Methicillin-Resistant *Staphylococcus aureus* (MRSA): Molecular Aspects of Antimicrobial Resistance and Virulence

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Awareness of the threat of MRSA is growing. Scientists have put a lot of effort into trying to divide and classify MRSA strains into groups to better understand it. This led to the discovery that the resistance gene, *mecA*, and surrounding DNA could be grouped into several types. It was also discovered that the MRSA strains that caused hospital-acquired (nosocomial) infections were different strains than those seen in the communities. Several studies led to the realization that the number of MRSA infections is increasing, that more *Staphylococcus aureus* infections are caused by MRSA strains, and that the community strains are now showing up in the hospital. There have been government initiatives to try to decrease MRSA infections, with the most perplexing issue being that of whether or not to perform surveillance cultures on as many people as possible to eradicate MRSA from the community, as well as the hospital.

INDEX TERMS: MRSA, antimicrobial resistance, virulence, Panton-Valentine leukocidin

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LEARNING OBJECTIVES

1. Describe how penicillin drugs function in a bacterial cell.
2. Discuss the different types of SCC*mec* elements and where they are found.
3. Differentiate between VISA and VRSA strains.
4. Discuss the virulence of methicillin-susceptible *S. aureus* and MRSA strains.

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When penicillin was first used against bacteria like *Staphylococcus aureus*, medical science thought that this bug had been conquered. Unfortunately, within a short space of time, penicillin-resistant strains of *S. aureus* were being isolated. The search for the cause of this resistance led to the discovery of the penicillin-binding proteins (PBPs), so named because of their role in how penicillin affects the bacteria. The PBPs are actually transpeptidases that are involved in the construction of the peptidoglycan portion of the bacterial cell wall. Their function is in catalyzing reactions that allow the cross-linking of the peptidoglycan subunits. When penicillin invades the bacterial cell, it binds to the PBPs, blocking their ability to function normally. As a result, the peptidoglycan cell wall layer is not able to be repaired, and new cell wall for cell division cannot be made. The bacteria were able to fight back by acquisition of a plasmid that contained a gene (*blaZ*) that produced a β-lactamase enzyme. The penicillin drugs all have a β-lactam ring at the core of their structure (Fig. 1). The β-lactamases (also known as penicillinases) are able to hydrolyze the peptide bond in the ring, causing the ring to open up, and rendering the penicillin useless for binding to PBPs.

Scientists fought back by developing modified ,-lactam drugs such as ampicillin and methicillin (Fig. 2). Their modified structure prevented methicillin from being hydrolyzed by β-lactamases. However, the bacteria didn't give up. They

were able to produce a new PBP, PBP2a (or PBP2"), that had a low-affinity for methicillin and other β-lactam drugs (i.e. the methicillin couldn't bind properly to the modified PBP). MRSA, then, is essentially considered to be resistant to all β-lactam antibiotics, such as oxacillin[1]. This resistance comes with a price however: the bacteria cannot replicate as quickly[2].

PBP2a is a high-molecular-weight PBP which has a multi-domain structure[3]. It is encoded by the *mecA* gene, which is part of a genetic element called the staphylococcal cassette chromosome *mec* (SCC*mec*). Each SCC*mec* element contains two required components; the *mec* gene complex, and the *ccr* gene complex which contains site-specific recombinase genes. The elements are classified into five types (I, II, III, IV, V) based on the combination of *mec* and *ccr* gene complexes that they contain. The elements also differ in the antibiotic resistance genes that they carry. Types I, IV, and V generally do not contain any other antibiotic resistance genes in addition to *mecA*. Types II and III may contain several other antibiotic resistance genes. This cassette typing helps to explain the difference seen in the antibiotic resistance of the HA-MRSA and CA-MRSA strains. Most HA-MRSA strains contain SCC*mec* types I, II, or III. Most CA-MRSA strains contain types IV or sometimes V[4,5].

In addition to resistance to ,-lactam drugs, *S. aureus* (and MRSA) strains have been isolated that are resistant to many classes of antibiotics. The resistance mechanisms may be acquired, or produced via mutations in genes, or induction of certain genes. Aminoglycoside antimicrobials, such as amikacin, gentamicin, and tobramycin, act by binding to the bacterial ribosome and preventing protein synthesis. Resistance is due to the production of the aminoglycoside-modifying enzyme acetyltransferase (encoded by the *aac* gene) and phosphotransferase (encoded by the *aph* gene), which modify the drugs by acetylation or phosphorylation. The modified drugs have a greatly reduced ability to bind to the ribosome[6].

Quinolone antimicrobials, such as ciprofloxacin, levofloxacin, and norfloxacin act by inhibiting the gyrase or topoisomerase IV enzymes in *S. aureus*, which halts DNA replication and transcription. Resistance is due to mutations in either the GyrA subunit of gyrase, or the GrlA subunit of topoisomerase IV, which reduce quinolone affinity for those targets. The mutations may be either single amino acid mutations or the result of an accumulation of multiple mutations, which increases the level of resistance. Another mechanism of resistance is by induction of the NorA efflux pump, which enables the bacteria to expel the drug from the cell[7]. As of 1999, nearly 89% of MRSA isolates from bloodstream infections in the U.S. were resistant to ciprofloxacin[8].

