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Chapter 2

MRSA and MSSA: The Mechanism of Methicillin Resistance and the Influence of Methicillin Resistance on Biofilm Phenotype of *Staphylococcus aureus*

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Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/65452

Abstract

Staphylococcus aureus (*S. aureus*), which is one of the most common causes of indwelling device–associated, nosocomial, and community-acquired infections, can produce biofilm as a virulence factor. Methicillin-resistant *S. aureus* (MRSA) that is resistant to β-lactam antibiotics causes life-threatening infections. Biofilm producer strains of *S. aureus* that causes indwelling device–associated infections resist to antimicrobials and immune system. The combination of methicillin resistance and the ability of biofilm formation of *S. aureus* makes treatment difficult. Methicillin resistance of *S. aureus* can affect biofilm phenotype of *S. aureus*; the *mec*A gene of MRSA increases biofilm production by inactivating accessory gene regulator (agr) quorum sensing regulator system, which is a two-component regulator system of virulence factor production. The aim of this review is to determine virulence factors of *S. aureus*, resistance mechanisms of methicillin, and the influence of methicillin resistance on biofilm phenotype of *S. aureus*.

Keywords: *Staphylococcus aureus*, MRSA, MSSA, biofilm, methicillin resistance, virulence, influence of methicillin resistance on biofilm

1. Introduction

The biofilm has an important role in the pathogenesis of certain bacterial infections such as staphylococcal indwelling device–associated infections, wound infections, chronic urinary tract infections (UTI), cystic fibrosis pneumonia, chronic otitis media (OM), chronic rhinosinusitis, periodontitis, and recurrent tonsillitis [1].

The biofilm infections such as *Staphylococcus aureus* (*S. aureus*) infections are the important problems in hospitalized and immunosuppressed patients worldwide due to their tough and nonresponsive treatment by antibiotics. Biofilm-producing bacteria are resistant to immune defense, antibiotics, and many antimicrobial agents [2, 3].

The *mecA* gene, which is located in the staphylococcal chromosomes, enhances virulence of *Staphylococcus* by causing resistant to methicillin antibiotics. Methicillin-resistant *S. aureus* (MRSA) causes hospital-associated (HA-MRSA) and community-associated (CA-MRSA) infections. Methicillin resistance of *S. aureus* causes treatment of *S. aureus* tough by antibiotics due to its resistance to all β-lactam antibiotics. Mechanisms of resistance to β-lactam antibiotics such as methicillin are regulated by regulatory genes in the presence of such antibiotics. *S. aureus* biofilm formation is regulated globally by the accessory gene regulator (agr) quorum sensing system that is also inactivated by the *mec*A gene of MRSA [2–4]. The virulence of *S. aureus*, mechanisms of methicillin resistance, role of methicillin resistance on biofilm, and alteration of biofilm formation of *S. aureus* in methicillin resistance are discussed in this review.

2. *Staphylococcus aureus* and virulence

2.1. Staphylococcus aureus

S. aureus, a Gram-positive coccus, produces catalase enzyme and coagulase enzyme, which coagulates blood by reacting with prothrombin, which converts fibrinogen to fibrin [5]. While *S. aureus* is a commensal bacterium and colonizes primary anterior nares of healthy staphylococcal nasal carrier individuals, *S. aureus* causes a wide range of infections such as skin infections, including abscesses, impetigo, and necrotizing fasciitis; tissue infections, including osteomyelitis and endocarditis; and toxinoses, including toxic shock syndrome, when immunity of the staphylococcal nasal carrier is suppressed [6, 7]. If MRSA is colonized in nares of healthy person, 29% potential risk appears for MRSA infections [8].

While antibiotics such as methicillin are used frequently in patients, antibiotic-resistant strains may develop. After penicillin usage had become widespread to treat infections, penicillin-resistant *S. aureus* strains arose. Only a few years after following the usage of penicillin, penicillin-resistant *S. aureus* strains had arisen, and penicillinase-resistant methicillin usage had introduced for the treatment of penicillin-resistant *S. aureus* strains. After methicillin usage was introduced in 1961, MRSA strains that were also multidrug-resistant arose within a year. Methicillin that has been providing widespread of MRSA and becomes useless drug has not being used in recent years [7].

