Journal of Islamic Pharmacy. Online ISSN: 2527-6123
Volume 8 (2) 2023; p78-82
DOI: 10.18860/jip.v8i2.24060

REVIEW ARTICLE

mec Genes as Marker in Methicillin-Resistant *Staphylococcus aureus*

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ABSTRACT

Methicillin-resistant Staphylococcus aureus (MRSA) manifests antibiotic resistance, especially for the β-lactams antibiotic group. MRSA bacteria are a common cause of infection in humans. The antibiotic resistance characteristic comes from the mecA, mecB, and mecC genes in the bacterial chromosome. mecA is the most common gene found in MRSA. Therefore, it is essential to know the role of the mecA gene in antibiotic resistance. This paper searched the literature about MRSA bacteria, the mec gene, and their relationship to cause resistance. The results showed that the mec gene found in MRSA bacteria causes antibiotic resistance in penicillin groups. Methicillin-resistant Staphylococcus aureus (MRSA) manifests antibiotic resistance, especially for the β-lactams antibiotic group. MRSA bacteria are a common cause of infection in humans. The antibiotic resistance characteristic comes from the mecA, mecB, and mecC genes in the bacterial chromosome. mecA is the most common gene found in MRSA. Therefore, it is essential to know the role of the mecA gene in antibiotic resistance. This paper searched literature about MRSA bacteria, the mec gene, and their relationship to cause resistance. The results showed that the mec gene found in MRSA bacteria causes antibiotic resistance in penicillin groups.

Keywords: β-lactam antibiotic, Methicillin-Resistant *Staphylococcus aureus*, *mec* gene

Submitted: November 02nd 2023 | 1st Revision: 5th December 2023 | Accepted: December 08th 2023 | Published: December 31st 2023

Introduction

Antimicrobial resistance (AMR) threatens the effective prevention and treatment strategies of an increasing range of bacterial infections. In 21st century we are facing the real possibility that minor injuries and common infections can lead to death. A detailed understanding of the evolutionary processes occurring in nature that lead to resistance development is thus essential for anticipating its emergence and to restrain its spread.

One of the best models of resistance development is the emergence of methicillin resistance in *staphylococci* not only due the fact that it is extremely well documented, but mainly because it gave rise to methicillin-resistant *Staphylococcus aureus* (MRSA) pandemics – presently a major public health concern [1]. Due to their high efficacy and low toxicity, β-lactams are the most widely used class of antibiotics. They inhibit bacterial cell wall biosynthesis through irreversible binding to the transpeptidase domain of penicillin binding proteins (PBPs) [2].

Staphylococcus aureus is a common colonizing agent that causes subclinical to severe infections. It is able to acquire different antimicrobial resistance mechanisms, and MRSA is a relevant human and animal pathogen. MRSA is a significant antimicrobial-resistant bacteria due to its resistance to beta-lactam antibiotics, such as methicillin. The development of antimicrobial resistance in MRSA is crucial for understanding the mechanisms behind antibiotic resistance and the evolution of drug-resistant strains. All MRSA contain a copy of an exogenous mec gene that codifies for PBPs with low affinity for β-lactams (*mecA, mecB*, and *mecC*) [3].

Some of the key mechanisms contributing to MRSA's antimicrobial resistance such as beta-lactamase production where MRSA primarily develops high-level resistance to beta-lactam antibiotics due to the production of beta-lactamase, an enzyme that breaks down the beta-lactam ring in antibiotics [4]. mecA gene mutations in MRSA strains, resistance to ceftobiprole and ceftaroline is caused by mutations in the mecA gene, which is closely related to the mechanism of methicillin resistance. PBP2A expression which is strains possessing PBP2A show resistance to all therapeutic beta-lactam antibiotics except ceftobiprole and ceftaroline, and they are referred to as MRSA. The mechanism of methicillin resistance is closely related to PBP proteins involved in peptidoglycan synthesis [5].

