THREAT POSED BY MULTI-DRUG RESISTANT *STAPHYLOCOCCUS AUREUS*
AND POSSIBLE NEW TREATMENT OPTIONS

A Seminar Style Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Science in Biology: Concentration in Clinical Microbiology

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THREAT POSED BY MULTI-DRUG RESISTANT *STAPHYLOCCUS AUREUS* 

AND POSSIBLE NEW TREATMENT OPTIONS

By Sarah Dahlke

We recommend acceptance of this thesis in partial fulfillment of the candidate's requirements for the degree of Master of Science in Biology, Clinical Microbiology Concentration.

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ABSTRACT

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*Staphylococcus aureus* is a common commensal inhabitant of human mucus membranes and skin. Despite its role as a commensal, *S. aureus* also cause a wide range of infections in patients of all health backgrounds. Throughout the years, multi-drug resistant strains, often referred to as methicillin-resistant *S. aureus* (MRSA), have increased in prevalence and successful treatment is quite complicated. Even more disconcerting, development of new antimicrobials to combat rising infection rates/resistance rates has been slow. This review provides a general discussion of the evolution of MRSA, mechanisms of resistance to antimicrobial agents, current antimicrobials available for combating infection with MRSA, and future prospects for more effective treatment regimens.
ACKNOWLEDGEMENTS

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INTERRODUCTION

*Staphylococcus aureus* is a ubiquitous Gram-positive cocci that commonly colonizes human skin and mucous membranes and is carried by approximately 25% to 50% of normal individuals\(^1\)-\(^2\). Asymptomatic colonization occurs intermittently and person-to-person transmission occurs via direct contact. In addition, injection drug users\(^3\), insulin-dependent diabetics\(^4\), children\(^5\), health-care workers\(^6\), and patients with long-term intravascular devices\(^7\) are more commonly colonized.

Despite its role as a commensal organism, *S. aureus* also produces multiple virulence factors that contribute to its pathogenicity. For example, teichoic acids in the bacterial cell wall promote adherence to damaged epithelial tissue\(^8\), and multiple other proteins foster binding to fibrinogen\(^9\)-\(^10\), fibronectin\(^11\), and collagen\(^12\). The species can also produce a number of extracellular enzymes that promote dissemination, including coagulase\(^13\), protease\(^8\), collagenase\(^13\), lipase\(^8\), hyaluronidase\(^14\), and staphylokinase\(^15\). In addition, the species may secrete multiple toxins that promote invasion by damaging host tissues. For example, pathogenic strains of *S. aureus* typically produce multiple hemolysins and leukocidins, including Panton-Valentine leukocidin, which can effectively lyse erythrocytes, leukocytes, or fibroblasts\(^16\)-\(^19\). Lastly, *S. aureus* also has numerous mechanisms for evading host immune responses. Primary among these is the expression of protein A, a cell wall protein that hinders phagocytosis by binding to the Fc region of circulating host IgG antibodies\(^20\).
The virulence factors produced by *S. aureus* contribute to a number of possible infections ranging from relatively mild skin infections, such as boils or folliculitis, to severe life-threatening complications, such as pneumonia or sepsis. *S. aureus* is also a major nosocomial pathogen and is the most common pathogen recovered from surgical site infections or hospital-acquired pneumonias. Moreover, *S. aureus* has been implicated as a major cause of nosocomial endocarditis, bacteremia, as well as eye and throat infections.

Overuse of antibiotics has promoted resistance to multiple first- and second-line antimicrobial agents and multi-drug resistant strains, like methicillin-resistant *S. aureus* (MRSA) have become commonplace. Despite the rise in drug resistant strains of *S. aureus*, new drugs and other treatment options have not kept pace. This review provides general discussion of the evolution of MRSA, common mechanisms of resistance to antimicrobial agents, current strategies for eliminating an infection with MRSA and future prospects for more effective treatment options.
HISTORICAL PERSPECTIVE

Prior to the 1950s, β-lactam antibiotics, such as penicillin-G, provided effective treatment for *S. aureus* infections that could otherwise be fatal. The antibiotics bind specifically to penicillin-binding proteins (PBPs), which then prevents cross-linkage of peptidoglycan precursors necessary for peptidoglycan synthesis. However, most *S. aureus* strains have become resistant to penicillin because they produce penicillase (β-lactamase) that inactivates the antibiotic by cleaving the β-lactam ring. In fact, penicillinase-producing strains became so prevalent that semi-synthetic penicillins, notably methicillin and oxacillin, were developed. Each has altered side chains that sterically hinder degradation by penicillinase.