Oxazolidinone antimicrobials, such as linezolid (2001), act by binding to the ribosome near where the two main subunits interface, which inhibits protein synthesis. Resistance is due to the bacteria changing the target site of the drug via a mutation, or mutations, in the *rrn* gene, which encodes a component of the 50S rRNA subunit. These mutations greatly reduce the ability of the drugs to bind to the ribosome[9].

Figure 1. Penicillin structure

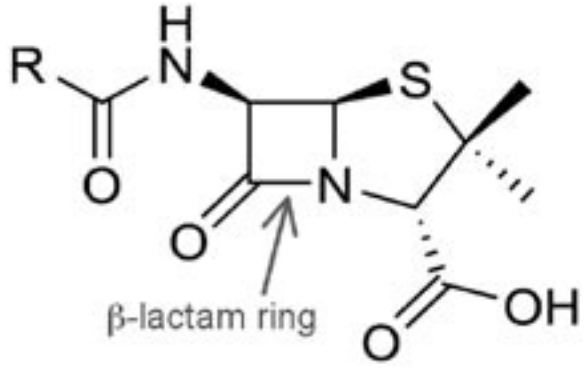
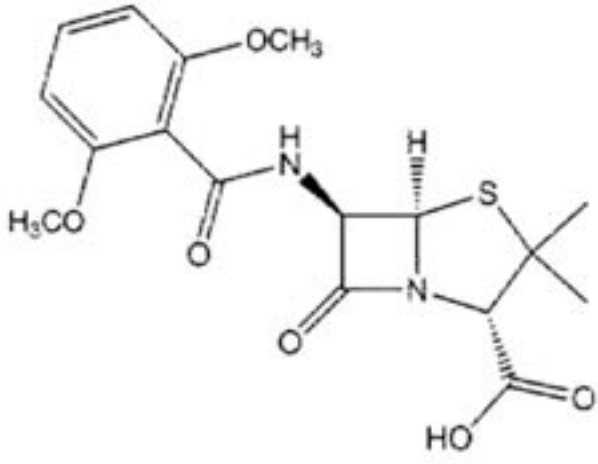


Figure 2. Methicillin structure



Combination drugs have not fared much better. Certain drugs have been used in combination to produce a synergistic bactericidal effect. Trimethoprim-sulfamethoxazole (Bactrim) has been in use for several decades as a treatment for a number of infections (originally mainly against gram-negative bacteria, especially in urinary tract infections). This drug combination targets the folate biosynthesis pathway in bacteria. Each drug targets a different step in the pathway. This pathway is vital for production of nucleic acids and essential proteins. Sulfamethoxazole is a structural analog of para-amino benzoic acid (PABA), which is the substrate for one of the reactions in the pathway, and competes with PABA for the active site of the enzyme which catalyzes the reaction. Trimethoprim is an analog of a portion of another substrate in the pathway, dihydrofolic acid, and competes with it for the enzyme that catalyzes its conversion to tetrahydrofolic acid. Resistance to sulfamethoxazole occurs due to a single amino acid substitution in the gene for the dihydropteroate synthase enzyme, *dhps*. This allows the enzyme to effectively allow binding of PABA, but has a greatly reduced affinity for the drug. Trimethoprim resistance is due to an amino acid substitution in the gene for the dihydrofolate reductase enzyme, *sulA*, which again causes a reduced affinity for the drug[10, 11]. Resistance to this drug combo has been slower to develop than for some other drugs. As of 1999, only 26% of bloodstream infection isolates were resistant[8].

The drug combination of quinupristin-dalfopristin (Synercid) acts synergistically by inhibiting protein synthesis due to binding to the ribosome and blocking the elongation step during translation. These drugs are streptogamins which contain two component types, A and B, which are represented by dalfoprisitin and quinupristin respectively. Resistance to streptogamins is mediated by a mechanism which also causes resistance to macrolide (such as erythromycin) and lincosamide (such as clindamycin) drugs, as these drugs have the same antimicrobial activity. The resistance is via three related genes, *ermA*, *ermB*, and *ermC*, which produce methylases that alter the targeted ribosome by methylating it. This leads to a reduced ability of the drug to bind to the ribosome and a greatly reduced antimicrobial effect[12]. As of 1999, resistance to erythromycin was at nearly 93%, and resistance to clindamycin was at over 79%[8].