MRSA has become epidemic not only in nosocomial infections but also in community-associated infections [9]. MRSA that has been a common cause of nosocomial infections worldwide also has been arising in the community in recent years [10]. Invasive infections of MRSA have high morbidity and mortality rates [11]. Most of invasive staphylococcal and community-acquired MRSA (CA-MRSA) infections are related to the nasal carriage of *Staphylococcus* [6].

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2.2. Biofilm and pathogenesis

Biofilm plays a role in the pathogenesis of staphylococcal infections. When microorganisms exposed to stress conditions, gene expression of biofilm is induced as a stress response. The biofilm that is a slime-like glycocalyx causes bacteria to survive in the stress conditions, causes bacterial attachment and colonization on biotic or abiotic surfaces such as prosthetic surfaces that may act as a substrate for microbial adhesion, and causes bacterial spread to whole body [12–14]. The biofilm producer *S. aureus* causes chronic infections such as indwelling device-related infections and chronic wound infections. Indwelling device-associated infections are mainly caused by biofilm producer *Staphylococci* including *S. aureus* and *Staphylococcus epidermidis*. The treatment of biofilm-embedded bacteria that are not eliminated completely by antimicrobials even at the high doses is tough and irresponsive. The patients whose indwelling device is infected by biofilm producers have higher risk of mortality. Infected implants that cannot be treated by antibiotics are removed out of the body to prevent biofilm-related infections [14].

2.3. Virulence of *S. aureus* Biofilm

Biofilm that is a slime-like glycocalyx embedded sessile community of microorganism inside. Polysaccharide matrix, staphylococcal surface proteins, extracellular DNA (eDNA), and teichoic acids construct biofilm of *S. aureus* that is an extracellular polymeric substance. Surface proteins of *S. aureus* also contribute biofilm formation, whereas polysaccharide intracellular adhesin (PIA) is the main component of biofilm formation in *S. aureus*. Extracellular DNA (eDNA) that plays a role in resistance and channels that store antibiotic-degrading enzymes such as β-lactamases construct extracellular polysaccharide matrix [14].

3. Mechanisms of biofilm formation and regulation by MRSA and MSSA

Biofilm is produced by distinct mechanisms in MRSA and Methicillin-sensitive Staphylococcus aureus (MSSA). Fitzpatrick et al. revealed that biofilm formation of the *icaADBC* operon deleted MRSA mutants was not affected, whereas biofilm formation of the *icaADBC* operon deleted MSSA mutants was impaired. This study showed that *ica*-independent biofilm formation is strain specific [15].

Biofilm is constructed not only by polysaccharide intracellular adhesin (PIA) but also by surface proteins. In the catheter infection, biofilm formation of clinical isolates of *S. aureus* of which *ica* operon is mutated is not reduced [13]. Biofilm of MSSA is formed in *ica*-dependent manner (PIA-dependent) by PIA that is encoded by *icaADBC* gene, whereas biofilm of MRSA is formed in *ica*-independent manner (PIA-independent) by surface proteins containing LPXTG anchoring domain that are anchored to peptidoglycan by sortase as a transpeptidase coded by *srtA* gene. Adherence to surfaces and intercellular aggregations of MSSA and MRSA cells are contributed by PIA in *ica*-dependent manner and surface proteins in *ica*-independent manner, respectively [4, 14]. Initial adherence of *S. aureus* to surfaces is contributed by Autolysin Atl that lyses cell causes release of eDNA and accumulation of cells in *ica*-inde-

pendent manner [14, 16]. Especially, clinical MRSA adheres to polystyrene abiotic surfaces with Atl [16]. Intercellular accumulation of HA-MRSA and CA-MRSA is formed by FnBPA, FnBPB, Bap proteins, SasG, and protein A [4, 17, 18].

Three stages of *ica*-dependent and *ica*-independent biofilm formation that are adherence (adhesion, attachment), aggregation (maturation, accumulation), and detachment (dispersal) are regulated by *ica* operon and accessory gene regulator (agr) quorum sensing two-component signal transduction system, respectively [14].

Not only biofilm formation but also virulence factors such as phenol-soluble modulins (PSMs), toxins, and degradation enzymes production are regulated by agr quorum sensing two-component regulatory system [14, 19, 20]. Activation of *agr* system causes reduction in biofilm production due to the production of phenol-soluble modulins (PSMs) as surfactants, proteases, and nucleases that disperse microorganisms embedded in biofilm by enzymatic degradation of the biofilm matrix [14, 21, 22].