Semi-synthetic penicillins provided reliable treatment for a time, but resistant strains have also become prevalent. The primary mechanism of resistance is expression of a novel PBP, termed PBP2a, which confers resistance to all β-lactam antibiotics because the drugs do not bind to PBP2a. Subsequent investigations demonstrated that PBP2a was encoded by the *mecA* gene, which is contained within a mobile genetic element termed the staphylococcal chromosomally encoded (SCCmec). As a result, isolates that have SCCmec integrated into the chromosome are now termed methicillin-resistant *S. aureus* (MRSA).

Initial infections with MRSA occurred almost exclusively in large urban health care settings, and were primarily observed in immunocompromised patients who had been hospitalized for extended periods of time. The infections were termed hospital-
acquired MRSA (HA-MRSA) and additional risk factors for infection included cardiac catheterization, surgery, or the presence of in-dwelling percutaneous devices\textsuperscript{33}. HA-MRSA strains were also typically resistant to other non-β-lactam antibiotic classes due to the selective pressures present in healthcare settings. In addition, molecular analysis demonstrated that hospital-acquired strains originated from two distinct clones designated USA100 and USA200\textsuperscript{34}.

In more recent times, however, MRSA infections have been observed in patients who did not have traditional risk factors and who were not hospitalized\textsuperscript{35}. These types of infections gained further national attention when several healthy children died from complications of MRSA infections\textsuperscript{36}. Shortly thereafter, outbreaks of community-associated MRSA (CA-MRSA) were documented in daycare centers\textsuperscript{37}, athletes\textsuperscript{38-39}, and prison inmates\textsuperscript{40}. Further epidemiologic studies\textsuperscript{34,41-42} also demonstrated that CA-MRSA arose from distinct clonal lineages, designated USA300 and USA400. Moreover CA-MRSA strains were typically resistant to only β-lactams and macrolides\textsuperscript{27,43} and were also more likely to express Panton-Valentine leukocidin, a toxin associated with necrotizing pneumonia\textsuperscript{18-19}.

The emergence of MRSA in the community has forced clinicians to rely heavily upon other non-β-lactam antibiotics. This practice has caused selection for other resistance phenotypes in addition to β-lactam resistance. For example, there have been increasing reports of CA-MRSA [USA 300] strains resistant to a variety of mechanisms to antibiotics such as erythromycin, clindamycin, gentamicin, and fluoroquinolones\textsuperscript{44-45}. Some of the most common resistance mechanisms seen in MRSA are discussed below.
ADDITIONAL MECHANISMS OF ANTIBIOTIC RESISTANCE

Expression of erythromycin ribosomal methylase (Erm)

In addition to β-lactam resistance, MRSA strains commonly show resistance to macrolide, lincosamide, and streptogrammin (MLS) antibiotics (e.g. erythromycin, clindamycin). These antibiotics represent chemically discrete drug classes that inhibit protein synthesis by binding to the 50S ribosomal subunit, and each has been used to effectively treat staphylococcal infections\(^46\). However, MRSA strains frequently acquire \(ermA\) or \(ermC\) genes that encode erythromycin ribosomal methylase (Erm). The Erm protein then provides resistance by methylating an adenine residue of the 23S rRNA component of the 50S ribosomal subunit. Methylation subsequently causes a conformational change that prevents the drugs from binding to the ribosome\(^47-49\). In addition, Erm-positive strains can be resistant to all MLS antibiotics or only macrolides. Infections caused by \(S.\ aureus\) with resistance to only macrolides can be misleading because expression of Erm is weakly induced by clindamycin \textit{in vitro} giving the appearance that strains are susceptible when routine susceptibility testing is performed. However, subsequent clindamycin therapy then selects for isolates that robustly express Erm, which ultimately causes treatment to fail\(^50\). Thus, prudent use of clindamycin and more accurate antimicrobial susceptibility testing by using additional tests, such as the erythromycin induction test (e.g. D-test), are necessary.
Expression of aminoglycoside modifying enzymes (AME)

Aminoglycosides such as gentamicin and tobramycin can also effectively eliminate staphylococcal infections by binding the 30S ribosomal subunit and inhibiting protein synthesis. These antibiotics are also effective because they can act synergistically with β-lactams and glycopeptides. However, most MRSA strains have acquired the ability to produce several aminoglycoside modifying enzymes (AME), such as aminoglycoside-6’-N-acetyltransferase/2”-O-phosphoryltransferase, aminoglycoside-3”-O-phosphoryltransferase III, and aminoglycoside-4’-O-phosphoryltransferase I, that alter the amino and hydroxyl groups of the aminoglycoside as it is transported into the bacterial cell. The modifications produce a conformational change in the drug structure that prevents the antibiotics from binding to the 30S ribosomal subunit51.

