

## Antimicrobial resistance mechanisms of *Staphylococcus aureus*

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The miracle of antibiotic discovery has been threatened by the emergence of superbugs. One of those superbugs, methicillin-resistant *Staphylococcus aureus* (MRSA) is now responsible for more deaths per year in the United States than HIV. The added healthcare costs for fighting MRSA infections is in the billions of dollars per year worldwide. Plus, the danger of not having effective antimicrobial therapy available is imminent. This added threat by these organisms includes the emergence of vancomycin-resistant *S. aureus* (VRSA), among other resistant strains (such as vancomycin-intermediate *S. aureus* – VISA, and borderline oxacillin-resistant *S. aureus* – BORSA). The MRSA strains, especially, have a growing diversity in the structure of the genetic element that confers resistance; the *Staphylococcal cassette Chromosome mec* (SCC*mec*), which carries the methicillin-resistance gene, *mecA*. These genetic elements also frequently carry resistance genes for other antimicrobials such as aminoglycosides (gentamicin, tobramycin) erythromycin and tetracycline. Certain strains of MRSA (hospital-acquired) are likely to carry antimicrobial resistance to additional drugs, including the fluoroquinolones.

**Keywords** antimicrobial resistance; *Staphylococcus aureus*

### 1. Introduction to *Staphylococcus aureus*

In the world of the microscopic, *Staphylococcus aureus* is one of the most versatile organisms. It is found world wide and is a leading cause of disease. Even though it is not classified as a true pathogen (an organism that is expected to always cause disease in humans), but an opportunistic pathogen, it has a diverse repertoire of possible infections. Normally, it is a transient colonizer of the skin and body entry portals (ears, eyes, nasal passages, etc.), and an estimated 20% of humans are carriers (asymptomatic permanent colonization). However, any break in the skin, or colonization of individuals with compromised immune systems can provide an opportunity for this bacteria to cause infection. The disease process can be mediated via two possible mechanisms: 1) production of toxins, and 2) colonization that causes tissue invasion and destruction. This bug comes naturally armed with a long list of virulence factors (see Table 1), and becoming resistant to antimicrobial drugs is just an added bonus.

**Table 1** Virulence Factors of *Staphylococcus aureus*

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Surface proteins that promote colonization
clumping factor (bound coagulase) – binds to and directly converts fibrogen to fibrin
collagen binding protein
fibronectin binding protein
capsular polysaccharide adhesin
protein A – bind to antibodies to prevent opsonization
Secreted proteins that allow invasion of and damage to host cells and tissues
α-toxin – membrane pore-forming hemolysin
β-toxin (sphingomyelinase C) – hydrolysis of cell wall lipids
δ-toxin – wide spectrum of cytolytic activity
γ-toxin – wide spectrum of cytolytic activity
Panton-Valentine leukocidin – membrane pore-forming
exfoliate toxins – ETA and ETB – causes sloughing off of epidermis
staphylococcal enterotoxins – SE-A, B, C1-3, D, E, G, H, I – gastrointestinal toxins
toxic shock syndrome toxin – TSST-1 – causes leakage of endothelial cells
coagulase – reacts with thrombin-like molecule to indirectly convert fibrinogen to fibrin
deoxyribonuclease – hydrolyzes DNA
hyaluronidase – hydrolyzes connective tissue
lipases – hydrolyze lipids
staphylokinase – lyses fibrin

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Most infections with *S. aureus* are localized at the area of entry and are self-limiting and not life threatening. However, increasingly, this bacteria is found to invade deeper into the body causing more serious, and even life threatening infections. Because of the many virulence factors it possesses, *S. aureus* is well adapted for causing serious infections, even without the threat of antimicrobial resistance. Now that *S. aureus* has become resistant to so many antimicrobial agents, the infections caused by this bacteria have become even more dangerous.

