GLOBAL JOURNAL OF BIOLOGY, AGRICULTURE & HEALTH SCIENCES
(Published By: Global Institute for Research & Education)
www.gifre.org

MECHANISM, DETECTION AND PREVALENCE OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* IN SAUDI ARABIA

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Abstract

Methicillin-resistant strains of *Staphylococci* were identified immediately upon the introduction of methicillin into clinical practice. Resistance was termed "intrinsic" because it was not due to destruction of the antibiotic by β-lactamase. Further studies revealed that methicillin resistance requires the presence of the *mec* gene. The *mec* gene is absent from susceptible strains and present in all resistant strains. Mec gene, encodes the penicillin binding protein 2a (PBP2A) that establishes resistance to methicillin and other semisynthetic penicillinase-resistant beta-lactams. Penicillin-binding proteins are peptidase enzymes located in the bacterial membrane that catalyze the transpeptidation reactions of peptidoglycan during cell wall synthesis. The most accurate methods to detect methicillin-resistant *S. aureus* (MRSA) are polymerase chain reaction (PCR), for detection of the *mecA* gene and latex agglutination tests for the protein product of *mecA*, penicillin binding protein 2a. Cultures are also important tool for surveillance from body sites (mostly the anterior nares) that are frequently colonized with MRSA. The majority of patients with asymptomatic MRSA colonization will be detected by screening culture from the anterior nares. Traditional methods used to process surveillance cultures take 48 to 72 hours to yield results. However, newly available techniques shorten the amount of time required to detect MRSA in surveillance cultures. A chromogenic selective agar containing cefoxitin detects a majority of MRSA isolates within 24 hours. Reports of MRSA infections are increasing worldwide, In Saudi Arabia, many institutions have reported an increase in the incidence of MRSA in recent years; other institutions have reported a variation in incidence. In one study from Saudi Arabia, MRSA, has been detected as high as 55.3%, whereas earlier studies conducted in the Jeddah hospitals showed a lower prevalence, with only minor variation between 6.5% and 8.9%.

Key words: Methicillin-resistant strains of *Staphylococci* (MRSA); β-lactamase; *mec* gene; Penicillin-binding proteins; Saudi Arabia.

1. Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) is an important cause of nosocomial infections worldwide. However, the epidemiology of MRSA is changing as the isolation of MRSA is no longer limited to hospitalized patients or persons with predisposing risk factors (Gorak *et al*., 1999). Outbreaks of community-associated MRSA (CA-MRSA) have been reported worldwide in diverse community populations (Kluytmans-Vandenbergh and Kluytmans, 2006). CA-MRSA strains are now recognized as distinct clonal entities that differ from traditional hospital acquired MRSA (HA-MRSA) strains. By definition, both CA-MRSA and HA-MRSA are resistant to methicillin (and all beta-lactam antibiotics), but important differences exist in epidemiology, microbiologic characteristics, clinical aspects of infection, and management strategies between the two.

Methicillin was introduced in 1959 to treat infections caused by penicillin-resistant *Staphylococcus aureus.* In 1961 the first *S. aureus* isolates that had acquired resistance to methicillin (methicillin-resistant *S. aureus*, MRSA) were reported from the United Kingdom (Jevons, 1961) and were soon recovered from other European countries, and later from Japan, Australia, and the United States. MRSA is a major pathogen in hospitals worldwide and has become gradually more difficult to treat due to increasing resistance (Hussain *et al*., 2000; Centers for Disease Control and Prevention Morbid. Mortal, 1999). There is a wide range in the prevalence of MRSA strains between different countries and even between hospitals in the same country. The extent of the spread of these organisms from hospital to hospital also shows variation (Struelens *et al*., 1994). The aim of this study was to determine the prevalence of MRSA strains isolated from nosocomial infections in Saudi Arabia.

2. Molecular Mechanism of Methicillin Resistance

Methicillin resistance requires the presence of the *mec* gene; strains lacking a *mec* gene are not methicillin resistant. Methicillin resistance is defined in the clinical microbiology laboratory as an oxacillin minimum inhibitory concentration (MIC) ≥4 mcg/mL (Benner and Kayser, 1968). Other methods of detection, such as the use of the cefoxitin disk or one of several polymerase chain reactions (PCRs) to detect the *mec* gene, are also used. Isolates resistant to oxacillin or methicillin are also resistant to all beta-lactam agents, including cephalosporins (with the exception of ceftaroline, a fifth-generation cephalosporin). MICs of 4 to 8 mcg/mL are considered to represent borderline or low level resistance.

