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Review Article

Understanding Methicillin Resistant *Staphylococcus aureus* Infection: The Cell Walls Perspective

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Abstract

Methicillin-resistant *Staphylococcus aureus* MRSA is any strain of *Staphylococcus aureus* that has acquired *mecA*, the gene that encodes a mutant form of the transpeptidase, penicillin-binding protein 2a (PBP2a). This mutant form PBP2a is unable to interact with β -lactam moieties. This enables *Staphylococcus aureus* cell wall synthesis in the presence of the β -lactam antibiotics. The detection of *mecA* gene can be directly using molecular methods or by phenotypic test for the presence of the *mecA* gene in *Staphylococcus aureus* using antibiotics disk diffusion test for cefoxitin. This involves incubating a lawn of the test isolate on Mueller Hinton agar +2% sodium chloride under standardized conditions with a cefoxitin disk (30 μ g). A zone size <22 mm indicates that the *mecA* gene is present and the isolate is reported as MRSA. Vancomycin, linezolid, quiupristin/dalfopristin, daptomycin and tigecycline are drugs now used to treat MRSA infections with care to avoid developing resistance. Acquiring such a resistance strain of *Staphylococcus aureus* is prevalent in hospitals, prisons, and nursing homes, where patients with open wounds, invasive devices and weakened immune system are at greater risk of nosocomial infection than the general public. Testing patients for MRSA upon admission, isolating MRSA-positive patients, decolonization of MRSA-positive patients, and terminal cleaning of patients' rooms and all other clinical areas they occupy is the current best practice protocol for nosocomial MRSA.

Introduction

Methicillin-resistant *Staphylococcus aureus* MRSA is any strain of *Staphylococcus aureus* that has developed resistance to beta-lactam antibiotics. Such resistance makes MRSA infection more difficult to treat with all antibiotics agents that contain beta lactam in their molecular structures, characterized by their four- membered Nitrogen ring (Figure 1); that is the penicillins (its derivatives methicillin, dicloxacillin, nafcilline, oxacillin, etc.) and cephalosporins.



Beta lactam antibiotics

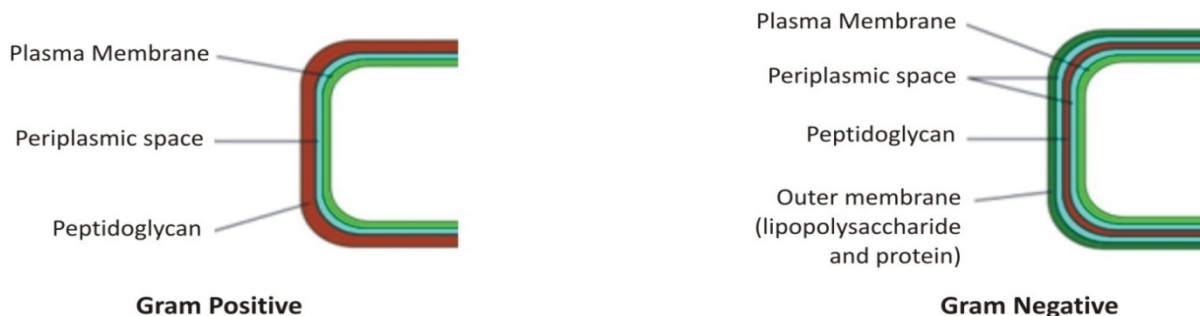
Figure 1: Beta Lactam Antibiotics.

Acquiring such a resistance strain of *Staphylococcus aureus* is prevalent in hospitals, prisons, and nursing homes, where patients with open wounds, invasive devices, and weakened immune systems are at greater risk of nosocomial infection than the general public. Though MRSA began as a hospital-acquired infection, it has developed limited endemic status and is now sometimes community-acquired. Hence the terms HA-MRSA (healthcare-associated MRSA) and CA-MRSA (community-associated MRSA) reflect this distinction.

Beta Lactam Mode of Action

Cell Wall Structure and Synthesis

Bacteria are classified as Gram positive or negative on the basis of the cell wall staining pattern with crystalline violet after counter staining with safranin or fushine. The Gram-positive bacteria have a thicker cell wall which lies over the plasma cell membrane, while in Gram-negative bacteria the thinner cell wall is sandwiched between an inner cytoplasmic cell membrane and a bacterial outer membrane (Figure 2).