Accessory gene regulator (*agr*) system, which includes *agr* locus, regulates cell density, virulence, and biofilm formation of bacteria. RNAII and RNAIII are transcribed by binding of activated AgrA to P2 and P3 promoters in *agr* operon (*agr*BDCA), respectively. RNAII transcript that contains *agrB*, *D*, C, *A* genes encodes AgrB, D, C, A as a component of agr system, whereas RNAIII transcript that contains the *hld* gene encodes the δ-PSM (δ-phenol-soluble modulins or termed δ-hemolysin). RNAIII regulates virulence factors such as surface proteins that cause biofilm formation and exotoxins (RNAIII dependent control). In RNAIII independent control of *S. aureus*, synthesis of α-PSMs and β-PSMs is regulated by binding of AgrA to promoters of α-PSMs and β-PSMs in *psm* operon [14].

Supplementations of certain chemicals to growth media affect biofilm formation of *S. aureus* strains by regulating of gene expressions or breaking bonds that construct biofilm. Sodium chloride (NaCI) that induces expression of *ica* operon increases biofilm formation of MSSA [4, 23, 24]. Sodium metaperiodate that degrades polysaccharide bonds decreases biofilm formation of MSSA, whereas biofilms of MRSA are not affected. Proteinase A does not affect biofilm formation of MSSA [24, 4], whereas biofilm formation of MRSA is affected. pH of growth media that is decreased by glucose degradation represses agr regulator system. So, glucose supplementation of growth media that represses agr regulator system increases biofilm formation [4, 25]. Phenyl-methylsulfonyl fluoride (PMSF) that is a serine protease inhibitor increases biofilm formation by preventing *agr*-related biofilm detachment [21] and enhancing secretion of autolytic enzymes [26]. In early biofilm formation of HA-MRSA, biofilm formation is inhibited by polyanethole sodium sulfanate of which effect is not only preventing autolytic activity but also maintaining growth [16].

4. *Staphylococcus aureus* genome

Staphylococcus aureus genome contains core genome, accessory component, and foreign genes. Core genome that constructs backbone of genome has main metabolic function. Core genome

http://dx.doi.org/10.5772/65452

is highly conserved, and similarity of genes among isolates is ~98–100%. Accessory component that constructs 25% of *S. aureus* genome contains mobile genetic elements (MGEs) such as transposons (Tn), chromosomal cassettes, pathogenicity islands (PIs), genomic islands, and prophages acquired horizontally between strains [5] (**Figure 1**). MGEs carry virulence genes that are acquired horizontally by other strains (bacterial horizontal gene transfer (HGT)) [7, 27].

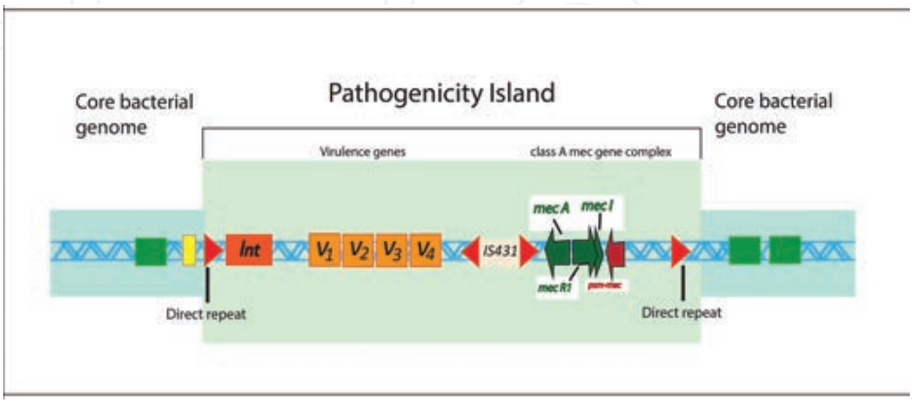

Figure 1. Staphylococcal genome.

Each strain of *S. aureus* has virulence varied according to having mobile genetic elements (MGEs) of which genes encode for varied virulence factors and toxins [9]. Genes of many secreted virulence factors such as exfoliative toxin A and B, superantigen toxins (SaPIs), toxic shock syndrome toxin (TSST), and enterotoxins are located in accessory genetic elements such as transposons, plasmids, prophages, and pathogenicity islands (PIs) that are also referred as MGEs, whereas genes that encode toxins such as α-toxins present in whole *S. aureus* strains and are located in core genome [7, 9]. Phenol-soluble modulins (PSMs) that are surfactant and encoded in core genome lyse immune cells such as neutrophils in inflammation and disperse biofilm [9, 14]. Cytolytic activity is present in shorter PSM-α type, while longer PSM-β type does not have cytolytic activity. It is seen that virulence of *S. aureus* is reduced by removing *psm-α* operon [7].