There is another mechanism such as plasmid-mediated drug resistance, decreased outmembrane permeability, active efflux system, and cellular enzymes. The mechanism of MRSA resistance is mainly due to plasmids or drug-resistant gene transmission mediated by plasmids, which can expand the resistance capabilities of MRSA. Decreased outmembrane permeability caused by the loss of specific outer membrane proteins (OMPs) can lead to drug resistance in *S. aureus*. Active efflux systems play a role in MRSA resistance, as they can pump out antibiotics from the cell, reducing their effectiveness. Cellular enzymes can also contribute to drug resistance in S. aureus, as they can modify the antibiotic or its target site, making the antibiotic less effective [6].

The *mecA* gene is a gene that mediates MRSA strains [7].

Other *mec* genes have been identified that are associated with β-lactam resistance, namely *mecB* in *Macrococcus caseolyticus* and *mecC* in *S. aureus*. The *mecB* are the most distant from *S. aureus mecA*, having, respectively, a nucleotide identity with *mecA* that is equal or lower than 62%, whereas *mecC* has 69% nucleotide sequence identity [2,3]. In this review we will focus on genes of methicillin resistance mediated by *mecA, mecB,* and *mecC*.

Methicillin-Resistant Staphylococcus aureus (MRSA)

Antimicrobials are medicines used to prevent and treat infectious diseases in humans. AMR occurs when bacteria, viruses, fungi and parasites no longer respond to antimicrobial medicines. As a result of drug resistance, antibiotics and other antimicrobial medicines become ineffective and infections become difficult or impossible to treat, increasing the risk of disease spread, severe illness, disability and death [8].

MRSA is the second most common cause of antibiotic-resistant bacterial infections in the European Union (EU) and European Economic Area (EEA) [9]. MRSA is associated with acquisition of a large transmissible element known as staphylococcal cassette chromosome *mec* (SCC*mec*), an event that occurred in *Staphylococcus aureus* prior to the isolation of the first MRSA strain in 1961 [10].

There are three distinct genotypes of MRSA were present in the 1980s, and two of them are still prevalent in the world as multidrug-resistant healthcare-associated MRSA (HA-MRSA) and MRSA with non-multidrug-resistance community-associated or acquired MRSA (CA-MRSA) [11]. For MRSA, the prevalence of resistance was <5% in 4 out of 20 (20%) antibiotic resistance surveillance systems in the Netherlands (ISIS-AR), United Kingdom (BSAC), Finland (FIRE) and Sweden (SVEBAR); 5-15% in 6 out of 20 (30%) antibiotic resistance surveillance systems in Switzerland (ANRESIS), Australia (AURA), EARS-NET, Bulgaria (BulSTAR), Croatia (ISKRA), Germany (ARMIN), and Japan (JANIS); >15% in 8 out of 20 (40%) antibiotic resistance surveillance systems in South Korea (KOR-GLASS), Argentina (WHONET-Argentina), Germany (SARI), Greece (WHONET-GREECE), France (ONERBA), CAESAR, Thailand (NARST), and Philippines (ARSP) [12].

Many of MRSA originate from a limited number of historically dominant clonal lineages. To analyse MRSA transmission and to decrease the incidence of new infections, international epidemiological research is crucial, and this research depends on MRSA surveillance programmes [13]. MRSA surveillance programmes exist worldwide, but only a few are multinational [12]. One European multinational programme is the European Antimicrobial Resistance Surveillance Network (EARS-Net) [14].

EARS-Net is co-ordinated by the European Centre for Disease Prevention and Control (ECDC) and depends on national surveillance systems [13]. Data from the European Antimicrobial Resistance Surveillance Network (EARS-Net) are relevant when monitoring trends in the European Union (EU) and European Economic Area (EEA), but do not give the full epidemiological picture, in particular for monitoring the effect of the European action plan [9]. There are several challenges when estimating the burden of disease associated with infections due to antibiotic-resistant bacteria. For example, sampling and microbiological procedures for testing of the isolates, data collection processes, and the structures of surveillance systems might vary between and within countries [9].

Non European multinational MRSA surveillance programmes mostly depend on national networks using different methodologies. Examples are the Asian Network for Surveillance of Resistant Pathogens (ANSORP), the Latin American Network for Antimicrobial Resistance Surveillance (ReLAVRA), the SENTRY Antimicrobial Surveillance Program and the Tigecycline Evaluation and Surveillance Trial (T.E.S.T), now embedded in the Antimicrobial Testing Leadership and Surveillance (ATLAS) database [15].