*S. aureus* is commonly found in most environments and may survive on dry surfaces for long periods. These bacteria are susceptible to high temperatures, and many disinfectants and antiseptics. It would seem that we should be able to control this bacteria fairly easily, but with the ability to become resistant to many antimicrobial agents, this isn't the case. It is now known that *S. aureus* is the most commonly transferred bacteria among health care workers due to poor hand washing techniques. Studies have shown that by simply improving the hand washing compliance in hospitals, not only are *S. aureus* infections decreased, but antimicrobial susceptible may also be improved [1, 2].

## 2. Increased healthcare costs, morbidity and mortality

Because infections with *S. aureus* are so prevalent, the healthcare costs involved (hospitalization and treatment) can be staggering. In New York City, in 2005, there were an estimated 13,550 cases of infection with *S. aureus*, which resulted in an estimated cost of over 435 million dollars [3]. In addition, infections with methicillin-resistant *Staphylococcus aureus* (MRSA) increase these costs even more. In the European Union (EU), the estimated additional costs of MRSA infections are €380 million annually; and with a result of 1 million extra days of hospitalization [4]. Studies in the United States show an increase in costs for treating a patient with a MRSA infection compared to a methicillin-susceptible *Staphylococcus aureus* (MSSA) infection range from \$3836 - \$13,901 per patient, per incident [5, 6]. Mortality rates for MRSA vs. MSSA infections are also increased. Studies compared from several countries showed that the mortality rate for patients with MRSA infections was generally 2 to 3 times higher than that for patients with MSSA infections [7].

## 3. Antimicrobial therapy history for *Staphylococcus aureus*

There is a wide variety of antimicrobial drugs that have been used to treat *S. aureus* infections, and most of these are still available. Some of these (such as the sulfa drugs) were in development at the same time as the early penicillins. Since antimicrobial resistance is often based on the mechanism of activity of the antimicrobial drug, we will briefly mention the ones that have been used, or are most likely to be used for therapy in *S. aureus* infections. The antimicrobial agent groups based on mechanism of action are listed in Table 2.

**Table 2** Antimicrobial Groups Based on Mechanism of Action

Inhibit Cell Wall Synthesis	<ul style="list-style-type: none"> <li>β-Lactams</li> <li>Carbapenems</li> <li>Cephalosporins</li> <li>Monobactams</li> <li>Penicillins</li> <li>Glycopeptides</li> </ul>
Depolarize Cell Membrane	Lipopeptides
Inhibit Protein Synthesis	<ul style="list-style-type: none"> <li>Bind to 30S Ribosomal Subunit</li> <li>Aminoglycosides</li> <li>Tetracyclines</li> <li>Bind to 50S Ribosomal Subunit</li> <li>Chloramphenicol</li> <li>Lincosamides</li> <li>Macrolides</li> <li>Oxazolidinones</li> <li>Streptogramins</li> </ul>
Inhibit Nucleic Acid Synthesis	<ul style="list-style-type: none"> <li>Quinolones</li> <li>Fluoroquinolones</li> </ul>
Inhibit Metabolic Pathways	<ul style="list-style-type: none"> <li>Sulfonamides</li> <li>Trimethoprim</li> </ul>

### 3.1. $\beta$ -lactam drugs

Before antibiotics were discovered, infections with *S. aureus* had a nearly 80% mortality rate. When penicillin became available in the 1940s, the medical community thought that death from *S. aureus* infections was a thing of the past. However, within a few years the *S. aureus* began to show resistance to penicillin. Now, an estimated 80% of all *S. aureus* isolates are penicillin-resistant [8]. Other antimicrobials related to penicillin ( $\beta$ -lactam drugs) were then developed: such as methicillin, oxacillin, and ampicillin. A few years after the development of methicillin, resistant strains were observed (methicillin-resistant *Staphylococcus aureus* – MRSA), and eventually methicillin was removed from the market. MRSA strains are considered to be resistant to all the penicillin (and most  $\beta$ -lactam) drugs, and since methicillin is no longer produced, oxacillin is used for susceptibility testing. Because of this, the name ORSA (oxacillin-resistant *Staphylococcus aureus*) is sometimes used instead of MRSA, but refers to the same strains. Ampicillin, which is a more broad spectrum drug, has not fared well against *S. aureus* isolates. By the 1990s most isolates of *S. aureus* were resistant to ampicillin [9]. Many more penicillin drugs were developed (such as amoxicillin, piperacillin, and ticarcillin); some were directed at *S. aureus*, and some are more broad spectrum and aimed at the gram negative bacteria as well.

