aureus* are *tet(K), tet(L), tet(M), tet(O).*

***tet (K) gene*** is a mobile genetic element originally detected in *S. aureus* plasmids of pT181 family [35]. It is a 4.45-kb plasmid protein consisting of 459 amino acids and belongs to the incompatibility group inc3 [36]. PT181-like plasmids have also been detected either integrated in the large plasmids or in the bacterial chromosome. They are always flanked by directly repeated insertion sequences of the type IS257 [37].

***tet (L) gene*** carrying plasmid pSTE1 was identified in *Staphylococcus hyicus* in 1992. In 1996, tet(L) was also found to be carried on the naturally occurring plasmid pSTS7 of *Staphylococcus epidermidis* [38]. It is the second most prevalent tetracycline resistant gene in Streptococci and Enterococci [39]. It consists of 458 amino acids.

***tet (M) gene*** is the most widely distributed tetracycline resistant gene in gram-positive bacteria [40]. It was first identified in *Streptococcus spp*. Subsequently, it has been isolated in a large number of gram-positive and gram-negative bacteria, including Mycoplasmas and Ureoplasmas [40]. The *tet(M)* gene is frequently associated with conjugative transposons of the Tn916-Tn1545 family [41, 42]. which also carry additional antibiotic resistance genes. According to the study of Schmitz et al. [34], *tet(M)* is the most prevalent single tetracycline resistance determinant in MRSA (Methicillin Resistant *Staphylococcus aureus*). The majority of *tet(M)*-positive *S. aureus* isolates also carry *tet(K).* Hence, MRSA isolates are typically of *tet(M) or tet(K,M)* genotype [43].

***tet (O) genes*** also have been detected very rarely in Staphylococci.

## 2.4 Mechanisms of macrolide resistance

Macrolides inhibit protein synthesis by stimulating dissociation of the peptidyl-tRNA molecule from the ribosomes during elongation. This results in polypeptide chain termination and a reversible stoppage of protein synthesis. The first described mechanism of Macrolide resistance was due to post-transcriptional modification of the 23S rRNA by the adenine-N6 methyltransferase. These enzymes add one or two methyl groups to a single adenine (A2058 in *Escherichia coli*) in the 23S rRNA moiety. Over the last 30 years, a number of adenineN6-methyltransferases from different species, genera, and isolates have been described. In general, genes encoding these methylases have been designated *erm* (erythromycin ribosome methylation), although there are exceptions, especially in the antibiotic-producing organisms. As the number of *erm* genes described has increased, the nomenclature for these genes has varied and has been inconsistent. In some cases, unrelated genes have been given the same letter designation, while in other cases, highly related genes (90% identity) have been given different names [33].

### *2.4.1 Macrolide resistance genes*

Although structurally unrelated to each other, Macrolides, Lincosamide, and Streptogramin, are often investigated simultaneously for microbial resistance, as some Macrolide resistance genes (*erm*) encode for resistance to two or all three of these compounds. In total, more than 60 different genes conferring resistance to one or more of the MLS antibiotics have been identified, including genes associated with rRNA methylation, efflux and inactivation.

The ***erm (A) gene*** is associated with the transposon, Tn554. It is integrated into SCCmec II elements, and is a non-conjugative or conjugative transposon. It is mostly seen in Methicillin resistant staphylococci [43].

The ***erm (B) gene*** is seen in transposons Tn917/Tn551. It is 2.3 and 4.4 kb in size and does not carry additional resistant genes [44].

The ***erm (C) gene*** is commonly located on small plasmids. It is widely spread in Methicillin susceptible strains [45].

The **msr (A)** gene is efflux- pump mediated, codes for 488 amino acids, ABC transporters system and is encoded by plasmid borne *msr (A)* genes [46]. It is an ATP-binding transport protein which mediates the active efflux of 14-membered ABC transporters system and confers resistance to Macrolides and B-compounds of the Streptogramins.