The Etiology of MRSA

MRSA is mostly mediated by the expression of an additional penicillin-binding-protein 2a (PBP2a) with low affinity for beta lactam antibiotics, except ceftobiprole and ceftaroline), encoded by the *mecA* gene or less frequently by the *mecC* gene [16]. *MecA* gene was linked to the nucleotide sequen promotor and truncated homolog of the regulator gene *blaR1* of beta lactamase (*bla*)-gene complex. Since mecA was found in the structure *blaR1*-(*p*)-*mecA*, they proposed the idea that *mec*-gene complex was produced by recombination event between *mecA* gene and *bla*-gene complex, by homologous recombination [11].

There were methicillin-resistant strains with and without the *mecR* locus, the *mecR* locus tend to be slow in the induced production of *mecA* by exposure to methicillin [11]. The *mecA* gene transcription is also repressed by *bla*-gene complex on a plasmid harbored by N315, but the bla regulators allowed much quicker induction of *mecA* on exposure to methicillin. There were also some strains with intact *mecI* gene but having a mutation in the operator region of *mecA* gene to which *mecI* repressor protein is supposed to bind. *Staphylococcal* Cassette Chromosome *mec* (SCC*mec*) elements were much larger and studded with apparently useless pseudogenes or truncated copies of transposons and insertion sequences [11].

SCCmec as a Vehicle of MRSA

There are two important clusters of genes were identified such as *mec*-gene complex (encoding methicillin resistance) and cassette chromosome recombinase (*ccr*) gene complex (encoding one or two site specific recombinases for the movement of the element [11].

The SCC*mec* type is defined by the combination of the type of *ccr* gene complex and the class of *mec* gene complex. Types I-III are older SCC*mec* types that are harbored by HA-MRSA, meanwhile types IV and V were recognized as new versions of SCC*mec* that were almost diagnostically harbored by CA-MRSA [11]. Types I-III relatively big in size and carry multiple antibiotic resistance determinants. Types IV and V they are short, and typically carrying no antibiotic resistance genes other than *mec* gene complex. The differences structure in SSC*mec* show in **Figure 1** [11].

mecA Gene

MRSA strains are known to contain the major antibiotic resistance gene *mecA*. *mecA* is one of the key factors in MRSA resistance to antibiotics. This gene is responsible for synthesizing a cell wall-forming transpeptidase, penicillin binding protein (PBP), which is carried on the *Staphylococcal* Cassette Chromosome mec (SCC*mec*) and induces resistance to

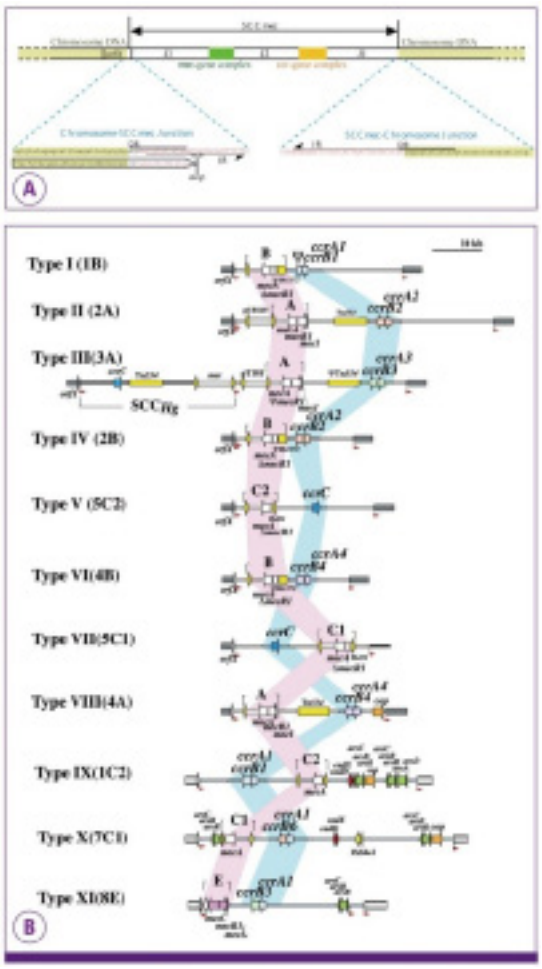

Figure 1. (A) Basic structure of SCC*mec*, (B) Various types of SCC*mec*

methicillin and other β-lactam antibiotics [17]. Class B PBP as knows as PBP2A which contains two domains, the C terminal which is known to have a transpeptidation function, and a N-terminal domain to which no function has been attributed, the socalled non-binding [18]. In *S. lentus* and *S. stepanovicii* so far no *mecA* homolog has been described [15].