In the 1970s, compounds with a  $\beta$ -lactam structure, but weak antimicrobial activity, were discovered. These compounds were not useful as antimicrobials when used alone, but were found to be  $\beta$ -lactamase (an enzyme produced by the microorganisms to fight the  $\beta$ -lactam drugs) inhibitors. Three of these inhibitors have been used in combination with penicillin drugs; amoxicillin/clavulanic acid, ampicillin/sulbactam, and piperacillin/tazobactam. These combination drugs improved the ability of the penicillin drugs to kill the microorganisms, but did not completely alleviate the resistance problem [10, 11].

Over the years, more  $\beta$ -lactam drugs have been discovered or developed synthetically. These include the cephalosporins, carbapenems, and monobactams. The cephalosporin drugs have gone through several generational evolutions over the years. The first drug (in the first generation) marketed successfully was cephalothin. The first generation cephalosporins were mostly effective against aerobic gram positive cocci. The successive generations added more activity against the gram negative enterobacteria and anaerobes. The fourth (cefepime) and fifth (ceftobiprole, ceftaroline) generations brought back more emphasis on effectiveness against gram positive cocci in an attempt to be useful against MRSA strains [12]. The carbapenem drugs, which are structurally related to the  $\beta$ -lactamase inhibitor drugs, are considered to have the best and broadest spectrum of activity against both gram positive and gram negative bacteria. The first carbapenem drug to be marketed was imipenem. Newer carbapenem drugs include: meropenem, ertapenem, and doripenem. As with other  $\beta$ -lactam drugs, the cephalosporins and carbapenems have had antimicrobial-resistance issues. Most of these drugs should not be used in monotherapy against MRSA strains [11, 13]. The monobactams, so named because they have no other ring fused to the  $\beta$ -lactam ring structure, have not had much success against gram positive cocci. The only monobactam currently on the market, aztreonam, is mainly used for *Pseudomonas aeruginosa* infections [11].

### 3.2. Other cell wall drugs

As Table 2 shows, the  $\beta$ -lactam drugs have activity against cell wall synthesis in microorganisms. This involves the peptidoglycan layer of the cell wall. Another group of drugs that have activity against cell wall synthesis are the glycopeptides; vancomycin being the main member of this group that has been used for *S. aureus* infections. With the increase in antimicrobial resistant *S. aureus* strains (such as MRSA), vancomycin has been considered the drug that could be counted on to control these infections. However, in the last 8 years, strains of *S. aureus* that are resistant to vancomycin have emerged [14]. There is also an antimicrobial group, the lipopeptides, that is involved in depolarization of the cell membrane. The only drug currently marketed from this group is daptomycin. Since it has only been on the market since 2003, there has not been a lot of resistance reported as of yet. But some low level resistance instances have been observed. Most disturbing is the ability of certain strains of *S. aureus* to exhibit decreasing susceptibility to daptomycin during treatment [15].

### 3.3. Drugs that inhibit protein synthesis

There are several groups of antimicrobial drugs that inhibit protein synthesis by binding to either the 30S or 50S ribosomal subunits of the microorganisms. These groups are the aminoglycosides (amikacin, gentamicin, tobramycin); the tetracyclines (tetracycline, minocycline, tigecycline); chloramphenicol (sole drug in this group); the lincosamides (clindamycin – used mainly for anaerobes); the macrolides (azithromycin, clarithromycin, erythromycin); the oxazolidinones (linezolid); and the streptogramins (quinupristin/dalfopristin). Instances of antimicrobial resistance have been reported for all of these drug groups: the aminoglycosides [16, 17]; the tetracyclines [18, 19]; chloramphenicol [18, 20]; the lincosamides [4, 19]; the macrolides [21-23]; and the streptogramins [24]. The oxazolidinones are a relatively new group, with Linezolid having been on the market only since 2000. There have been, however, some reports of low to moderate levels of resistance [25, 26].