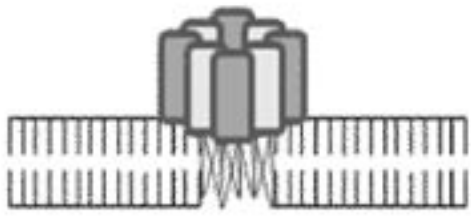
The latest antimicrobial that threatens to fall to resistance is vancomycin. This is the drug that doctors thought they could count on to treat infections with multidrug-resistant MRSA. Unfortunately, there have now been at least six confirmed cases of vancomycin-resistant *Staphylococcus aureus* (VRSA) infections in the US.[13]. Vancomycin is a glycopeptide that interferes with bacterial cell wall synthesis by binding to peptidoglycan subunit precursors and preventing their incorporation into the cell wall. The resistance problem is really two separate issues. Besides VRSA strains, there are MRSA that possess intermediate resistance to vancomycin (VISA). The resistance mechanisms are not the same. The VRSA resistance is mediated via the apparent acquisition of the *vanA* gene from vancomycin-resistant enterococci (VRE). This gene allows synthesis of modified peptidoglycan precursors that have decreased affinity (1000-fold) for vancomycin. In VISA, resistance is apparently mediated through genetic mutations (not defined as yet) that result in production of a much thicker cell wall that makes it very difficult for vancomycin to enter the cell[14].

Surprisingly, despite the huge problem of antimicrobial resistance, MRSA strains do not carry any special or added virulence factors beyond those already found in *S. aureus* strains, which naturally possess a lot of virulence factors. When the infections were easily treated, the virulence factors didn't pose so grave a threat. Now, with resistance becoming common, these natural virulence factors are able to cause considerable health issues. Table 1 lists the virulence factors of *S. aureus*. Not all strains possess all of these factors, but the surface proteins, especially, are expressed by the majority of strains.

Figure 3. Panton-valentine leukocidin

A. PVL Octamer top view

B. PVL forming a pore in a cell membrane



Interestingly, there is one major difference between virulence factors in CA-MRSA and HA-MRSA strains. CA-MRSA strains are the most likely to produce the Panton-Valentine leukocidin (PVL); furthermore, most CA-MRSA strains have that ability. One study of PVL prevalence showed that while 85% of strains of CA-MRSA that caused pneumonia produced PVL, 0% of pneumonia-causing HA-MRSA strains did[15]. The main CA-MRSA strain in the US, USA300, produces PVL, and is associated with necrotizing pneumonia, necrotizing fasciitis and highly virulent infections of skin and soft tissue. The genes that encode PVL are *lukF-PV* and *lukS-PV*. Each of these genes produces one of the two subunit types of PVL, LukF-PV and LukS-PV. The active PVL toxin is an octamer made up of four each, alternating, F and S subunits, arranged in ring-form (Fig. 3).

The subunits are produced and secreted singly from the bacterial cell. The subunits then assemble into the active ring-form on the membrane of a polymorphonuclear leukocyte (PMN) or macrophage. The assembled toxin produces a pore in the cell membrane, which leads to apoptosis[16]. The LukS-PV subunits bind to specific cell membrane receptors first. This causes a conformational change in the LukS-PV subunit. That change allows the LukF-PV subunits to bind to the membrane-bound LukS-PV subunits, to form the active toxin[17].

The very real danger of MRSA becomes apparent when you consider the fact that all *S. aureus* strains possess multiple virulence factors. Further, not only is there resistance to ,-lactams in all the MRSA strains, but also many HA-MRSA strains are resistant to multiple drugs. Further still, there are the CA-MRSA strains with the PVL toxin. CA-MRSA strains are now being transmitted nosocomially as well as the HA-MRSA strains. This all adds up to a major healthcare issue. Now throw in the threat of impending vancomycin-resistance. Pharmaceutical researchers are facing a challenge that they are in danger of losing. If it takes on average 10-15 years to get a new antimicrobial agent approved for use, how are we going to face the MRSA crisis? Unless proactive steps are taken, it is likely that VRSA will be as common in 10-15 years as MRSA is now.

Table 1. Virulence Factors of *Staphylococcus aureus*

Surface Proteins that Promote Colonization

- Clumping factor (bound coagulase)—binds to and directly converts fibrinogen to fibrin
- Collagen binding protein
- Fibronectin binding protein
- PNSG—capsular polysaccharide adhesin
- Protein A—binds to the Fc domain of IgG antibodies to prevent 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
- γ-toxin—wide spectrum of cytolytic activity
- γ-toxin—wide spectrum of cytolytic activity
- Panton-Valentine Leukocidin—membrane pore-forming
- Exfoliate toxins—ETA and ETB—cause epidermis sloughing
- Staphylococcal enterotoxins (SE-A, -B, -C1, -C2, -C3, -D, -E, -G, -H, -I)—gastrointestinal
- Toxic shock syndrome toxin (TSST-1)—causes leakage of endothelial cells
- Free coagulase—reacts with thrombin-like molecule, coagulase-reactive factor, to indirectly convert fibrinogen to fibrin
- Deoxyribonuclease—hydrolyzes DNA
- Hyaluronidase—hydrolyzes connective tissue
- Lipases—hydrolyze lipids
- Staphylokinase—lyses fibrin

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