*gyrA and norA mediated drug efflux*

Fluoroquinolones are a unique class of drugs that bind to DNA topoisomerases and gyrases, which are essential for DNA replication. These drugs were widely favored because of their broad spectrum of activity. However, due to the ability of *S. aureus* to rapidly develop resistance to fluoroquinolones and because there is a high level of resistance among strains, fluoroquinolones are no longer recommended to treat MRSA infections.

*Staphylococcus aureus* has become resistant to fluoroquinolones through two mechanisms52. The first and most common resistance mechanism is caused by mutations in the *gyrA* that encodes A subunit of DNA gyrase, a critical enzyme for DNA synthesis. Several studies have demonstrated low level exposure has created resistant isolates by
inducing point mutations that result in amino acid changes of either Ser84 to Leu or Ala, Ser85 to Pro, or Glu88 to Lys\textsuperscript{52-53}. Any of these changes alters the shape of the A subunit in such a way that fluoroquinolone binding is prevented, even when lethal concentrations of the drug are present in the bacterial cell.

The second mechanism of resistance is achieved by the acquisition of a transposon that contains \textit{norA}\textsuperscript{54-55}, which codes for a cell membrane associated efflux pump that pumps fluoroquinolones out of the cell. Production of the efflux pump alone confers only low-level fluoroquinolone resistance, but the combination of an efflux pump with the \textit{gyrA} mutation enables \textit{S. aureus} to achieve a high level of resistance to fluoroquinolones\textsuperscript{55}. 
CURRENT INFECTION CONTROL AND TREATMENT STRATEGIES

Infection control

Although the scenario with multi-drug resistant MRSA appears grim, there are a number of ways that infection rates can be controlled. Nasal carriage or skin colonization with MRSA is a principal risk factor for infection in many patient demographics, including especially surgical patients, intensive care unit (ICU) patients, persons undergoing renal dialysis or organ transplants, and HIV positive patients. In response, hospitals have adopted a proactive approach for monitoring MRSA nasal carriage in these patient demographics. For example, real-time polymerase chain reaction (RT-PCR) assays or culturing of nasal swabs prior to or upon hospital admission has allowed hospital staff to identify MRSA carriers. Infection control measures such as maximum sterile placement of central lines and isolation precautions can then be implemented in a timely manner. In fact, one study found proactive approaches such as these have reduced HA-MRSA bacteremias by up to 67%. Moreover, prophylactic decolonization of surgical patients with mupirocin ointment and chlorhexidine gluconate body wash can also significantly reduce the number surgical site infections. Thus, there is little doubt more that widespread adoption of these types of preventive measures is essential to curb spread.

Antibiotic Strategies

When infection control strategies fail there are still treatment regimens that can be used to successfully eliminating MRSA infections. Their success, however, depends
primarily on the health status of the patient and the location of the infection. For example, mild infections, such as folliculitis or carbuncles, can often be treated successfully with topical antimicrobials such as bacitracin or mupirocin\textsuperscript{60-61}. In addition, localized abscesses and wounds in otherwise healthy individuals can often be successfully treated by drainage or debridement.

However, supplemental antibiotic therapy is typically necessary if the infected patient is very young or very old, has a pre-existing illness, or if additional systemic symptoms (e.g. fever or swelling) are present\textsuperscript{60}. In these instances, clindamycin, trimethoprim-sulfamethoxazole (TMP-SMX), rifampin, tetracyclines, linezolid, or vancomycin are most commonly used\textsuperscript{60}. However, with the exception of vancomycin, each drug can have significant side effects or can rapidly induce resistance (Table 1). For example, tetracyclines cause discoloration of teeth in young children and skeletal hypoplasia in developing fetuses\textsuperscript{25,60-61}, clindamycin can cause severe diarrhea, and prolonged linezolid therapy has been associated with thrombocytopenia\textsuperscript{25,62}. Additionally, rifampin must be used as a co-therapy because \textit{S. aureus} rapidly develops resistance. Moreover, treatment with TMP-SMX can cause complications in pregnant women and young children.