4.1. Prophages

Prophages have an effective role in pathogenicity of *S. aureus* due to causing horizontal gene transfer (HGT) by transferring virulence genes of which products are staphylokinase, enterotoxin A, G, K, Panton-Valentine leukocidin (PVL), and exfoliative toxin [5].

4.2. Pathogenicity islands (PIs)

The gene of superantigen toxins (SaPIs), which is one of the secreted virulence factors of *S. aureus*, is located in pathogenicity islands (PIs) that is also located in chromosome. SaPIs contain bacteriophage-associated genes that encode helicases and terminases involved in replication, integrases involved in integration, recombination and excition of MGE, and certain direct repeats [5, 28, 29].

The most known PI of *S. aureus* is SaPI1 that contains the *tst* gene encoding for TSST [7]. High-frequency transduction of SaPI1 is mediated by encapsulating SaPI1 by staphylococcal phage 80*α* that is own phage of *S. aureus* to transfer its genes in transduction process. Enterotoxin B is encoded by SaPI3 that is one of the SaPIs and encapsulated by phage 29 that is phage of *S. aureus* to transfer its genes in transduction process [5, 28].

S. aureus not only carry SaPIs but also carry vSa family genomic islands that encode ~50% of toxin and virulence factors of *S. aureus*. Conserved genes are present in this family. Among this family, vSa1 contains genes encoding for enterotoxin such as *seb*, *tsst*, and *ear,* whereas vSa2 contains genes encoding for enterotoxin such as *sec* and TSST (*tss*t). vSa*α* and vSaβ that are also present among vSa family genomic islands contain leukocidin genes [5].

4.3. Insertion sequence (IS) and transposons (Tn)

Insertion sequences (ISs) contain inverted repeats at their terminals and the integrases gene that causes transposition. Transposons (Tn) not only contain the transposase gene but also may contain ISs that induce movement of Tn and certain genes such as antibiotic resistance genes [5]. These elements provide a mechanism to transfer of virulence and resistance genes such as antibiotic resistance genes from place to place within the same cell or to other cell. These movable elements are excised from paired inverted repeats by transposase enzyme. While these elements are excised and inserted to new location such as within a gene that may be located within the same cell or other cell, the gene is disrupted [30].

4.4. Plasmids

Plasmids that are extrachromosomal genetic elements carry resistance genes causing antibiotic or heavy metal resistance, and virulence genes encoding for virulence factors, rather than genes involved in metabolic processes having vital functions [5]. There are three types of plasmids of *S. aureus* according to their size. Type I plasmids that are the smallest plasmids contain just one antibiotic-resistant determinant. Type II plasmids of which sizes are intermediate contain β-lactamase gene. The largest one is type III plasmids containing multiple resistant determinants such as gentamycin, trimethoprim, and ethidium bromide resistance [31]. Conjugative plasmids that are also type III plasmids are transferred horizontally to other cell by their own *tra* genes [5].

4.5. SCCmec

MGEs contain the *mecA* gene causing methicillin and other β-lactam resistance and occur in chromosome of methicillin-resistant *Staphylococcus* such as MRSA, and methicillin-resistant *S. epidermidis* MRSE is called staphylococcal cassette chromosome *mec* (*SCCmec*) [7, 9]. Inverted repeats that are localized at both terminals of *SCCmec* are the recognizing sequences for *SCCmec*-specific recombinase in the processes of excision of *SCCmec* from chromosome and integration of *SCCmec* to either other parts of chromosome or chromosome of other strain (**Figure 1**) [32].

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SCCmec is composed of variable and conserved genetic elements. *SCCmec* carries *mec* operon that contains *mecA*, and regulatory genes such as *mecI* and *mecRI*, and cassette chromosome recombinase genes *ccrA*, *ccrB*, and *ccrC* that are localized in *ccr* locus and contribute excision from *SCCmec* and integration to chromosome. All these elements are highly conserved among *Staphylococcus*. J-region that is a variable region of *SCCmec* composed of genetic elements integrated such as ISs, Tns, and plasmids. In addition to methicillin resistance that is caused by *mecA* in a strain, if these integrated elements include additional genes encoding for antibiotic resistance, rather than methicillin, multiple resistance arises in this strain [5]. Just a year later on the first usage of methicillin for treatment of MRSA, clinical MRSA isolates that have multiple resistant to antibiotics were reported [33].