The only *mecA* homologue that confers resistance to β-lactams is the *mecA* in *S. fleuretti*. In other hand, *mecA*1 was founded in *S. sciuri* and *mecA*2 homologue in *S. vitulinus* [18]. *S. sciuri* carries the most ancestral form of *mecA* (*mecA*1) which has 85% homology in nucleotide sequence with S. *aureus mecA* meanwhile *S. vitulinus* harbors an intermediary form (*mecA*2) with 94% homology. All of *S. fleurettii*, one of the oldest *staphylococcal* species. and some S. vitulinus have a *mecA* form that is almost identical to that of S. aureus *mecA* (*mecA*f, *mecA*v; 99% homology) [17]. As shown in **Figure 2**, the chromosomal locus containing the complete *mecA*-gene complex as well as the surrounding J regions bracketed by two mobile genetic elements Tn554 and IS431 of type-II SCC*mec* was found in *S. fleurettii* strain SFMP07 [19].

Subsequent whole genome sequencing of the strain revealed that the region was about 240-Kb apart from the *orfX*, and there were multiple SCCs in the vicinity of *orfX* but no copy of it found around the *mecA* gene complex [15]. This indicated that *mecA* was an intrinsic component of the chromosome of *S. fleurettii*. It must have been an important PBP for the ancestral staphylococci to survive the environment soaked with ß-lactam antibiotics produced by fungi and actinobacteria.

The *mecA* gene must have been transmitted vertically during the initial steps of *staphylococcal* speciation. However, after a while, it became decayed (with mutations) or deleted out from the chromosomes of the descendants as exemplified by the emergence of methicillin-susceptible *S. aureus* (MSSA) as a human colonizer. It is curious why *mecA* gene was lost from the *staphylococcal* chromosome during speciation. The divergence time of *S. sciuri* group from the major *staphylococcal* clade including *S. aureus* is calculated to be 200- 300 million years ago, which corresponds to the geological age of the emergence of mam-malian animals [19].

Since then, *staphylococcal* species evolved with mammalians as the colonizers of diverse mammalian animals [19]. Our hypothesis is that the descendants of *staphylococci* became methicillin-susceptible, because they became protected from the threat of ß-lactam-producing fungi or actinobacteria by the immune system of mammalian hosts.

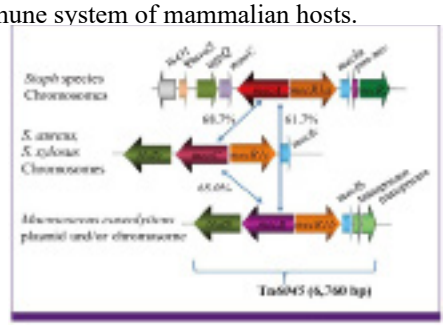

Figure 2. Novel *mecA* gene homologs with *mec B* and *mecC*

The *mecA* gene is inducible and encodes the high-molecular-weight, 78-kD PBPa polypeptide. It occurs in both MRSA and methicillin resistant coagulase-negative *staphylococci*, and is highly conserved. Analysis of the nucleotide sequence of *mecA* and its operator region revealed that sequences contained within the 5' end were similar to sequences within the β-lactamase gene, blaZ, of *S. aureus* [20]. Several studies have demonstrated that acquisition of *mecA* confers to *staphylococci* a competitive advantage in the hospital, community and veterinary environments. Introduction of the *mecA* determinant into the *S. aureus* genome on multiple occasions, has led to the emergence and worldwide dissemination of several methicillin-resistant *S. aureus* (MRSA) clones [17].