As a result, vancomycin has been increasingly relied upon because it can eliminate MRSA from most complicated infections without significant side effects\textsuperscript{25}. In fact, vancomycin is often the only remaining effective antibiotic for eliminating MRSA from major abscesses (e.g. near major organs), major surgical or traumatic wounds, endocarditis, osteomyelitis, or bacteremia\textsuperscript{60-61}. 
EMERGING RESISTANCE TO VANCOMYCIN

Vancomycin is a glycopeptide antibiotic that kills Gram-positive organisms by binding to the D-alanine-D-alanyl portion of nascent peptidoglycan molecules to prevent cross linkage by PBPs. The drug has been the most effective at treating serious MRSA infections without major side effects. However, a number of MRSA strains known as glycopeptide intermediate S. aureus (GISA), have developed partial resistance to vancomycin and a few isolates, termed vancomycin-resistant S. aureus (VRSA), are completely resistant. This is especially disconcerting since there are few other viable alternative treatment options available. In response, the Centers of Disease Control and others have published recommendations to curb unnecessary usage of vancomycin, but there is increasing concern that vancomycin-resistant MRSA strains will continue to proliferate.

An additional resistance mechanism, where the organism acquires the vanA operon from vancomycin-resistant enterococci (VRE), however is much more concerning since operon contains genes for an enzyme to synthesize alternative D-Lac peptidoglycan precursors resistant to the effects of vancomycin and an enzyme that hydrolyzes nascent D-alanine-D-alanyl peptidoglycan. Acquisition of the vanA operon occurs via conjugal transfer from vancomycin-resistant Enterococcus spp., which are natural reservoirs for the plasmid that contains the gene. Not surprisingly, most VRSA isolates recovered to date have been from patients co-colonized with VRE and MRSA, but there is
increasing concern that overuse of vancomycin will continue to select for these organisms.
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<th>Drug</th>
<th>Advantages</th>
<th>Disadvantages</th>
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<td>Clindamycin</td>
<td>Can be used for infants and children Oral administration</td>
<td>Inducible resistance concerns, Antibiotic associated diarrhea</td>
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<td>Tetracycline</td>
<td>Oral administration Cost</td>
<td>Not recommended for pregnant women or young children (&lt; 8 years) Resistance concerns</td>
</tr>
<tr>
<td>TMP-SXT</td>
<td>Cost Oral administration Can be used for children</td>
<td>Resistance concerns Interference with renin-angiotensin inhibitors, Risk for hyperkalemia, Not recommended for infants &lt; 2 months old or pregnant women in 3rd trimester</td>
</tr>
<tr>
<td>Rifampin</td>
<td>Reaches high intracellular levels Penetrates biofilms</td>
<td>Adjunct therapy only due to resistance development Lack of data on dosing regimens</td>
</tr>
<tr>
<td>Linezolid</td>
<td>Excellent tissue penetration Available in oral formulation Can be used for children</td>
<td>Very expensive to use Myelosuppression Risk for serotonin syndrome Resistance concerns</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>Well studied Can be used for infants and children Relatively mild side effects</td>
<td>Resistance concerns Slowly bactericidal Relatively poor tissue penetration</td>
</tr>
<tr>
<td>Daptomycin</td>
<td>Relatively good tissue penetration Acceptable substitute for vancomycin</td>
<td>Resistance concerns Development of myopathy Cost (I.V. administration)</td>
</tr>
<tr>
<td>Quinupristin/Dallopstin</td>
<td>Rapidly bactericidal Relatively good tissue penetration Acceptable substitute for vancomycin</td>
<td>Resistance concerns Side effects Cost (I.V. administration)</td>
</tr>
<tr>
<td>Tigecycline</td>
<td>Excellent tissue penetration Acceptable substitute for vancomycin</td>
<td>Use linked to fatality? Not recommended for pregnant women or young children (&lt; 8 years) Cost (I.V. administration)</td>
</tr>
<tr>
<td>Telavancin</td>
<td>Activity against VISA Rapidly bactericidal Acceptable substitute for vancomycin</td>
<td>Cost (I.V. administration) Risk of nephrotoxicity</td>
</tr>
</tbody>
</table>
NEW GENERATION ANTIBIOTICS

The emergence of vancomycin resistance in *S. aureus* has increased research efforts to identify new antibiotics to combat MRSA and VISA/VRSA infections. As a result, there are now several FDA approved antibiotics that can be used as alternatives to vancomycin. In addition, several other antibiotics are in the later stages of pharmaceutical development.