2.1 mec gene

The presence of the *mec* gene is an absolute requirement for *S. aureus* to express methicillin resistance. The *mec* gene is absent from susceptible strains and present in all resistant strains (Crisóstomo *et al*., 2001; Enright *et*

al., 2002). The structural component of the *mec* gene, *mecA*, encodes the penicillin binding protein 2a (PBP2A) that establishes resistance to methicillin and other semisynthetic penicillinase-resistant beta-lactams.

The mechanism of oxacillin resistance may be different in borderline-resistant strains, in which the *mec* gene is not present or is present in a very small resistant subpopulation.

The complete genomes for several strains of methicillin-resistant *S. aureus* (MRSA) have been published; three classes of pathogenicity islands and diverse possible super antigens have been identified (Robinson *et al*., 2005; Li *et al*., 2009; Francois *et al*., 2007). The *mec* gene consists of a structural component, *mecA*, and, in many cases, two regulatory components that control expression of the gene:

- *mecR1-mecI* is a negative regulator of *mecA* transcription. Mutations in this set of genes results in more highly resistant strains.
- The beta-lactamase genes (*blaI*, *blaRI*, and *blaZ*) control expression of beta-lactamase and, because of sequence similarity to the *mecR1-mecI* genes, also can downregulate *mecA* gene transcription (Clinical and Laboratory Standards Institute, 2006). However, beta-lactamase produces resistance by a mechanism different from *mec*; it hydrolyzes the beta-lactam ring. Because this negative regulation is not tightly controlled, expression of resistance following exposure to beta-lactams is relatively rapid. The looser control allows the *mec* gene to synthesize protein, which, under conditions of greater regulatory control, would not be permitted.

In addition to these regulatory genes, there is a series of five auxiliary genes that can modify expression of methicillin resistance. These are the *fem* (factor essential for the expression of methicillin resistance) A to E genes.

2.2 Penicillin-binding protein 2a

Penicillin-binding proteins are peptidase enzymes located in the bacterial membrane that catalyze the transpeptidation reactions of peptidoglycan during cell wall synthesis. *MecA* encodes penicillin binding protein (PBP) 2a, an inducible protein that establishes resistance to the semisynthetic penicillinase resistant beta-lactams: methicillin, nafcillin, oxacillin, and all cephalosporins (Inglis *et al*., 1988; Tesch *et al*., 1988). In contrast to the other four PBPs (1-4), PBP2a has a low affinity for beta-lactam antibiotics.

In susceptible Staphylococcal isolates, the beta-lactams covalently bind to PBPs 1-3, thereby inactivating enzyme activity, preventing transpeptidation, and ultimately contributing to bacterial death. PBP2a, with its low affinity for the beta-lactams, can substitute for the enzymatic activity of these PBPs and allow completion of cell wall assembly. The resulting peptidoglycan has a structurally different muropeptide composition, a change that does not appear to affect cellular function (Kuroda *et al*., 2001).

2.3 Staphylococcal Chromosomal Cassette mec (SCCmec)

The *mec* gene is part of a 21 to 67 kb mobile chromosomal element called the Staphylococcal cassette chromosome (SCC*mec*). The majority of healthcare-associated (mostly nosocomial) MRSA clones is associated with SCC*mec* types I, II, and III and are multidrug resistant (Baba *et al*., 2002). In contrast, most community-associated MRSA (CA-MRSA) strains have type IV or V SCC*mec* and were formerly susceptible to other antibiotic families; this is no longer the case (Li *et al*., 2009; Diep *et al*., 2006; Zhang *et al*., 2001; Hartman and Tomasz, 1984; Utsui and Yokota, 1985; de Jonge and Tomasz, 1993).

Methicillin resistance may reduce the virulence of healthcare-associated MRSA by interfering with agr quorum sensing (Michel and Gutmann, 1997). In one study, healthcare-associated MRSA strains carrying SCC*mec* type II produced reduced amounts of cytolytic toxins as measured by an *in vitro* T cell survival assay and *in vivo* murine bacteremia model. Alteration of the cell wall appeared to affect the agr quorum sensing system, resulting in diminished virulence. This effect has not been observed among community-associated MRSA isolates and may help explain the failure of hospital-acquired MRSA (HA-MRSA) to spread into the community.