Variants of *mec* operon that are located in *SCCmec* are present according to whether *mecI* and *mecRI* genes are intact or having deletions. The variants of *mec* complex are class A, B, C, D, and E *mec*. IS431 that is related to the *mecA* gene are present in all *mec* operon classes. All classes except A consist of deleted portions that are happened in *mecI* and may run through to a portion of *mecRI* gene. Eight types of *SCCmec* were found according to having combinations of distinct variants of *mec* and *ccr* [5]. Multidrug-resistant strains have *SCCmec* type II and III that contain additional resistance genes. While MRSA is characterized by containing *SCCmec* type I or III and II in recent years, CA-MRSA strains are characterized by containing *SCCmec* type IV. Other *SCCmec* types are seen in strains very rare [7].

Methicillin-resistant strains of *Staphylococcus* have *mec* operon, whereas methicillin-sensitive strains of *Staphylococcus* do not have *mec* operon [34]. HGT of *mecA* from one to another strain is proved by researchers; the *mecA* gene of MRSA and *mecA* homolog of *Staphylococcus sciuri* revealed 88% identity. But *S. sciuri* containing *mecA* homolog is susceptible to methicillin. This supported that MRSA strains are descendents of ancestral strains in evolutionary process [35, 36]. *Staphylococcus haemolyticus* (*S. haemolyticus*) genome carries intact IS*1272* element, whereas the gene of *S. aureus* and *S. epidermidis* carries IS*1272* element deleted [37]. This revealed that horizontal gene transfer (HGT) is happened by acquisition of IS1272 from *S. haemolyticus* to *S. aureus* and *S. epidermidis*. HGT of *mecA* that is happened from *S. epidermidis* to *S. aureus* causes arising of MRSA during treatment with antibiotic [38]; *mecA* is transferred to methicillin-resistant *Staphylococcus* by the way that having inverted repeats at terminals of the *mec* gene complex and IS*431* of which location is especially within gene complexes encoding various resistance factors such as the *mec* gene complex.

Methicillin resistance is not only seen in isolates of *S. aureus* but also seen more common in isolates of *S. epidermidis*. Approximately, 70% of whole hospital-acquired methicillin-resistant *Staphylococcus* isolates is S. *epidermidis* [5].

5. The relationship between methicillin resistance and biofilm formation

The association between methicillin resistance and biofilm phenotype is taken attention according to studies executed [39–41]. Researchers determined that biofilm formation of HA-

MRSA BH1CC strain is decreased by removing *SCCmec* that results up-regulation of protease activity [4, 42, 43].

Biofilm formation of MRSA is enhanced by both phenol-soluble modulin mec (PSMmec) encoded by *psm-mec* and penicillin-binding protein 2a (PBP2a) encoded by *mecA* that also repress virulence of MRSA [42].

5.1. psm-mec

SCCmec not only contains genes encoding methicillin resistance and recombination but also contains genes encoding other antibiotics and heavy metal resistance; the *psm-mec* gene that is located near *mecA* in *SCCmec* especially type II, III, and VIII encodes PSMmec peptide. The PSMmec that is a cytolysin is the only staphylococcal toxin of which the gene is colocated with antibiotic-resistant determinant in MGEs of *S. aureus* rather than core genome; the *psm-mec* gene is conserved region of class A *mec* gene complex (**Figure 1**) [9].

Like many virulence toxins of *S. aureus* such as α-toxin and other PSMs, expression of PSMmec is also regulated by Agr two-component signal transport system [14, 44]. Many virulence toxins are regulated by RNAIII-dependent manner, whereas other PSMs and *psm-mec* are regulated by RNAIII independent manner (**Figure 2**) [14].