**Daptomycin**

One of the newer FDA approved drugs for treating complicated skin and soft tissue infections (cSSTIs) is daptomycin. The antibiotic is an injectable lipopeptide that forms pores and causes rapid depolarization of the cell membrane via insertion of the drug’s lipophilic tail\(^{70-71}\). In support of its efficacy, several studies have demonstrated that treatment with daptomycin is as effective as treatment with vancomycin for eliminating *S. aureus* from animals with wound infections or sepsis\(^{72-74}\). In addition, treatment with daptomycin can eradicate staphylococcal biofilms from catheters\(^{75}\) and the combination of daptomycin and rifampin can successfully eliminate MRSA from foreign implants placed into guinea pigs\(^{72}\). Moreover, few serious side effects have been observed in patients treated with daptomycin however, myopathy has been observed in patients undergoing prolonged therapy, so creatine phosphokinase levels must be monitored throughout therapy\(^{25,76}\).
One area of concern, however, is that clinical S. aureus isolates with decreased susceptibility to daptomycin have been recovered from patients who were switched to the drug after treatment with vancomycin had failed. The mechanism(s) responsible for the partial resistance remains unclear, but loss of membrane proteins, altered surface charge, morphologic change, and gene mutations have been postulated as possible causes.

**Quinupristin/Dalfopristin**

Another new drug is quinupristin/dalfopristin (QD). QD is an injectable combination of two unique streptogrammins that prevent protein synthesis by binding separate areas on the 50S ribosomal subunit. Together the drugs have excellent bactericidal activity in vitro, and animal studies have confirmed their efficacy for eliminating staphylococcal infections from thighs, abscesses, lungs, and heart valves. More significantly, QD can effectively treat cSSTIs, and is, in fact, FDA-approved for treating adults and children 16 years and older. It should also be noted, however, that isolates that are resistant to other streptogrammins will also be resistant to treatment with QD, so antimicrobial susceptibility testing prior to therapy is critical. In addition, QD is recommended only after treatment with vancomycin has failed, because arthralgia, myalgia, and thrombophlebitis are relatively common side effects. Additionally, the requirement for intravenous administration greatly increases the cost of therapy to the patient.

**Tigecycline**

A third drug that was recently granted FDA approval is tigecycline, a derivative of minocycline. The drug inhibits protein synthesis in bacteria by binding to the 30S
ribosomal subunit and blocking the entry of aminoacyl-tRNA to the A-site in the
ribosome\textsuperscript{93}. The antibiotic is bacteriostatic\textsuperscript{94}, but animal studies have confirmed that the
drug can effectively eliminate MRSA from blood, bone, and tissue infections caused by
implanted devices\textsuperscript{95-98}. However, widespread use of the antibiotic is hindered by several
shortcomings. For example, tigecycline has a high tropism for tissue, but does not
achieve high concentrations in the human bloodstream\textsuperscript{61} so it is inappropriate for treating
staphylococcal bacteremia. More significantly, the FDA recently suggested that
tigecycline should be used with extreme caution due to reports of increased risk of
fatality with prolonged usage\textsuperscript{99}.

**Linezolid**

Perhaps the most successful new generation therapy for treating MRSA is
linezolid, a bacteriostatic oxazolidinone antibiotic that inhibits protein synthesis by
preventing assembly of an essential tRNA-mRNA complex\textsuperscript{100}. The antibiotic has
excellent *in vitro* activity against MRSA\textsuperscript{101}, and promising findings from phase II and III
clinical trials\textsuperscript{102-105} resulted in FDA approval for cSSTIs and pneumonia\textsuperscript{25}. Moreover,
pharmacokinetic studies have confirmed excellent penetration into numerous tissues
including bone, fat, muscle, hematomas and synovial fluid, which suggests the antibiotic
may be an effective treatment option for septic arthritis, osteomyelitis, and as a
prophylactic therapy for arthroplasty\textsuperscript{106-107}. An important caveat, however, is that
treatment with linezolid is recommended only after vancomycin therapy has failed
because it is costly to use\textsuperscript{25}, causes thrombocytopenia during prolonged use\textsuperscript{62}, and like
other drugs, acquisition of resistance is possible\textsuperscript{108}. 
Telavancin

The most recent drug approved by the FDA is telavancin, a synthetic glycopeptide (similar to vancomycin) that contains additional hydrophobic side chains\textsuperscript{109-110}. The drug inhibits cell wall synthesis by binding the D-alanine-D-alanyl portion of peptidoglycan precursors in Gram-positive bacteria\textsuperscript{63} and also destabilizes cell membranes by intercalating into phospholipids\textsuperscript{109-110}. Telavancin is rapidly bactericidal against MRSA and GISA \textit{in vitro}, but displays poor activity against VRSA\textsuperscript{111}. In fact, researchers\textsuperscript{110-116} have provided convincing evidence that treatment with telavancin can be more effective than treatment with linezolid or vancomycin, even with only a once daily dosing regimen\textsuperscript{110, 113}. However, prolonged televancin therapy can cause kidney damage so use of the antibiotic is only recommended in instances where vancomycin or daptomycin therapies have failed\textsuperscript{61, 109}. 

