mec Genes as Marker in Methicillin-Resistant Staphylococcus aureus

Zahra Umami*, Asri Indahning Warni, Dewi Wahyuni

Faculty of Pharmacy, Airlangga University, Surabaya, East Java, Indonesia

*E-mail: zzahramami@gmail.com

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

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 to 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 β-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 cefotibprobole and cefatoline 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 cefotibprobole and cefatoline, 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 out membrane 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 out membrane 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 (SCCmec), 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 (ReLA VRA), 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 cefotiboprole and cefaloridine), 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. *Staphylococcus* Cassette Chromosome mec (SCCmec) 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 (crr) gene complex (encoding one or two site specific recombinases for the movement of the element) [11].

The SCCmec 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 SCCmec types that are harbored by HA-MRSA, meanwhile types IV and V were recognized as new versions of SCCmec 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 SCCmec 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 (SCCmec) and induces resistance to...
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 mammalian 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 β-lactam-producing fungi or actinobacteria by the immune system of mammalian hosts.

Figure 2. Novel mecA gene homologs with mecB 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 the mecA resistance involved alterations in the promoter of mecA.[17]. 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, cefotolozane, 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 \textit{mecA} gene was found in family of \textit{Staphylococcus}, such as \textit{S. fleurettii}, \textit{S. epidermidis}, \textit{S. haemolyticus} and \textit{S. xylosus} [13].

The intact \textit{bla}-gene complex has the structure blal-blalR1-(p)-blaZ, where \textit{p} stands for the di- vergent promoters for \textit{blaZ} and \textit{blaR1}. Since \textit{mecA} was found in the structure ΔblaR1-(p)-\textit{mecA}, they proposed the idea that mecc-gene complex was produced by recombination event between \textit{mecA} gene and \textit{bla}-gene complex, by homologous recombination [13].

\textbf{mecB Gene}

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

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

\textbf{mecC Gene}

\textit{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 \textit{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). \textit{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 \textit{mecC} MRSA [27].

\textbf{Conclusion}

\textit{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 \textit{mec} gene that codifies for PBP2A with low affinity for β-lactams (\textit{mecA}, \textit{mecB}, and \textit{mecC}). \textit{mecA} is the most common gene responsible for methicillin resistance in MRSA, carried on the staphylococcal cassette chromosome (SCC\textit{mec}). On the other hand, \textit{mecB} and \textit{mecC} are alternative genes associated with methicillin resistance. \textit{mecB} is rarely found, while \textit{mecC} is distinct from \textit{mecA}.

\textbf{References}


[22] Schwendener S, Perretet V. The integrase of the Macrococcus caseolyticus resistance island mecD (McRImecD) inserts DNA site-specifically into *Staphylococcus* and *Bacillus* chromosomes. Mol Microbiol. 2018;110(3):455–68.