Sequencing SCC*mec* from many MRSA strains has resulted in identification of 11 different SCC*mec* types (I-XI) that vary in genetic makeup and size (Daum *et al*., 2002; Ma *et al*., 2002; Naimi *et al*., 2003). Transfer of SCC*mec* from MRSA into well-adapted strains of methicillin-susceptible *S. aureus* (MSSA) has occurred on a number of occasions, resulting in new MRSA isolates that spread rapidly in healthcare institutions. In 2011, a novel *mecA* homologue was identified in isolates obtained from both cows and humans (Salmenlinna *et al*., 2002). This paper (as well as others) identified livestock as a reservoir of MRSA with the potential for transmission to humans (Sattler *et al*., 2002).

2.4 Origin in Coagulase-negative Staphylococci

It is believed that the *mec* gene was acquired from these closely related Staphylococcal species via a limited number of genetic events. The *mec* gene is essentially the same in all Staphylococcal species. Several studies point to coagulase-negative *Staphylococci* (CoNS) as the origin of methicillin resistance in *Staphylococcus aureus*. Segments of DNA from an insertion sequence found in CoNS (IS 1272) have been identified in MRSA (Rudkin *et al*., 2012; Oliveira *et al*., 2001). Insertion sequence elements are DNA segments that encode enzymes that allow for site-specific recombination. In addition, one study reported 88 percent amino acid homology for the MRSA *mec* gene in *S. sciuri*, another species of CoNS (Ito *et al*., 2004). The presence of different insertion sequence fragments within the *mec* gene makes transposition a likely mechanism of transfer for the gene between species (SCCmec, 2014).

Sequencing of the MRSA strain USA300, an epidemic clone of community-acquired MRSA, has shown that additional virulence and resistance genes have also been acquired from CoNS (Francois *et al*., 2007). These genes include molecular variants of enterotoxin Q and K and a mobile element (the arginine catabolic mobile element, ACME) that encodes an arginine deaminase pathway and an oligopeptide permease system. The authors hypothesize these genes enable the strain to evade host immune responses and contribute to its ability to survive and spread in host tissue. The virulence of USA300 appears to be linked to the differential expression of selected virulence determinants that were already present in the progenitor strain (USA500) of USA300 (Centers for Disease Control and Prevention Morbid. Mortal, 1999).

2.5 Expression of Methicillin Resistance

Despite the presence in methicillin-resistant *S. aureus* (MRSA) of the *mec* gene, the phenotypic expression of methicillin resistance varies. There are three different forms for the expression of methicillin resistance: homogeneous, heterogeneous, and borderline. Most clinical isolates of MRSA from a given patient are heterogeneous in their expression of methicillin resistance.

As an example, under routine growth conditions (37ºC, unsupplemented media), ≥99.9 percent of MRSA appear to be susceptible to the beta-lactams (e.g. oxacillin 4mcg/mL). However, if the cells are grown at 30 to 35ºC or in the presence of 6.5 percent sodium chloride, they become more homogeneously resistant and express beta-lactam resistance at a much higher frequency (SCCmec, 2014). In addition, growth of heterogeneous strains in the presence of a beta-lactam results in the selection of a homogeneous phenotype. Serial passage of these cells in the absence of antibiotic leads to slow reversion back to the heterogeneous state.

A similar phenomenon has been observed in experimental endocarditis. Treatment of rabbits infected with MRSA with a beta-lactam results in a greater percentage of the total bacterial population being resistant than in untreated controls (García-Álvarez *et al*., 2011). There was also a correlation between the potential efficacy of the antibiotic and its affinity for binding to PBP2a *in vitro.*

2.6 fem genes

The *fem* genes and other genes are necessary for the homogeneous expression of resistance (van Cleef *et al*., 2011). These auxiliary genes affect different steps in the synthesis of peptidoglycan. Inactivation of these genes can convert a homogeneously resistant strain to a heterogeneous resistant one (Archer and Niemeyer, 1994).

2.7 Borderline Resistance

Borderline resistance refers to isolates that are at the margin of resistance with a minimum inhibitory concentration (MIC) to methicillin of 4 to 8mcg/mL. This type of resistance may be due to one of several mechanisms.

- Some strains with borderline resistance possess the *mecA* gene. In these strains, the resistant subpopulation may be extremely small and therefore more susceptible to beta-lactams.
- In strains that lack the *mecA* gene (and therefore PBP2a), there may be alterations in or overexpression of the other PBPs, resulting in reduced affinity for beta-lactams or the availability of more enzyme for peptidoglycan synthesis. Overproduction of beta-lactamase with slow hydrolysis of the beta-lactam antibiotic has also been hypothesized as a potential mechanism for borderline resistance in *mecA*-negative strains (SCCmec, 2014, Archer *et al*., 1994; Chambers, 1997).