EXPERIMENTAL ANTIBIOTICS

Modified cephalosporins

In response to the increasing prevalence of MRSA and limitations associated with the newer FDA approved drugs, a number of modified cephalosporins (e.g. β-lactams) with high binding affinity for PBP2a are being evaluated. One of the leading candidates is ceftobiprole. Numerous studies have confirmed excellent bactericidal in vitro activity against a wide variety of S. aureus strains including numerous MRSA and VISA isolates\textsuperscript{117-120}. In addition, ceftobiprole was more effective than vancomycin at eliminating MRSA from the bloodstream or abscesses in laboratory mice\textsuperscript{117}. Furthermore, treatment with ceftobiprole can effectively clear staphylococci from infected heart vegetations\textsuperscript{121-123} and phase III clinical trials have confirmed efficacy for treating of cSSTIs\textsuperscript{124-125}.

Another promising cephalosporin is ceftaroline, which effectively kills MRSA, VISA, VRSA, and daptomycin-non susceptible MRSA in vitro\textsuperscript{127-130} and also eliminates MRSA infections in several animal models\textsuperscript{131-132}. More promising, human clinical trials have confirmed effective treatment of cSSTIs\textsuperscript{133-134} and community-acquired pneumonia (CAP)\textsuperscript{135-136} with minimal side effects.

New generation oxazolidinones

In contrast to the original bacteriostatic oxazolidinones (e.g. linezolid), the new generation oxazolidinones have modified chemical structures to increase efficacy. Prominent among these is tedizolid, which has tetrazolyl and pyridin substitutions at C9
in place of a morpholin ring form (e.g. linezolid) (Figure 1) and the result is excellent bactericidal activity in vitro\textsuperscript{137-141}, even against isolates that have gained resistance to linezolid\textsuperscript{140}. In addition, the high level of activity in vitro suggests small dosages will necessary for effective in vivo treatment\textsuperscript{142-143}. For example, recent clinical studies have demonstrated that the effectiveness of a single daily 200 mg dosage of tedizolid was comparable to twice daily 600 mg dosages of linezolid for eliminating MRSA from infected tissues\textsuperscript{142-143}.

**Diaminopyrimidines**

Diaminopyrimidines are another class of antibiotics that are being re-investigated. The drugs selectively inhibit dihydrofolate reductase (DHFR), an enzyme required for de-novo synthesis of purines, thymidylic acid, and certain amino acids\textsuperscript{144}. Iclaprim is a modified diaminopyrimidine that has rapid bactericidal activity against \textit{S. aureus}, including MRSA and also works synergistically with sulfamethoxazole or sulfadiazine\textsuperscript{145}. However, iclaprim has been somewhat difficult to study in traditional rodent models of infection due to naturally high concentrations of exogenous thymidine within the mice\textsuperscript{146}. This antagonizes the action of diaminopyrimidines because the bacteria use thymidine kinase to acquire exogenous thymidine and bypass the DHFR step in \textit{de novo} purine synthesis\textsuperscript{146-147}. Therefore, accurate results from rodent studies are likely dependent on the use of thymidine kinase deficient MRSA mutants because the mutants are unable to acquire exogenous thymidine\textsuperscript{146}.
Figure 1. Structural comparison of linezolid (A).
Despite this shortcoming, however preliminary clinical trials have shown effectiveness for treatment of community-acquired pneumonia caused and cSSTIs (Table 2). For example, a phase II clinical trial revealed that iclprim was similar to vancomycin in treating cSSTIs caused by *S. aureus*, including MRSA\(^\text{149}\). Furthermore, a phase III trial also showed that iclaprim was comparable to linezolid in the treatment of *S. aureus* cSSTIs (clinicaltrials.gov NCT00299520). Unfortunately, due to financial constraints, iclaprim has yet to reach the market and its potential as a MRSA therapeutic may never be fully realized.