## 2.5 Aminoglycosides resistant *S. aureus*

Aminoglycosides are broad spectrum antibiotics that inhibit protein synthesis of the bacteria. They were first isolated from the Actinomycetes spp. namely

*Streptomyces griseus* and introduced for clinical use in 1944. They were used as the first-line drugs worldwide but were replaced by Cephlaosporins, Carbapenems and Flouoroquinolones due to lesser toxicity and broader coverage than Aminoglycosides [47]. Members of these groups include Neomycin, Amikacin, Gentamicin, Netilmicin, Tobramycin, Kanamycin etc. The novel Aminoglycosides recently developed, namely Arbekacin and Plazomicin were meant to overcome the Aminoglycoside resistance mechanisms [48]. Clinical studies reported a higher incidence of nephrotoxicity in patients on Aminoglycosides. Hence, screening the patients for serum urea and creatinine after injection of Aminoglycosides is important to monitor the severity of the toxic effects. Aminoglycosides have got a substantial activity against *S. aureus* infections including MRSA, VISA, and VRSA [47].

Entry of Aminoglycosides inside the bacteria mostly comprises of three distinct stages [49]:

1. **Increase in permeability of bacterial cell membrane:** Binding of polycationic Aminoglycoside antibiotics to the bacterial membrane which has negative charged components such as phospholoipids and teichoic acids occurs by electrostatic attraction. This leads to disruption of the outer membrane of the bacterial cells.

2. **Energy dependent:** Entry of Aminoglycoside antibiotics into cytoplasm is mediated by slow, energy dependent and electron transport mechanisms.

3. **Mistranslation of protein synthesis and inhibition of protein synthesis**: This occurs once the Aminoglycoside molecules enter into the cytoplasm. Mistranslation leads to cytoplasmic damage and facilitates rapid uptake of more Aminoglycosides inside the bacterial cell.

Aminoglycoside resistance mostly occurs by

1. Enzymatic modification

2. Target site modification

3. Efflux pump proteins on bacterial cell.

1. **Enzymatic methylation of the rRNA**: Methylation at N7 of guanine residues of the 16 s rRNA produces high level resistance, but this has not been reported among clinically important bacteria.

The major mechanism of aminoglycoside resistance among both gram negative and gram positive clinical isolates is the enzymatic modification of amino or hydroxyl group of these antibiotics. Three families of enzymes are responsible in performing co-factor dependent drug modification:

i. Aminoglycoside phosphotransferases (APHs)

ii. Aminoglycoside acetyltransferases (AACs)

iii. Aminoglycoside nucleotidyltranferases (ANTs)

These are further subdivided into many types (designated by Roman numerals). AAC (6′)-I enzymes are aminoglycoside acetyltransferases, modifying the antibiotic at position 6′ [50, 51].

Aminoglycoside resistance in clinical strains of *S. aureus* is due to the acquisition of cytoplasmic Aminoglycoside Modifying Enzyme (AME) by plasmids. For example, Gentamicin and Neomycin resistance is confered by bifunctional Acetyl Transferase –Phosphotransferase (aac-aphD) encoded by Tn4001.

Neomycin resistance occurs by aphA encoded adenyl transferase which is encoded by PUB 110 or Tn 5405. It is seen in SSC II mec [52].

2. **Modifications of the target** include mutational changes in the ribosomal proteins or 16S rRNA. The mutational changes are mostly seen in Streptomycin

3. **Efflux pump proteins on bacterial cell** is an intrinsic aminoglycoside resistance mechanism in various pathogens. In the opportunistic pathogen, *P. aeruginosa*, intrinsic low-level resistance to Aminoglycosides, Tetracycline and Erythromycin is mediated by the expression of the multiple efflux (Mex) XY-OprM system. In *S. aureus*, efflux pump proteins causing resistance to aminoglycosides have not been identified [46].

## 2.6 Linezolid

### *2.6.1 Mechanism of action*

It is an Oxazolidinone, useful in treatment of resistant gram positive coccal and bacillary infection. It is primarily bacteriostatic but can exert bactericidal action against some *Streptococci, Pneumococci and B. fragilis* [53, 54].

It acts mainly by inhibiting bacterial protein synthesis, acting at an early step. It binds to the central loop of domain V in the 23S fraction (P site) of the 50S ribosome and interferes with the formation of tertiary N-formylmethionine- tRNA- 70S initiation complex. Hence it stops protein synthesis before it starts.

### *2.6.2 Mechanism of resistance*

Since Linezolid is a synthetic drug, natural resistance to this drug does not occur; hence mutations are mostly acquired.