3. Laboratory Detection

The most accurate methods to detect methicillin-resistant *S. aureus* (MRSA) are polymerase chain reaction (PCR) for detection of the *mecA* gene and latex agglutination tests for the protein product of *mecA*, penicillin binding protein 2a (Chambers *et al*., 1990; Harbarth *et al*., 2006; Miller *et al*., 2005; Sakoulas *et al*., 2001). When these tests are not available, traditional microbiology laboratory techniques are acceptable, such as oxacillin-salt agar screening plates and cefoxitin disk diffusion tests.

4. Culture

Surveillance cultures are performed at body sites (mostly the anterior nares) that are frequently colonized with MRSA. The majority of patients with asymptomatic MRSA colonization will be detected by screening culture from the anterior nares (sensitivity 73 to 93 percent) (Sanford *et al*., 1994; Eveillard *et al*., 2006; Huang *et al*., 2007). The sensitivity can be increased by also screening open lesions, such as surgical wounds, pressure (decubitus) ulcers, and areas of skin breakdown.

In patients without open lesions, a one-year study of multisite screening (nares, rectum, and axilla) found that nares culture alone missed 27 percent of MRSA carriers (Eveillard *et al*., 2006). Throat swabs may also be helpful. In a screening study of almost 3000 individuals for *S. aureus* carriage, 37 percent had nasal carriage and 13 percent were colonized only in the throat, increasing the sensitivity of screening by 26 percent (Mertz *et al*., 2007). A similar increase in yield with throat swabs (22 percent) was seen in the small subset of MRSA carriers.

Traditional methods used to process surveillance cultures take 48 to 72 hours to yield results. However, newly available techniques shorten the amount of time required to detect MRSA in surveillance cultures. A chromogenic selective agar containing cefoxitin detects a majority of MRSA isolates within 24 hours, while commercially available real-time PCR tests for *mecA* can detect MRSA within two hours (Chambers *et al*., 1990).

5. Antimicrobial Susceptibility Testing

In the past, most healthcare-associated MRSA strains were multidrug resistant. Isolates that are resistant to oxacillin but remain susceptible to most non-beta-lactam agents (e.g. trimethoprim-sulfamethoxazole, clindamycin, and ciprofloxacin) are usually community-associated MRSA. Such strains should be tested using a confirmatory test such as a *mecA* probe, PCR assay for *mecA*, oxacillin-salt agar screening plates, or cefoxitin disk diffusion tests.

Antibiotic susceptibility testing for methicillin resistance has been modified to enhance the detection of these isolates, a large number of which are heterogeneously resistant to methicillin. Susceptibility testing now includes use of the more stable oxacillin rather than methicillin disk, incubation at ≤35ºC for 24 rather than 18 hours, and the incorporation of 6.5 percent sodium chloride into the media. It is important to remember that isolates resistant to oxacillin are also resistant to all beta-lactam agents including cephalosporins.

Specific recommendations exist for the different methods of susceptibility testing such as automated turbidometric or disk diffusion assays (National Committee for Clinical Laboratory Standards, 1998). In the future, it is likely that clinical isolates will be screened for methicillin resistance by PCR or with probes specific for segments of the *mec* gene. Kits for the detection of the *mec* gene are becoming more common. Alternative approaches include screening with cefoxitin disks and the MRSA latex agglutination test (Felten *et al*., 2002).