Penicillin and other β-lactams used in many countries in food production animals not only to enhance animal growth, but also to treat infections and as a prophylactic. Penicillin resistance emerged due to the acquisition of β-lactamases that were able to hydrolyse and inactivate penicillin. Further developments to overcome resistance to penicillin included the synthesis of penicillinase-resistant penicillins, such as methicillin in 1960. An additional mechanism driving β-lactam resistance involved alterations in the promoter of *mecA* homologues: either deletions around the RBS site or alterations in -10 and -35 regions [17].

New antibiotics have been developed to overcome antibiotic-resistant. Unfortunately, resistance to these new antibiotics has already been reported. Six approved medications, including cefoperazone, mezlocillin, cefpiramide, ceftolozane, piperacillin, and ertapenem, exhibited a high binding affinity against the *MecA* protein [17].

mecA has a low affinity than for β-lactam antibiotics than the native PBPs that are the targets of this class of antibiotics others so its many studies have carried out *mecA* detection

from several isolates. The *mecA* gene was found in family of *Staphylococcus*, such as *S. fleurettii*, *S. epidermidis*, *S. haemolyticus* and *S. xylosus* [13].

The intact *bla*-gene complex has the structure *blaI-blaR1-(p)-blaZ*, where p stands for the di- vergent promoters for *blaZ* and *blaR1*. Since *mecA* was found in the structure Δ*blaR1-(p)-mecA*, they proposed the idea that *mec*-gene complex was produced by recombination event between *mecA* gene and *bla*-gene complex, by homologous recombination [13].

mecB Gene

Besides *mecA*, other mec genes have been identified that are associated with β-lactam resistance, namely *mecB* and *mecD* in *Macrococcus caseolyticus* [22]. The *mecB* and *mecD* are the most distant from *S. aureus mecA*, having, respectively, a nucleotide identity with *mecA* that is equal or lower than 62%, whereas *mecC* has 69% nucleotide sequence identity. Both *mecB* and *mecC* were carried within mobile genetic elements structurally similar to SCC*mec* that were inserted in the *orfX* region [23].

The *mecB* was recently found within a plasmid in a single *S. aureus* human carriage strain belonging to ST7 [24]. The exact evolutionary link between *mecA, mecB, mecC* and *mecD* forms is still undetermined. Among all *mec* genes, *mecA* is apparently, the most successful in *Staphylococcus*. The *mecB* was recently found within a plasmid in a single *S. aureus* human carriage strain belonging to ST7 [24].

mecC Gene

mecC MRSA has been reported in a wide range of other host species, including livestock, wildlife and companion animals in many European countries. As in human isolates, those isolated from animals are associated more with the clonal complex 130 (CC130) and with ST425 to a lesser degree [25].

Several studies have shown that *mecC*-positive MRSA is relatively common in dairy cattle, suggesting that cattle provide an infection reservoir and farmers in contact with dairy cattle may be at risk of acquiring these isolates (26). *mecC* MRSA is currently rare in humans, but there are interesting geographical differences in terms of prevalence; in particular, the latest increase in prevalence in Denmark underlines the need to monitor *mecC* MRSA [27].

Conclusion

Staphylococcus aureus is a common colonizing agent that causes subclinical to severe infections. It is able to acquire different antimicrobial resistance mechanisms, and MRSA is a relevant human and animal pathogen. The increasing prevalence of MRSA infections in the hospitals, other care centers and lately in the community has become a worldwide phenomenon. The wide spread dissemination of multiple - drug resistant strains and antibiotic clones of the bacterium facilitated by inherent or acquired molecular/genetic element is worrying as it complicates diagnosis. This antibiotic resistance occurs in the β-lactam antibiotic group, where resistance occurs due to the presence of the PBP2A protein. All MRSA contain a copy of an exogenous mec gene that codifies for PBP2A with low affinity for β-lactams (*mecA*, *mecB*, and *mecC*). *mecA* is the most common gene responsible for methicillin resistance in MRSA, carried on the staphylococcal cassette chromosome (SCC*mec*). On the other hand, *mecB* and *mecC* are alternative genes associated with methicillin resistance. *mecB* is rarely found, while *mecC* is distinct from *mecA*.

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