1. Mutations in the 23srRNA subunit domain V region of ribosomes lead to alteration of peptidyltransferase center (PTC), where conserved regions of ribosome interact directly with Linezolid. Gram positive bacteria passes 4 to 6 allelic copies of 23S rRNA; hence, development of Linezolid resistance requires more than one allele to be mutated.

2. Mutations in the genes of ribosomal proteins L3 (rplC gene), L4 (rplD gene), and L22 (rplV) gene arefound in some gram positive bacteria.

3. Acquired resistance by Natural cfr (Chloramphenicol –Florfenicol Resistance) gene from Chloramphenicol resistant bacteria, which is a plasmid mediated gene, encodes a protein to catalyze the post transcriptional methylation of the C-8 atom (A2503) in the 23S rRNA. Methylation by the cfr leads to development of multidrug resistance to Linezolid, Lincosamide and Streptomycin [52].

Genes encoding for Ribosomal proteins have been analyzed by PCR and Amplicon sequencing.

Whole molecular background is elucidated by PCR- Amplicon sequencing and whole genome sequencing [56].

## 2.7 Mupirocin (MUP)

### *2.7.1 Mechanism of action*

Mupirocin is a mixture of several pseudomonic acids. It binds to its target site of the enzyme isoleucyl-tRNA synthetase and inhibits protein synthesis. However it does not bind to the mammalian enzyme counterparts, making it non-toxic for human beings. The synthesis of bacterial isoleucine tRNA gets depleted which leads to cessation of protein and RNA synthesis in the bacteria. At the concentrations near Minimum Inhibitory Concentration (MIC) Mupirocin is bacteriostatic and at higher concentrations it becomes bactericidal. It is mainly used against the gram positive bacteria [57].

### *2.7.2 Mechanism of resistance*

Mupirocin-resistant (mupR) *S. aureus* was first reported in the United Kingdom in 1987.

Mupirocin resistance is classified into two types.

1. **Low Level MUP resistance**- MIC value of 8-64 mcg/ml is mainly due to chromosomal point mutations in the native ileS1 gene leading to a Val-to-Phe change in the MUP- binding site.

2. **High Level MUP resistance**- At a MIC of 128- 256 μg/ml. there is plasmid mediated resistance, which occurs by two mechanisms:

    1. Acquiring an alternate isoleucine - tRNA synthetase i.e. by acquisition of a plasmid mediated mupA or isleS2 gene.

    2. Acquisition of mupB gene [58, 59].

## 2.8 Fusidic acid

### *2.8.1 Mechanism of action*

It was isolated from a strain of *Fusidium coccineum*, which is a steroid like antibiotic. It is mainly bacteriostatic in nature but may become bactericidal at higher concentrations. It acts by binding with Elongation factor G i.e. Translocase which is necessary for translocation on the bacterial ribosome after peptide bond formation during protein synthesis. However eukaryotes have another enzyme which is not affected by the drug. This specific mode of action explains the absence of intrinsic cross- resistance between Fusidic acid and other antibiotics. It has a limited spectrum of activity, mainly against Gram positive bacteria i.e. *Staphylococcus aureus, S.epidermidis, Clostridium spp. and Corynebacterium*. However, *Streptococci* are moderately susceptible. But most Gram Negative Bacteria are resistant to it [60].

### *2.8.2 Mechanism of resistance*

Two major Fusidic acid resistance mechanisms are discovered in *S. aureus*:

1. Alteration of the drug target site which is due to the mutations in fusA gene (encoding elongation factor G, EF-G), rplF or fusE (encoding ribosome protein L6)

2. Point mutation in fusA gene occurs in domain III of EF-G.

Other resistant mechanisms include:

i. Fusidic acid resistant small colony variant (SCV) isolates, referred to as fusA-SCV class mostly occur due to mutations in domain V of EF-G

ii. Acquired Fusidic acid resistance of *Staphylococcus* spp. includes *fusB*, *fusC*, and *fusD.* The genes *fusB (found in plasmid pUB 101 in S. aureus)* and *fusC* were found in *S. aureus* and coagulase-negative Staphylococci

iii. *fusD* is an intrinsic factor causing Fusidic acid resistance in *Staphylococcus saprophyticus* [61].