6. Prevalence of methicillin-resistant *Staphylococcus aureus* in Saudi Arabia

Between 1990 and 1996, the National Nosocomial Surveillance System (USA) identified *S. aureus* as the most common etiological agent of nosocomial infection, principally causing nosocomial pneumonia and surgical wound infections (Rubin *et al*., 1999). Reports of MRSA infections are increasing worldwide. In Saudi Arabia, many institutions have reported an increase in the incidence of MRSA in recent years; other institutions have reported a variation in incidence. In one study (Atif *et al*., 2014) it has been found that 55.3% of *S. aureus* isolated were MRSA, whereas earlier studies conducted in the Jeddah hospitals showed a lower prevalence, with only minor variation between 6.5% and 8.9% at King Khalid National Guard Hospital (Zaman and Dibb, 1994; Al-Anazi, 2009). MRSA was also most frequently isolated from the ICU (39%), followed by the outpatient department (19%) and surgical ward (16%) (Atif *et al*., 2014). In Korea, MRSA from tertiary hospitals accounted for 60–70% of all *S. aureus* isolates (Chong and Lee, 2000) and MRSA was more prevalent in an ICU setting than in non-ICU settings (Sista *et al*., 2004). This is because antibiotic usage is greatest in the ICU settings. In one study (Tyagi *et al*., 2008), it was found a high occurrence of MRSA in surgical wound infections, especially in neurosurgical and orthopaedics patients, a result of overcrowding, high workload, and understaffing of wards. The majority of patients with MRSA were men (60%) (Atif *et al*., 2014). This is in agreement with the finding of (Khanal and Jha, 2010), who explained that men might be more likely to contract community-acquired infections because of their involvement in outdoor activities. Lack of knowledge, comparative over-use of antibiotics without prescription, and failure to complete the prescribed treatment course among the men might have led to more men having MRSA than women. In a study (Atif *et al*., 2014) carried from Saudi Arabia, it was found, 25.7% of patients with MRSA infections were over 60 years old, 15% were 41–50 years old, and 14.1% were children less than 1 year old. This is in agreement with the findings of Madani (Madani, 2001), who reported that 36% of MRSA infections at King Fahad Hospital (KFH) in Jeddah occurred among patients over 60 years old. The studies conducted at Makkha (Atif *et al*., 2014) 27.6% of MRSA infections occurred in the 31–60 age group. Studies from Jeddah (Madani, 2001), reported higher numbers at King Abdulaziz University Hospital (KAUH) and KFH in Jeddah (33% and 46%, respectively). Of the MRSA strains isolated, 41% were from wound and skin swabs, 26% from sputum and tracheal aspirates, and 19% from blood samples (19%). The high rate of MRSA infection in wounds might be due to the increased chance of MRSA infection in deep-seated lesions (Shittu *et al*., 2011). Alanazi (Al-Anazi, 2009), reported that 28% of MRSA isolates were cultured from the skin and tissues. The epidemiology of infections caused by MRSA is rapidly changing. In the past 10 years, MRSA infections have emerged in the community. The MRSA clones in many countries have mostly been associated with skin and soft tissue infections (Deresinski, 2005). The majority of patients with MRSA were Saudi (78.2%), followed by Pakistani (5.8%) and Yemeni (2.9%). Similarly, Madani *et al*. (Deresinski, 2005), found that number of Saudi patients with MRSA was more than two times the number of non-Saudi patients. This is explained by the fact that most patients admitted to the hospitals during the study periods were Saudi men. The study showed that the resistance rates among MRSA isolates were 100%, 100%, and 0.9% for penicillin G, oxacillin, and vancomycin, respectively. Thus, β-lactam antibiotics such as penicillin are ineffective against *S. aureus*. Most clinical MRSA isolates were susceptible to vancomycin, whereas sensitivity to other drugs was poor. This indicates that vancomycin resistance may also spread. Therefore, among carriers, patients, and health care workers, it is important to continue to isolate and identify MRSA so that the effectiveness of newer glycopeptides such as teicoplanin can be monitored regularly. Statistically, an extremely significant correlation between the resistance to oxacillin and the resistance to gentamicin and erythromycin ($p > 0.0001$) was found. It also supports a relationship between oxacillin and aminoglycoside resistance in MRSA (Kim *et al*., 2004). Historically, gentamicin and erythromycin have had wide clinical application; they are inexpensive and available from diverse sources. In Makkah and other parts of Saudi Arabia, they are sold with or without prescription. Misuse and overuse of these antibiotics may have contributed to increasing antibiotics resistance in Saudi Arabia. Therefore, to prevent treatment failures in the absence of data on antibiotic susceptibility, public awareness of the ineffectiveness of gentamicin and erythromycin against MRSA infections is urgently needed. No resistance to linezolid, teicoplanin, tigecycline, and mupirocin was detected in the MRSA isolates in study conducted in Saudi Arabia (Atif *et al*., 2014). The glycopeptides vancomycin and teicoplanin and the oxazolidinone, linezolid have been considered the drugs of choice for the treatment of MRSA infections (Zarakolu *et al*., 2009). Gram positive cocci are rarely resistant to linezolid. A recent study examined the linezolid susceptibility of 1930 MRSA isolates collected from different regions of the United States; 99.9% were susceptible to linezolid (Jones *et al*., 2008). Tigecycline represents an exciting new class of glycylcycline antimicrobial agents for the treatment of multi drug resistant gram-positive bacteria (Squires and Postier, 2006).

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