The Bacterial cell envelope

Figure 2: The bacterial cell envelope.

Peptidoglycan which is also known as murein is the building block of the cell wall. It is a polymer consisting of sugars and amino acids. The sugar component consists of alternating residues of β -(1,4) linked N-acetylglucosamine and N-acetylmuramic acid. Attached to the N-acetylmuramic acid is a peptide chain of three to five amino acids. The peptide chain is cross-linked to the peptide chain of another strand forming the mesh-like cell wall layer (Figure 3).

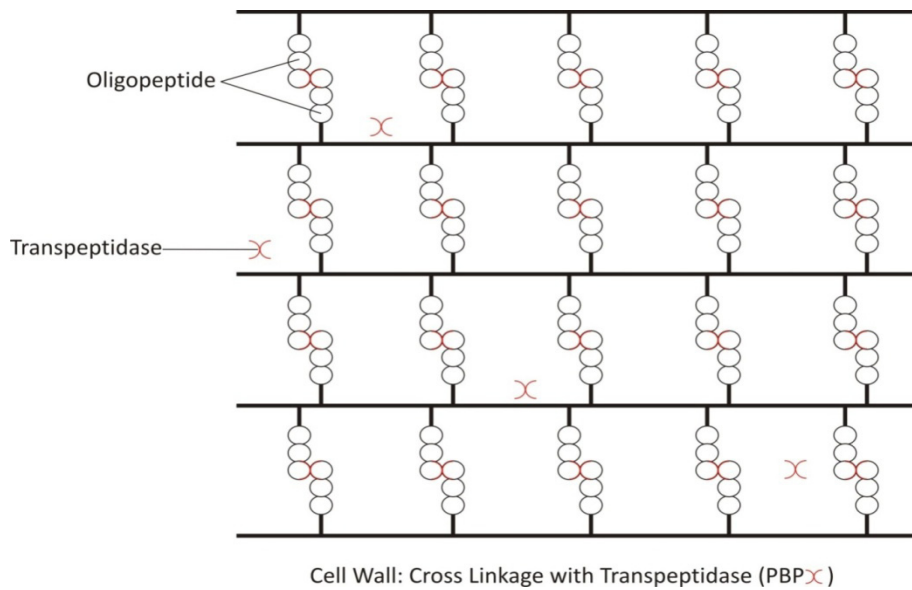


Figure 3: Cell Wall Cross Linkage with Transpeptidase.

The protein transpeptidase is the enzyme that enables this cross-linking (Hogan 2010), (Figure 4).

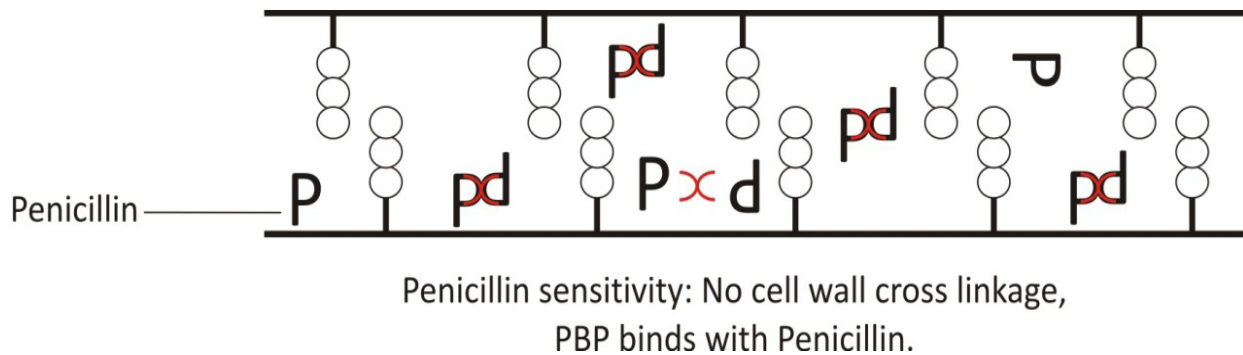


Figure 4: Penicillin Sensitivity.

Beta Lactam Sensitivity

Transpeptidase can also bind to the antibiotics penicillin hence it is called “penicillin- binding protein.” In beta lactam sensitivity, the β -lactam antibiotics bind to the transpeptidase, penicillin binding to transpeptidase will interfere with the synthesis of cell wall.