## 3. Alternative to antibiotic therapy

### 3.1 Spread of antibiotic resistant

*3.1.1 Antimicrobial peptides*

Antimicrobial peptides or host defense peptides are biologically active molecules produced by variety of organisms [62]. AMPs have board spectrum of antimicrobial activity against pathogenic microorganisms and are the first line defense against the foreign attacks [63]. AMPs also serve as immune-modulators in higher animals [64]. AMP'S are expressed by specific genes and their expression is by either constitutive or specific external factors [64]. AMPS are classified into several types based on the source, activity Amino acid sequences and structural characteristics. AMPS are usually 1. Cationic and Hydrophophic in nature with helical polypeptides of short amino acid sequences mostly lysine and arginine amino acids. 2. Some are Cationic and Amphiphilic (Both hydrophobic and Hydrophilic).

*3.1.2 Membrane target mechanism*

Amphiphilic peptides are alpha helix and their amphiphilicity interacts with bacterial cell membrane. These alpha helices peptides are folded and adsorbed with both hydrophilic and hydrophobic sides of lipid bilayer membranes. Positive charged AMPS interact with negative charged cell membranes by electrostatic interactions and undergo conformational changes of the cell membrane.

*3.1.3 Non membrane target mechanism*

AMPS bind to hydrophobic and negative charged cell membrane of lipid bilayer at their N-terminal ends containing basic amino acids and their C-terminal ends are amidated with neutral hydrophobicity. The number of positive net charge are related to the antibacterial activity and their hemolytic activity is related to the hydrophobicity of the peptides. Multiple models to explain the action of these peptides, include the toroidal pore model, the barrel-stave model, and the carpet model etc. [65].

*3.1.4 Advantages of AMPs*

1. AMPs have rapid germ killing abilities with low bactericidal concentration

2. No toxic effects

3. Hard to induce bacterial resistance

4. AMPs have broad spectrum antimicrobial activity

5. AMPs have good thermal stability and good water stability

6. AMPs are small molecules with low synthetic cost

7. AMPs show inhibitory ability to cancer cells [66].

*3.1.5 Disadvantage of AMPs*

AMPs have mostly L-amino acids; are sensitive to protease degradation and rapid renal clearance.

AMPs are not specific to microorganisms and display systemic toxicity

Oral administration of AMPS can lead to proteoloytic degradation by gastric enzymes such as trypsin and pepsin.

Systemic administration results in short half life time in vivo and cytotoxicity in blood

Chemical modification of AMPS and the use of drug delivery vehicles such as Nanoparticles, lipid system can improve the properties of AMPS for their clinical use [26].

**3.2 Nanoparticles**

Nanoparticles are smaller in size (less than 10 nm in diameter) that exhibit high surface area to volume ratio [27]. Nano particles have significant application in the medical fields. Nano-drugs or Nanoparticles can act individually or synergistically with antibiotic components against the multi-drug resistant pathogens. Nanoparticles are used as drug delivery vehicle that improve the therapeutic efficacy and enhance their physicochemical characteristics [28]. Metal and metal oxide Nanoparticles such as gold, silver, titanium, copper, zinc etc. are the most studied Nanoparticles against the multi-drug resistant pathogens [28].

*3.2.1 Interaction and penetration of nanoparticle to bacteria*

Electric charges present on the nanoparticles are the most important property in terms of antimicrobial effect. Interactions of nano-particles with bacteria membrane depend on the different factors such as electrostatic interactions, hydrophobic interactions, receptor ligand interaction and Van der Val forces [29].

The phosphates present in the teichoic acids of gram positive bacterial cell wall are responsible for bacterial negative charge and acts as binding site of divalent cation ions. Gram Negative bacteria consists of plasma or cytoplasmic membrane followed by peptidoglycan layer and hydrophobic lipid bilayer consisting of lipopolysaccharides (Phosphates and Carboxylates) which are responsible for negative charge of gram negative bacterial cell wall. The interaction of NPs with membrane structure leads to blebbing, tubule formation and other membrane defects [67].