**Lipoglycopeptides**

Similar to telavancin, oritavancin and dalbavancin are lipoglycopeptides that contain additional hydrophobic side chains. Both drugs inhibit cell wall synthesis in Gram-positive organisms\(^\text{63}\) and also destabilize the cell membrane\(^\text{142-143}\). Several studies have demonstrated that both drugs have potent bactericidal activity *in vitro* against MRSA and GISA\(^\text{150-153}\) but oritavancin also has *in vitro* activity against VRSA\(^\text{151}\). In addition, both drugs have had similar success at eliminating a variety of MRSA infections in animal models\(^\text{153-154}\) and in human clinical trials\(^\text{155-156}\). Moreover, both drugs have preliminary pharmacokinetic profiles suggestive of once weekly or one-time dose per treatment, which may be of benefit to patients because it would reduce overall drug costs.
Table 2. Summary of experimental drugs for MRSA

<table>
<thead>
<tr>
<th>Drug</th>
<th>Research Highlights</th>
<th>Reference</th>
</tr>
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| Ceftobiprole | Activity against MRSA, GISA  
As efficacious as vancomycin in animal models  
Efficacy for cSSTIs in clinical trials | 117-25                     |
| Ceftaroline  | Activity against MRSA, GISA, VRSA  
Efficacy for cSSTIs and CAP in clinical trials | 126-36                     |
| Tedizolid    | Activity against MRSA, linezolid resistant *S. aureus*  
Lower effective dose compared to linezolid in clinical trials  
Efficacy for cSSTIs in clinical trials | 137-43                     |
| Iclaprim     | Activity against MRSA  
Efficacy for cSSTIs and CAP in clinical trials | 144-6, 148-9, clinicaltrials.gov identifier NCT00299520 |
| Oritavancin  | *In vitro* and *in vivo* activity against MRSA, GISA, and VRSA  
Long half life—potential one dose per treatment  
Efficacy for cSSTIs in clinical trials | 151-2, 154-5                 |
| Dalbavancin  | *In vitro* and *in vivo* activity against MRSA, GISA  
Long half life—potential once weekly treatment  
Efficacy for cSSTIs in clinical trials | 150, 153, 156              |
POSSIBLE ALTERNATIVE TREATMENT OPTIONS

In addition to traditional chemotherapeutic antibiotics, a number of alternative antimicrobials are being examined for their efficacy against *S. aureus* and MRSA. These include fatty acids, plectasins, lantibiotics, and bacteriophage. All are in the early stages of development and continue to be examined for their efficacy in eliminating MRSA.

**Non-specific killing by fatty acids**

Fatty acids are naturally-occurring non-toxic byproducts of digested dietary fats that are used routinely as food preservatives\textsuperscript{157-159}. The compounds can also non-specifically kill a variety of microorganisms by intercalating into the phospholipid membranes and causing the membrane to rupture due to the resultant fluid imbalance\textsuperscript{160}. Medium-chain (C=10, 12) saturated fatty acids are the most adept at this as they are able to penetrate the cell wall to intercalate into the cell membrane. Moreover, the addition of a glycerol moiety to these fatty acids intensifies the potency\textsuperscript{157}. For instance, one such compound, termed monolaurin, kills numerous species of bacteria including *S. aureus in vitro*\textsuperscript{157-158, 160-161}. These findings have led to additional studies investigating the utility of monolaurin for as a topical microbicidal. For example, another study showed that women who used tampons containing a 14% monolaurin coating were significantly less likely to harbor *S. aureus*\textsuperscript{162}. The utility of monolaurin for treating wound or systemic infections is currently unclear however, it appears to be an effective means to eradicate staphylococcal colonization.
**Targeting with plectasins**

Antimicrobial peptides (AMPs) are integral facets of the innate immune response and a conserved group of AMPs, termed defensins, may also have relevance for eliminating MRSA\textsuperscript{163}. For example, plectasin is a defensin-like molecule produced by the ascomycete, *Pseudoplectania nigrella* that targets Lipid II in the cell wall of Gram-negative and Gram-positive bacteria, including MRSA. Several studies have demonstrated the ability of a plectasin derivative, termed NZ2114, to kill *S. aureus in vitro*\textsuperscript{164-166}. More significantly, treatment with NZ2114 was shown to clear MRSA from infected heart vegetations of rabbits\textsuperscript{166}.

**Lantibiotics**

Another antimicrobial peptide class is the lantibiotics. Lantibiotics are antimicrobial peptides characterized by lanthionine or methyllanthionine rings that are produced by Gram-positive bacteria to kill other bacteria\textsuperscript{167}. Longer lantibiotic peptides (34 amino acids) are able to bind to Lipid II and form pores in the cell membrane, whereas shorter lantibiotic peptides (19 amino acids) halt cell wall synthesis by sequestering Lipid II. Most significantly, studies examining the ability of lantibiotics to kill *S. aureus in vitro* have provided encouraging results\textsuperscript{167-169}. For example, one lantibiotic, termed mersacidin, has excellent *in vitro* activity against MRSA\textsuperscript{170} and topical therapy with the lantibiotic eradicated MRSA nasal colonization in mice\textsuperscript{171}. Additionally, a lantibiotic derivative termed NAI-107 is bactericidal to MRSA and GISA\textsuperscript{172} *in vitro*, and the compound easily accumulates at bactericidal levels in animal tissues after intravenous administration.
Killing by bacteriophage