The cell wall is required for bacterial survival and multiplication for it serves to maintain the structural strength of the cell, counter the osmotic pressure of the cytoplasm and binary fission [1].

Methicillin Resistance

In the antibiotics resistance, MRSA expresses a PBP that will not allow the antibiotics into their active site. There is acquisition of *mecA* the gene that encodes a mutant form of the transpeptidase, called penicillin-binding protein 2a (PBP2a). As a consequence, there is inability to interact with β -lactam moieties [2]. As such this mutant transpeptidase can continue to catalyze the transpeptidation reaction required for peptidoglycan cross-linking, enabling cell wall synthesis in the presence of the β -lactam antibiotics [3], (Figure 5).

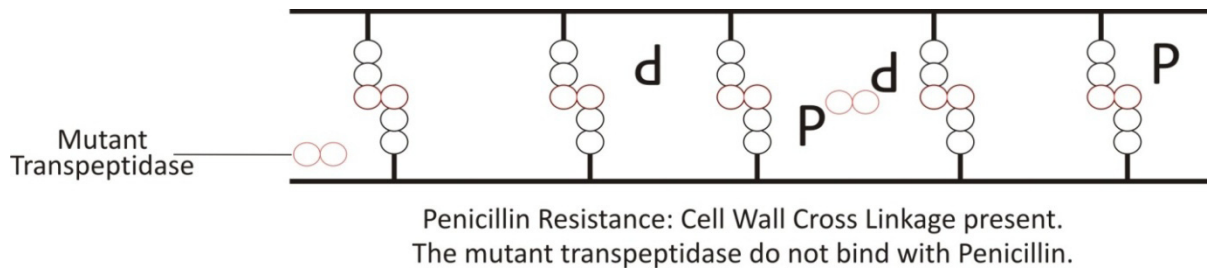


Figure 5: Penicillin Resistance.

Signs and Symptoms

Some CA-MRSA strains display enhanced virulence, spreading more rapidly and causing illness much more severe than traditional HA-MRSA infections.

About 75 percent of community-associated (CA-) MRSA infections are localized to skin and soft tissue [4]. The most common manifestations of CA-MRSA are simple skin infections, such as impetigo, boils, abscesses, folliculitis, and cellulitis. Rarer, but more serious, manifestations can occur, such as necrotizing fasciitis and pyomyositis (most commonly found in the tropics), necrotizing pneumonia, infective endocarditis (which affects the valves of the heart), and bone and joint infections [5]. Before the spread of MRSA into the community, abscesses were not considered contagious, because infection was assumed to require violation of skin integrity and the introduction of staphylococci from normal skin colonization. However, newly emerging CA-MRSA is transmissible (similar, but with very important differences) from HA-MRSA.

Diagnosis

The detection of *mecA* gene can be directly using molecular methods or by phenotypic test for the presence of the *mecA* gene in *Staphylococcus aureus* using antibiotics disk diffusion test.

Phenotypic Test for the Presence of the *mecA* Gene

Primary Culture

The diagnosis of MRSA is by culture of the bacteria from an infected area. This could be an area of the skin with pus, abscesses, blisters or blood in patients with sepsis or pneumonia. The culture medium in nutrient agar plates and *Staphylococcus aureus* is identified as a positive coagulase test.

Sensitivity

After *Staphylococcus aureus* bacteria are isolated, the bacteria are then cultured in the presence of methicillin (and usually other antibiotics). *Staphylococcus aureus* grows in the presence of methicillin, the bacteria are termed MRSA. Accurate detection of resistance can be difficult due to the presence of two subpopulations (one susceptible and the other resistant) that may coexist within a culture of staphylococci [6]. All cells in a culture may carry the genetic information for resistance, but only a small number may express the resistance *in vitro*. This phenomenon is termed heteroresistance.

Cells expressing heteroresistance grow more slowly than the susceptible population and may be missed at temperatures above 35°C [7]. Recommends incubating isolate being tested against antibiotics at 33-35°C (maximum of 35°C) for a full 24 hours before reading. When resistance was first described in 1961, methicillin was used to test and treat infections caused by *S. aureus*. However, oxacillin, which is in the same class of drugs as methicillin, was chosen as the agent of choice for testing staphylococci in the early 1990s because oxacillin maintains its activity during storage better than methicillin and is more likely to detect heteroresistant strains.