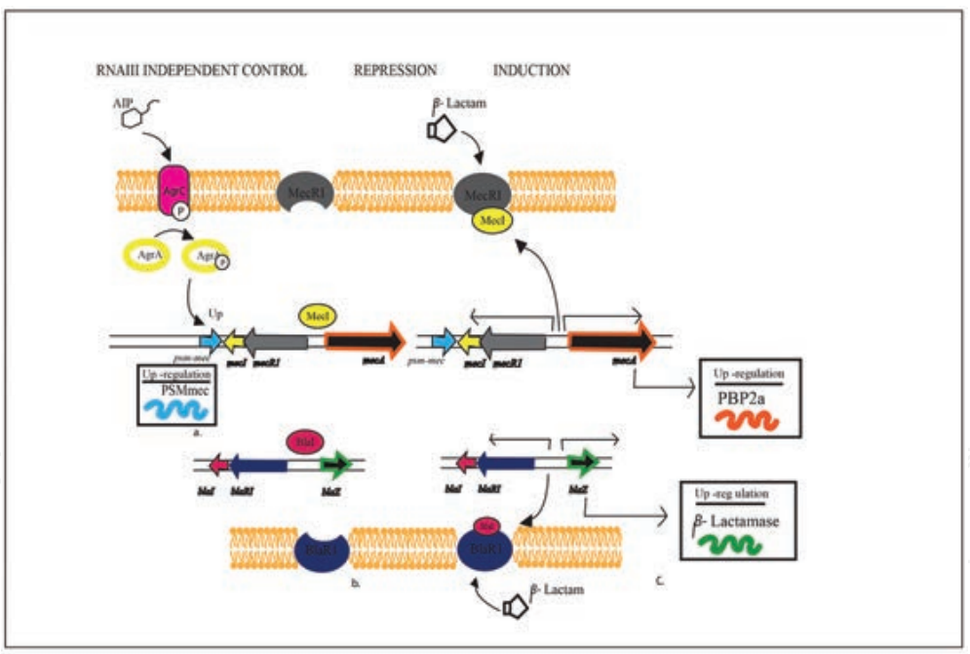

Figure 2. Mechanisms of *PSMmec*, PBP2a, and β-lactamase regulations. (a) Up-regulation of *psm-mec* by RNAIII independent Agr regulator system. (b) Repression of *mecA* and *blaZ* genes: In lack of β-lactams no transcription occurs. (c) Induction of genes: PBP2a and β-lactamase are transcribed by expression of *mecA* and *blaZ* in the presence of β-lactams, respectively.

Biofilm formation is increased by the repression of Agr system that downregulates *psm-mec* in MRSA [4] and PSMs [14]; the *psm-mec* gene of MRSA also has pleiotropic effect by changing biofilm phenotype and regulation of *psm* gene, decreasing toxin production, and the way

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decreasing virulence, and the *psm-mec* gene that is up-regulated by Agr regulator system promotes biofilm formation of MRSA by reducing expression of PSM*α* toxin that is encoded in chromosome. As a result of reduced expression of PSM*α* toxin, virulence of *S. aureus* is reduced by PSMmec (**Figure 3**) [45, 46].

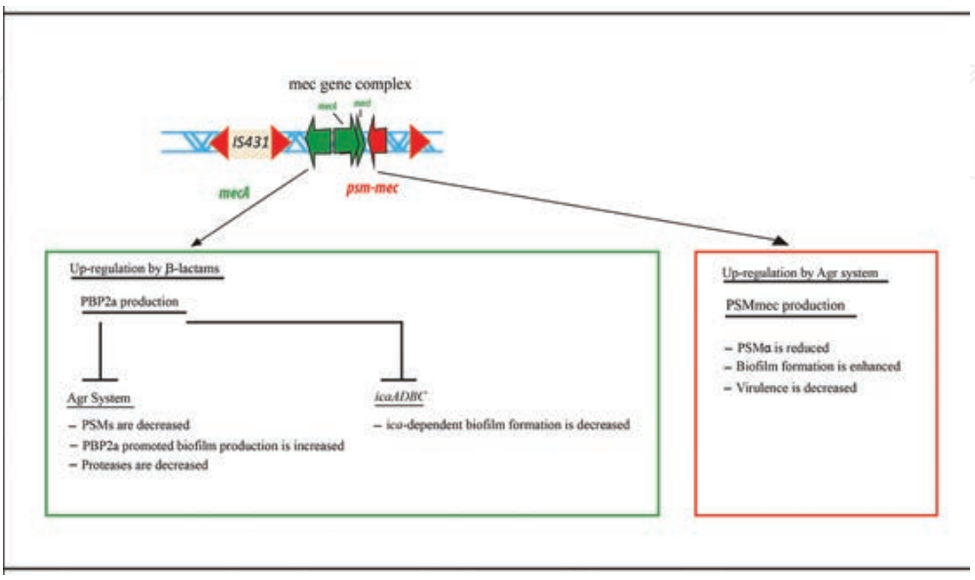

Figure 3. The effect of *mecA* and *psm-mec* induction of Staphylococcus aureus on biofilm formation and virulence.