### 3.4. Drugs that inhibit nucleic acid synthesis

There is one group of antimicrobials, the quinolones, that attack microorganisms by inhibiting nucleic acid synthesis. The group name quinolone refers to the first generation of these drugs. Subsequent generations are the fluoroquinolones. The second generation fluoroquinolones include ciprofloxacin, norfloxacin, and ofloxacin; the third generation includes levofloxacin; and the fourth generation includes gatifloxacin and moxifloxacin. All three generations of the fluoroquinolones have been reported to have resistance developing [22, 27, 28].

### 3.5. Drugs that are metabolic pathway inhibitors

Another antimicrobial mechanism is the use of compounds that inhibit microbial metabolic pathways. The most commonly used drugs focus on the folate biosynthesis pathway in microorganisms. The sulfa drugs (sulfonamides) inhibit part of this pathway. These drugs have a structure which closely resembles para-aminobenzoic acid (pABA) which is a required substrate in one step of this pathway. The sulfa drugs can dock in the active site of the enzyme responsible for catalyzing the reaction that prepares pABA for combination with glutamate. The sulfa drugs then effectively block the ability of pABA to dock, and stop the progression of the pathway at that point. A different point in the pathway is inhibited by the drug trimethoprim. This point is later in the pathway. The trimethoprim binds to another enzyme and inhibits that enzyme from being active. To insure that the pathway is completely blocked, a sulfonamide drug, sulfamethoxazole, and trimethoprim are used as a combination drug. There has been some resistance reported [27, 29].

## 4. Strains of *Staphylococcus aureus*

Most *S. aureus* strains are now penicillin-resistant. In addition, a large majority of strains ( $\geq 60\%$  in many areas) are also methicillin-resistant, so we will mainly address MRSA strains. Various methods have been used over the years (and varying methods in different countries, etc.) to categorize MRSA strains into epidemiologically related or clonal groups. These methods include the antibiogram, bacteriophage typing, pulsed field gel electrophoresis (PFGE), multilocus sequence typing (MLST), and *spa* typing [30]. The MLST typing placed most MRSA strains into five clonal complex (CC) groups; CCR, CC8, CC22, CC30, and CC45 [31]. In 2003 the Centers for Disease Control (CDC) in the U.S. assembled a typing database for MRSA isolates which was developed using a PFGE-based typing system validated with MLST and *spa* typing. This system established eight typing clusters, pulse-field type (PFT) USA100 through USA800 [32]. Outside of the US, designations for MRSA strains use the CC group along with a sequence type (ST) number [33]. The predominant ST clones are: ST80 in Europe, ST59 in Taiwan, and ST30 in Eastern Australia [34].

Another main difference in MRSA strains was observed because originally, MRSA was thought to be primarily a nosocomial infection issue. There seemed to be a clear delineation between MRSA infections acquired in the hospital setting (hospital-acquired or HA-MRSA), and those acquired in the community (community-acquired or CA-MRSA). The HA-MRSA strains were found to belong to the USA200, USA500, USA600, and USA800 groups. Whereas the CA-MRSA strains belonged to the USA 300 and USA400 groups. The USA700 group contained both CA-MRSA and HA-MRSA strains [30, 32]. These designations have been fairly accurate until recently when it was observed that more and more of MRSA infections in the hospital setting were found to belong to CA-MRSA groups [35-38].

The CA-MRSA and HA-MRSA strains also have been observed to differ in respect to the types of infections caused and drug-resistance characteristics. CA-MRSA strains are more likely to cause skin and soft tissue infections. HA-MRSA strains are more likely to cause pneumonia, bacteremia, endocarditis, osteomyelitis, and toxic shock syndrome. CA-MRSA strains are most often not resistant to other drugs, while HA-MRSA strains tend to be multi-drug resistant [30]. Both CA-MRSA and HA-MRSA express the same virulence factors as most *S. aureus* strains (see Table 1). The CA-MRSA strains, however, are most likely to carry an additional virulence factor, the Panton-Valentine leukocidin (PVL). This virulence factor is associated with invasive, destructive infections such as necrotizing pneumonia and necrotizing fasciitis [39].