Oxacillin sensitivity test is with a plate containing 6 µg/ml of oxacillin in Mueller-Hinton agar supplemented with 4% NaCl. MRSA sensitivity was later modified to cefoxitin since cefoxitin is a more potent inducer of *mecA* expression, and the test results are relatively easy to interpret. However, the acronym MRSA is still used by many to describe these isolates because of its historic role.

Cefoxitin test involves incubating a lawn of the test isolate on Mueller Hinton agar +2% sodium chloride under standardized conditions with a cefoxitin disk (30 mcg). A zone of growth inhibition around the cefoxitin disk of ≥ 22 mm rules out MRSA; a zone size < 22 mm indicates that the *mecA* gene is present and the isolate should be reported as MRSA [8]. (Plate 1)

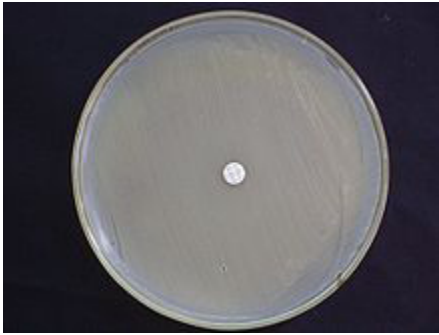


Plate 1: Mueller Hinton agar showing MRSA resistant to oxacillin disk.

In addition, there are chromogenic agars that can be used for MRSA detection. This is a Rapid culture which contains media substrates that change color in the presence of *Staphylococcus aureus*; selectivity for MRSA is achieved by incorporation of antibiotics into the agar. Use of such agar allows identification of MRSA from primary isolation plates within 24 to 48 hours, obviating the need for additional subcultures or biochemical tests [9]. (Plate 2).

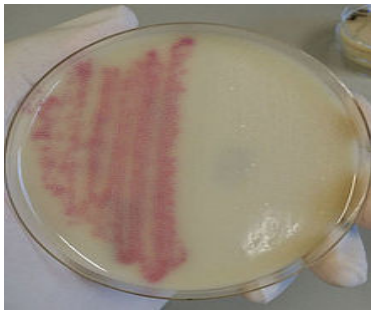


Plate 2: A selective and differential chromogenic medium for the qualitative direct detection of MRSA. Molecular methods.

The latex agglutination test for PBP2a, or Nucleic acid amplification tests, such as the polymerase chain reaction (PCR), can be used to detect the *mecA* gene.

Management Treatment

Antibiotics

Both CA-MRSA and HA-MRSA are resistant to traditional anti-staphylococcal beta lactam antibiotics. CA-MRSA has a greater spectrum of antimicrobial susceptibility, including to sulfa drugs (like co-trimoxazole/trimethoprim-sulfamethoxazole), tetracyclines (like doxycycline and minocycline) and clindamycin, but the drug of choice for treating CA-MRSA is vancomycin. HA-MRSA is resistant even to these antibiotics and often is susceptible only to vancomycin. Vancomycin is a glycopeptide antibiotics its oral absorption is very low; hence it must be administered intravenously to control systemic infections [10].

Several newly discovered strains of MRSA show antibiotic resistance even to vancomycin. These new evolutions of the MRSA bacterium have been dubbed Vancomycine intermediate- resistant *Staphylococcus aureus* (VISA) Sieradzki and Tomasz (1997) and Schito (2006).

Linezolid, quinupristin/dalfopristin, daptomycin, and tigecycline are used to treat more severe infections that do not respond to vancomycin [11]. Current guidelines recommend daptomycin for VISA bloodstream infections and endocarditis [12].

A new class of non-B-lactam antibiotics, oxadiazoles, was reported to be effective against MRSA infection in mouse models. The mechanisms of oxadiazoles' antibacterial effect are the inhibition of the penicillin binding protein, PBP2a and biosynthesis of the bacterial cell wall. It was found to have bactericidal activity against vancomycin- and linezolid-resistant MRSA and other Gram-positive bacterial strains [13].

Phage Therapy

An entirely different approach is phage therapy. Bacteriophages are much more specific than antibiotics, so they can hypothetically be chosen to be indirectly harmless not only to the host organism (human, animal, or plant), but also to other beneficial bacteria, such as gut flora, reducing the chances of opportunistic infections [14].