Biofilm formation (adherence to surfaces and intercellular aggregations) of MSSA and MRSA strains is contributed by PIA in *ica*-dependent manner and surface proteins in *ica*-independent manner, respectively [4, 14]. Interestingly, in spite of the biofilm of MSSA that is formed by *ica*-independent manner is not seen or seen less prevalent, the *psm-mec* gene of type II *SCCmec* enhances expression of Atl and FnBPA in MSSA isolates [42].

5.2. mecA

Agr system is repressed by expression of PBP2a that is encoded by *mecA*, as a result of oxacillin usage [10]. PSMs are downregulated, proteases and virulence are decreased, and PBP2a promoted biofilm formation enhanced by repressed Agr regulator system. In contrary to this, *ica*-dependent biofilm formation is decreased by *ica* that is repressed by PBP2a (**Figure 3**) [42].

6. β-Lactam, methicillin, and multidrug resistance

6.1. Peptidoglycan biosynthesis of *S. aureus*

Peptidoglycan, surface proteins such as protein A, clumping factor A, fibronectin-binding protein (FnBP), collagen-binding protein, and teichoic acids construct the cell wall of *S. aureus*. Peptidoglycan is constructed by polypeptides containing L-alanine, D-glutamic acid, L-lysin and D-alanine, respectively, and glycan polysaccharide strands [5].

At the beginning of peptidoglycan synthesis, UDP-*N*-acetylmuramyl-pentapeptide (UDP-NAM-pentapeptide) and UDP-*N*-acetylglucosamine (UDP-NAG) that are nucleotide sugar-linked precursors are synthesized in cytoplasm of *S. aureus*. Pentapeptide with the sequence of L-alanine, D-glutamic acid, L-lysin, D-alanine, and D-alanine, respectively, is linked to NAM in cytoplasm. Then, bactoprenol that is a membrane-bound lipophilic acceptor transfers UDP-NAM-pentapeptide and UDP-NAG that are hydrophilic precursors from cytoplasm to the outer surface of cell membrane, respectively [5].

Then, transglycosylation and transpeptidation reactions are catalyzed by penicillin-binding proteins (PBPs) of which 4 types (PBP1, PBP2, PBP3, PBP4) are present in *S. aureus* [5]. PBPs that are DD-peptidases are bound to membrane [36]. *N*-acetylglucosamines (NAG) and *N*-acetylmuramic acids (NAM) that are bound by β(1-4) glycosidic bond catalyzed by PBPs in transglycosylation process construct glycan strands that form backbone of peptidoglycan. Transglycosylation reaction is catalyzed by PBPs, especially penicillin-binding protein 2 (PBP2) and glycosyltransferase Mtg. In transpeptidation reaction that is catalyzed by PBPs, L-lysine that is the amino acid of polypeptide linked to NAM of one glycan strand is cross-linked to D-alanine that is the amino acid of polypeptide linked to NAM of other glycan strand by pentaglycine cross bridge synthesized by family of FemABX non-ribosomal peptide. The last D-alanine of pentapeptide of UDP-NAM-pentapeptide is cleaved during transpeptidation reaction that cross-links peptidoglycan (**Figure 4**) [5].