## 5. Molecular aspects of antimicrobial resistance

There are four general mechanisms that microorganisms use to fend off attack by antimicrobial agents. These are: limiting uptake of the drug, modification of the drug target, inactivation of the drug, and active efflux of the drug. All of the mechanisms that are discussed in this chapter will be variations on one of these mechanism themes. Acquisition of these mechanisms may be intrinsic (the bacteria already has genes on its chromosome which are natural to all members of a species – these genes just need to be activated), or acquired (received from another bacteria via plasmid, bacteriophage, or simple uptake of DNA which carries the necessary genes – present in only certain isolates of a species). Depending on the drug involved, the bacteria may use either or both of these types of gene acquisition. The resistance mechanisms and genes are summarized in Table 3.

**Table 3** Antimicrobial Resistance Genes and Mechanisms in *Staphylococcus aureus*

Antimicrobial Agents	Mechanisms of Resistance	Genetic Basis
$\beta$ -lactams		
Penicillins	$\beta$ -lactamases – inactivate drugs	<i>blaZ</i> – plasmid
Cephalosporins	Altered penicillin-binding protein (PBP2a) targets	<i>mecA</i> – acquired from ?
Monobactams		
Carbapenems		
Glycopeptides	VISA – cell wall thickens	gene unknown
Vancomycin	VRSA – modified target	<i>vanA</i> – from enterococci
Lipopeptides	Change in cell membrane charge – decreased drug binding	<i>mprF</i> gene mutation
Daptomycin		
Aminoglycosides		
Amikacin	Aminoglycoside modifying enzymes – modify target	<i>aac</i> – plasmid
Gentamicin		<i>ant</i> – plasmid
Tobramycin		<i>aph</i> – plasmid
Tetracyclines		
Tetracycline	Active efflux	<i>tetK</i> – plasmid
Minocycline	Ribosomal protection – competitive binding	<i>tetM</i> – plasmid
Tigecycline		
Chloramphenicol	Acetylation of drug – inactivation	<i>cat</i> – plasmid
Macrolides and Lincosamides		
Erythromycin	Methylation of ribosome – decreased binding	<i>ermA</i> , <i>ermB</i> , <i>ermC</i> – plasmid
Clindamycin		
Oxazolidinones	Mutation of ribosome	<i>rrn</i>
Linezolid	Methylation of ribosome	<i>cfr</i> – plasmid
Streptogramins		
Quinupristin/Dalfopristin	Methylation of ribosome	<i>ermA</i> , <i>ermB</i> , <i>ermC</i>
Fluoroquinolones		
Ciprofloxacin	Modified target – gyrase	<i>gyrA</i>
Norfloxacin	Modified target – topoisomerase IV	<i>grrA</i>
Levofloxacin	Active efflux	<i>norA</i>
Gatifloxacin		
Moxifloxacin		
Metabolic Pathway Inhibitors	Target enzyme modification	TMP – <i>dhfr</i>
Trimethoprim/Sulfamethoxazole		SMZ – <i>dhps</i>

SMZ – sulfamethoxazole, TMP – trimethoprim, VISA – vancomycin-intermediate *Staphylococcus aureus*, VRSA – vancomycin-resistant *Staphylococcus aureus*.