Nanoparticles can bind to cell wall by electrostatic interactions and disrupt cytoplasmic membrane leading to leakage of cytoplasmic content of the bacterial

cell. Nano particles also bind to intracellular components such as DNA and other enzymes responsible for normal cellular machinery causing disruption in cellular machinery by creating oxidizing stress, electrolytic imbalance and enzyme inhibition followed by cell death. For example, free copper ions (CU2+) from CU Nanoparticles generates reactive oxygen species that disrupts the amino acid synthesis and DNA [67].

*3.2.2 Nanoparticles as a drug delivery vehicle*

Nano-particles based drug delivery system provides increased drug retention time in blood. Reduced non-specific distribution at targeted site of infections, Opsonin proteins in blood rapidly attach to Nanoparticles, promoting macrophages to bind and remove NPs from blood circulation [68].

*3.2.3 Bacterial resistance to NPS*

Bacterial cells acquire resistant towards NPs by multiple mutations. NPs resistance to bacteria is a clinical concern but it is rare. Some studies suggest that bacteria develop resistance to Ag, Au, and Cu NPs after continuous exposure. For example: CU++ NPs sowed reduced susceptibility to TiO2 NPS after continuous exposure to *Schewanella oneidensis* [69].

Increased use of Ag NPS in clinical application raises the NP bacterial drug resistance to *K.pneumoniae and Enterobacter cloacae.* Hemeg et al. showed, $Al_2O_3$ NPs increased the expression of conjugation-promoting genes and are responsible for horizontal gene transfer of resistant genes [70].

**3.3 Probiotics**

Probiotics are living Microorganisms that confers a health benefit to the host when administered in adequate amount. For example, *Lactobacilli and bifidobacteria*. Probiotics bacteria have many beneficial properties:

1. Controlling the activity of pathogenic bacteria
2. Improving intestinal barrier function
3. Reducing adherence to pathogenic bacteria cells,
4. Co-aggregation
5. Production of organic acids which antagonize the pathogenic bacteria.
6. Many Probiotics produce antimicrobial compounds such as short chain fatty acids, Nitric oxide, bacteriocins [71].

*3.3.1 Spread of antibiotic resistant*

Gastrointestinal bacteria act as a major reservoir for resistance genes that can be acquired from ingested bacteria and it is responsible for transfer of resistant gene from one bacteria cell to another by plasmid mediated conjugation. Intrinsic resistance of probiotic bacteria is a major concern. Vancomycin, Tetracycline and Chloramphenicol antibiotic resistance have been reported in lactobacillus spp. intrinsically [71].

### 3.4 Vaccines

See **Table 1** [72].

| Target antigen | Clinical trails | Out come |
|---|---|---|
| CP 5and CP8 | Phase III | Failed |
| CP-5 CRM197, CP8-CRM and CIfA (SA3 ag) | Phase I | Significant antibody response |
| CP5-CRM 197, CP8-CRM197, MntC and CIfA (SA4 ag) | Phase I | robust immune response, safe, and well-tolerated and phase 2b is ongoing |
| Alpha toxin and Panton-Valentine leukocidin | Phase I | good toxin neutralizing sero-positive response |
| EsxA and Esx B | Preclinical | protection with improving survival of murine model |
| Surface Protein A (SpA) | Preclinical | protection in mouse model |
| D-alanine auxotrophic *S. aureus* | Preclinical | protection from the formation of abscesses and improved survival in immunized mice |
| AdsA | Preclinical | protection in the immunized mouse model |
| Coa (Hc-CoaR6) | Preclinical | strong T-cell response and protection in mice against lethal dose of *S. aureus* |
| Staphylococcal enterotoxin B | Preclinical | efficient protection in BALB/c mice |

**Table 1.**
*List of vaccines in clinical trials and outcomes (adapted from Ansari et al., [72]).*

## 4. Conclusion

Staphylococcus is an adaptable pathogen and has ability to develop rapid antibiotic resistance. After 1980s development of newer classes of antibiotics is very limited. Rapid development of resistance will reduce the availability of antibiotic in clinical practice and this will cause serious health problem in future. Development of newer molecules in expensive clinical trials, the huge investment in target based discovery with the structural biology did not yield the hope for newer break throughs. Microorganisms are very crucial in developing resistance to novel therapeutic agent rapidly. This will development of more strategies to combat the antibiotic resistance. Antibiotic stewardship policy is mandatory to control the development and spread of antibiotic resistance in community and hospital settings.

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