An additional promising alternative treatment for eliminating MRSA is the use of species-specific bacteriophages, especially since they generally do not affect mammalian tissues. Several studies have examined whether phage treatment could successfully target MRSA. For example, one study demonstrated that MRSA was highly susceptible in vitro to phage P-27/HP and treatment with the bacteriophage protected mice against subsequent challenge with *S. aureus*. In addition, treatment with linezolid and an anti-*S. aureus* phage successfully eliminated MRSA from infected foot ulcers in mice. Similarly, lytic enzymes produced by phage that target peptidoglycan bonds have been used to eliminate MRSA. For example, the lysin, ClyS, killed multiple *S. aureus* isolates (including MRSA) in vitro and treatment with ClyS and oxacillin protected recipient mice from staphylococcal sepsis. There was also no evidence that therapy with ClyS elicited an immune response that would hinder future applications.
SUMMARY

*Staphylococcus aureus* is a significant human pathogen capable of causing a variety of infections in patients of all health backgrounds. During the past several decades, the overuse of antibiotics has selected for multi-drug resistant strains, which has limited current treatment options. More worrisome, vancomycin, the first line treatment for severe MRSA infections, is losing efficacy. As a result, the pharmaceutical industry has been forced to re-examine existing antibiotics and broaden antimicrobial screens in an attempt to find relevant drugs. To date, several suitable antibiotics have been identified and some are currently available for treatment of MRSA infections. Moreover, a number of other potentially effective experimental antibiotics are in various stages of clinical development and several are also on track to gain FDA approval (Table 2). Other alternative antimicrobials such as plectasins, lantibiotics, *S. aureus* specific bacteriophage, and phage lysins may also be viable treatment options for MRSA if they can successfully pass the rigors of pharmaceutical development (e.g. clinical trials for the FDA). However, antibiotic resistance in *S. aureus* appears likely to remain a problem.

Efficient and innovative drug development remains essential to keep pace with antibiotic resistance. Many of the newer drugs being developed are from dated antibiotic classes (e.g. cephalosporins, glycopeptides, diaminopyrimidines etc) and the discovery of new drug classes has been slow. Moreover, treatment strategies will also need to be more aggressive to prevent the development of resistance. For example, it may necessary to use several different antimicrobials to target multiple essential *S. aureus* cell functions
simultaneously. This type of approach may greatly reduce the occurrence of drug resistant mutants that may occur during therapy. Lastly, stringent infection control and antibiotic stewardship will remain important in slowing the development and spread of antibiotic resistance.
REFERENCES


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94. Bradford PA, Weaver-Sands DT, Petersen PJ. *In vitro* activity of tigecycline against isolates from patients enrolled in phase 3 clinical trials of treatment for complicated skin


ingredient of the antibacterial prodrug TR-701, a novel oxazolidinone antibacterial

(Torezolid) against staphylococcal blood isolates collected in Spain. Antimicrob Agents

of the prodrug TR-701, against linezolid-resistant strains. Antimicrob Agents Chemother

tedizolid (TR-700) against Gram-positive clinical isolates from a phase 2 study of oral
tedizolid phosphate (TR-701) in patients with complicated skin and skin structure

142. Prokocimer P. Human pharmacokinetics of the prodrug TR-701 and TR-700, its active
moiety, after multiple oral doses of 200 and 400 mg TR-701, a novel oxazolidinone.

143. Prokocimer P, DeAnda C, Fang E, Mehra P, Das A. Tedizolid phosphate vs linezolid for
treatment of acute bacterial skin and skin structure infections: the ESTABLISH-1

144. Sader HS, Fritsche TR, Jones RN. Potency and bactericidal activity of iclaprim against

iclaprim, in combination with folate inhibitors and other antimicrobials with different

146. Entenza JM, Haldimann A, Giddey M, et al. Efficacy of iclaprim against wild-type and
thymidine kinase-deficient methicillin-resistant Staphylococcus aureus isolates in an in

147. Hamilton-Miller JMT. Reversal of activity of trimethoprim against Gram-positive cocci

fluid, alveolar macrophages, and bronchial mucosa after a single intravenous dose of 1.6
mg/kg of iclaprim (AR-100) in healthy men. J Antimicrob Chemother 2007;60(3):677-
80.
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