### 5.1. $\beta$ -lactam drugs

The issue of antimicrobial resistance to the penicillin drugs has been thoroughly researched over the years. An initial discovery was the existence of penicillin-binding-proteins (PBPs), which were found to have a role in how penicillin worked. These PBPs are actually transpeptidases; enzymes that are involved in construction of the peptidoglycan portion of the bacterial cell wall. They function to catalyze reactions that allow cross-linking of peptidoglycan subunits as these subunits are incorporated into the cell wall (either for repair of cell wall damage or for constructing new cell wall for cell division). Penicillin will gain entry into the bacterial cell where it will bind to the PBPs and block the ability of the PBPs to function normally. The bacteria fought back by acquiring a plasmid that contained a gene (*blaZ*) that encoded a  $\beta$ -lactamase enzyme. All penicillin drugs (and related  $\beta$ -lactam drugs) have a  $\beta$ -lactam ring at the core of their structure. The  $\beta$ -lactamase enzymes (also known as penicillinases) hydrolyze the peptide bond in the  $\beta$ -lactam ring, which opens up the ring and makes it impossible for the drug to bind to the PBPs.

The antimicrobial researchers then developed modified  $\beta$ -lactam drugs, such as ampicillin, methicillin and oxacillin, which had a modified structure to protect the  $\beta$ -lactam ring from attack by the  $\beta$ -lactamases. The bacteria were not stymied for long. They acquired (from a still unknown source) a new PBP, named PBP2a (or PBP2') that had a much lower affinity for methicillin and most other  $\beta$ -lactam drugs [40]. The gene that encodes the PBP2a protein is *mecA*. This gene is acquired through horizontal transfer of a mobile genetic element known as the staphylococcal cassette

chromosome *mec* (SCC*mec*). These genetic elements contain two required components; the *mec* gene complex, and the *ccr* gene complex (which contains site-specific recombinase genes). The SCC*mec* elements have been classified into eight types (I-VIII) based on the structure and combination of *mec* and *ccr* gene complexes present [41]. These elements also differ in what other antimicrobial resistance genes are carried on them. Types I, IV, V, VI, and VII generally do not carry other resistance genes. Types II, III, and VIII may contain one or more other resistance genes, such as *ermA* (erythromycin), *aadD* (tobramycin), and *tetK* (tetracycline). These types are also used to help distinguish CA-MRSA and HA-MRSA strains. Most HA-MRSA strains carry SCC*mec* types I, II, III, VI, and VIII; while most CA-MRSA strains carry types IV, with some carrying types V and VII [42, 43].

## 5.2. Other cell wall drugs

While there have only been a small number of reports of vancomycin resistant *Staphylococcus aureus* strains, this well may become a major medical issue within a few years. Bacterial resistance to vancomycin is actually manifested using two different mechanisms. The two types of resistant strains are named according to the level of resistance; vancomycin-intermediate *Staphylococcus aureus* (VISA) and vancomycin-resistant *Staphylococcus aureus* (VRSA). VISA resistance is mediated through thickening of the bacterial cell wall which makes it difficult for vancomycin to enter the cell. The mutations or mechanisms required for this change in the cell wall have not yet been determined [44, 45]. VRSA resistance is mediated through acquisition of the *vanA* gene from closely associated enterococci. The expression of this gene allows modification to the peptidoglycan precursors which decreases the binding affinity for vancomycin [46, 47].

The development of lipopeptide drugs is relatively recent, and daptomycin has been available since 2003. Possible resistance mechanisms have been studied and the most commonly seen involves a gain of function mutation in the *mprF* gene. This gene encodes an enzyme that is responsible for adding a lysine to cell membrane components that changes the negatively charged peptidoglycan to a positively charged moiety. This change in charge apparently decreases the ability of daptomycin to bind to the bacterial cell membrane [48, 49].

## 5.3. Drugs that inhibit protein synthesis

The drugs that inhibit protein synthesis work in a specific manner by binding to either the 30S or the 50S ribosomal subunit. Those that bind to the 30S subunit include the aminoglycosides and the tetracyclines. Those that bind to the 50S subunit include chloramphenicol, the lincosamides, macrolides, oxazolidinones, and the streptogramins. We will discuss the resistance mechanisms to these drugs based on that ribosomal subunit specificity.

### 5.3.1. Drugs that bind to the 30S ribosomal subunit

The main aminoglycoside drugs used to treat *S. aureus* infections are gentamicin and tobramycin. Resistance to these drugs has been observed since the 1960's. The resistance mechanism is similar for all aminoglycoside drugs; production of an aminoglycoside-modifying enzyme (AME) that chemically modifies the drug (quite often by transfer of an acetyl group), decreasing the drug's ability to bind to the 30S ribosomal subunit. The possible genes involved are the *aac*, *ant*, and *aph* genes. These genes are thought to be acquired via a plasmid [50, 51].