They would have a high therapeutic index, that is, phage therapy would be expected to give rise to few side effects. Because phages replicate *in vivo*, a smaller effective dose can be used. On the other hand, this specificity is also a disadvantage: a phage will only kill a bacterium if it is a match to the specific strain. Consequently, phage mixtures are often applied to improve the chances of success, or samples can be taken and an appropriate phage identified and grown. Experimental phage therapy tested in mice had a reported efficacy against up to 95% of tested *Staphylococcus* isolates [15].

Natural Products

Some *in vitro* studies with honey have identified components in honey that kill MRSA [16].

Some semi-toxic fungi/mushrooms excrete broad spectrum antibiotics shown to inhibit the growth of *Staphylococcus aureus* [17].

An *in vitro* study showed that the cannabinoids (components of *Cannabis sativa*), including cannabidiol (CBD), cannabinol (CBN), cannabichromene (CBC), tetrahydrocannabinol (THC) cannabigerol (CBG) and terpenoid pinene show activity against a variety of MRSA strains [18].

In vitro studies have shown that oakin, an oak extract, can kill MRSA [19]. Studies suggest that allicin, a compound found in garlic, may prove to be effective in the treatment of MRSA [20].

It has been reported that maggot therapy to clean out necrotic tissue of MRSA infection has been successful. Studies in diabetic patients reported significantly shorter treatment times than those achieved with standard treatments [21].

Risk Factors and Prevention

Hospital Patients

Healthcare provider-to-patient transfer is common, especially when healthcare providers move from patient to patient without performing necessary hand-washing techniques between patients [22,23]. An effective strategy is hands washing with running water and an anti-microbial cleanser with persistent killing action, such as Chlorhexidine, p-chloro-m-xyleneol, hexachlorophene, and povidone-iodine [24]. Used paper hospital gowns are associated with MRSA hospital infections, which could be avoided by proper disposal.

Exclusion from work for those with wound drainage that cannot be covered and contained with a clean, dry bandage and for those who cannot maintain good hygiene practices. Workers with active infections should be excluded from activities where skin-to-skin contact is likely to occur until their infections are healed [25]. glycopeptides, cephalosporins and in particular quinolones are associated with an increased risk of colonisation of MRSA. Reducing use of antibiotic classes that promote MRSA colonisation, especially fluoroquinolones, is recommended [22,23].

Staphylococcus aureus most commonly colonizes under the anterior nares the nostrils [26], and swab screening patients admitted to hospitals has been found to be effective in minimizing the spread of MRSA in hospitals [26,27]. Patient screening upon hospital admission, with nasal cultures, prevents the cohabitation of MRSA carriers with non-carriers, and exposure to infected surfaces.

In healthcare environments, MRSA can survive on surfaces and fabrics, including privacy curtains or garments worn by care providers. Complete surface sanitation is necessary to eliminate MRSA in areas where patients are recovering from invasive procedures. Alcohol is used as an effective surface sanitizer against MRSA; and Quaternary ammonium is used in conjunction to extend the longevity of the sanitizing action.

Comparisons have been made on the antimicrobial efficacies of copper and several non-copper proprietary coating products to kill MRSA [28]. At 20°C, the drop-off in MRSA organisms on copper alloy C11000 is dramatic and almost complete (over 99.9% kill rate) within 75 minutes. However, neither a triclosan-based product nor two silver-containing based antimicrobial treatments (Ag-A and Ag-B) exhibited any meaningful efficacy against MRSA. Faster antimicrobial efficacies were associated with higher copper alloy content. Stainless steel did not exhibit any bactericidal benefits.

Prison Inmates, Military Recruits, and the Homeless

Prisons, military barracks, and homeless shelters can be crowded, and confined, and poor hygiene practices may proliferate, thus putting inhabitants at increased risk of contracting MRSA [29].

Athletes

Locker rooms, gyms, and related athletic facilities offer potential sites for MRSA contamination and infection [30]. A study linked MRSA to the abrasions caused by artificial turf [31]. Three studies by the Texas State Department of Health found the infection rate among football players was 16 times the national average.

Livestock

A new variant of MRSA has emerged in animals and is found in intensively reared production animals (primarily pigs, but also cattle and poultry), where it can be transmitted to humans. Though dangerous to humans, CC398 is often asymptomatic in food-producing animals [32]. In a single study [33], MRSA was shown to originate in livestock and spread to humans, though the MRSA strain may have originated in humans and was transmitted to livestock [34].

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