Resistance to the tetracycline drugs has also been observed for many years. The resistance mechanisms are similar for all bacteria. There are two mechanisms used: active efflux of the drug (the *tetK* gene), and ribosomal protection by competitive binding to the 30S subunit by a ribosomal protection protein (RPP) encoded by the *tetM* gene. Binding of the RPP interferes with the binding of the drug. Both of these mechanisms are thought to be acquired via a plasmid [52, 53].

### 5.3.2. Drugs that bind to the 50S ribosomal subunit

Resistance to chloramphenicol is mediated by acquisition of a plasmid that carries a chloramphenicol acetyltransferase gene (*cat* gene). The enzyme produced by this gene inactivates chloramphenicol by acetylation (chloramphenicol can no longer bind to the 50S subunit) [54].

Resistance to the lincosamides (clindamycin), macrolides (erythromycin), and streptogramins (quinupristin/dalfopristin) is mediated by the same mechanism. Three related genes, *ermA*, *ermB*, and *ermC*, that encode for erythromycin resistance methylases are acquired via plasmids. These enzymes methylate a site on the ribosome resulting in a conformational change. This decreases the ability of these drugs to bind to the ribosome [55-57].

The oxazolidinones are newer drugs, and the only one currently in use is linezolid. Resistance to linezolid has found to happen via two mechanisms: mutation of the ribosomal RNA (*rrn* gene), and methylation of the ribosomal RNA (*cfi* gene). The *cfi* gene which encodes an RNA methyltransferase is plasmid-borne, and has also been shown to confer resistance to chloramphenicol, clindamycin, and streptogramin drugs [58, 59].

#### 5.4. Drugs that inhibit nucleic acid synthesis

The fluoroquinolone drugs act by inhibiting gyrase or topoisomerase IV which halts DNA replication and transcription. Resistance to these drugs is a result of mutations in either the GyrA subunit of gyrase (encoded by *gyrA* gene), or the GrlA subunit of topoisomerase IV (encoded by *grlA* gene) which reduce the ability of the drugs to bind to their targets. In addition, some moderate resistance to norfloxacin (and possibly ciprofloxacin) is caused by induction of the NorA efflux pump (*norA* gene) [60].

#### 5.5. Drugs that are metabolic pathway inhibitors

The combination drug sulfamethoxazole/trimethoprim targets the folate biosynthesis pathway in bacteria by competitive inhibition. Resistance to sulfamethoxazole is due to a mutation in the *dhps* (dihydropteroate synthase enzyme) gene which allows pABA to bind but has greatly reduced binding of sulfamethoxazole. Resistance to trimethoprim occurs by a mutation in the *dhfr* (dihydrofolate reductase enzyme) gene with reduced binding of trimethoprim [29, 60].

## 6. Conclusions

When antimicrobial agents were first being discovered it was thought that there would soon be an end to *S. aureus* infections. Unfortunately, that did not turn out to be the case. With each new antimicrobial agent discovered or developed, the bacteria found ways to fight each drug, either by mutation of its own genes, or by acquisition of genes from other bacteria. Now most *S. aureus* strains are resistant to the penicillin drugs, and many are resistant to all  $\beta$ -lactam drugs. All strains of *S. aureus* have a huge arsenal of virulence factors, and now many are adding multidrug resistance as well. We cannot seem to develop new drugs fast enough to keep up with developing resistance. There are a few newer drugs that currently are fairly effective against *S. aureus* strains (ceftaroline, ceftobiprole, dalbavancin, iclaprim, tigecycline), but how long will it be before these drugs fall to resistance mechanisms? Let us hope that pharmaceutical researchers are able to develop drugs fast enough to keep *S. aureus* (especially MRSA strains) from becoming a major cause of mortality worldwide.

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