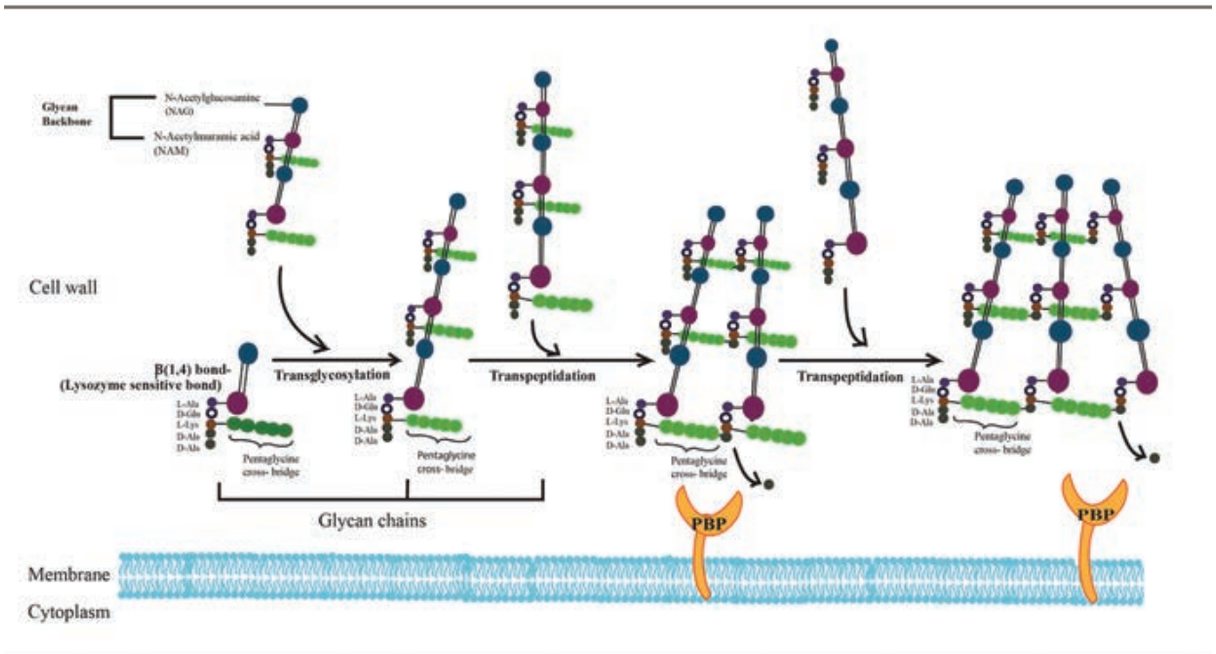

Figure 4. Biosynthesis of staphylococcal peptidoglycan. Peptidoglycan is constructed by transglycosylation and transpeptidation reactions catalyzed by penicillin-binding proteins (PBPs).

Teichoic acids that are polymers of glycerol phosphate or ribitol residues give negative feature to cell membrane and act as receptor of *S. aureus* phage [5].

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6.2. Effect of β-lactam antibiotics against cell wall

Binding of β-lactams to PBPs that have high affinity to β-lactams is lethal for *Staphylococcus* [36]. Transpeptidase domain of PBPs in peptidoglycan is inactivated by β-lactam agents such as penicillins, cephalosporins, and methicillin and oxacillin that are both penicillinase-insensitive β-lactams acting as substrate of PBPs, rather than D-alanyl-D-alanine. Before enzyme substrate complex of β-lactam and PBP is formed completely, they can be dissociated by disrupting noncovalent association between them at the beginning of this complex. Later on, irreversible complex is formed by covalently binding of β-lactam that is a structural analog of D-alanyl-D-alanine substrate of PBP to active site of PBP complex that is the site for the binding of D-alanyl-D-alanine as a substrate during transpeptidation reaction (**Figure 4**). By this way, transpeptidation reaction that is the last step of peptidoglycan biosynthesis is blocked by β-lactam antibiotics that inactivate PBP. *Staphylococcus* undergoes to death due to the inhibition of peptidoglycan biosynthesis [5, 47].

6.3. Mechanism of β-lactam resistance of *Staphylococcus aureus*

β-lactamase enzymes cause resistance of cell to β-lactam antibiotics by inactivating β-lactam antibiotics. β-lactamase inactivates β-lactam antibiotics by disrupting amide bond of β-lactam ring [5].

Expression of the *bla*Z gene that is located in plasmid or transposon and encodes β-lactamase is regulated by *bla*I and *bla*RI that are own regulators. In the lack of β-lactam antibiotic, BlaI that bound to promoter-operator region repress *blaZ* gene, *bla*I-*bla*RI operon, so transcription of *bla*Z is not happen. In the usage in treatment or supplementation of β-lactam antibiotic to growth media, β-lactam binds to BlaRI that is a β-lactam-sensing signal transducer, and then, intracellular zinc metalloprotease domain of BlaRI is separated and cleaves BlaI that is already bound to operator. By this way, in the presence of β-lactam, *blaZ* is transcribed to β-lactamase that permits MRSA to grow by inactivating β-lactam (**Figure 2**) [5].

A study that showed the association between the antibiotic susceptibility patterns and the antibiotic resistance genes in staphylococcal isolates obtained from various clinical samples of patients revealed that 93.5% of *S. aureus* clinical isolates and 86.8% coagulase negative *Staphylococci* strains carry the blaZ gene [48].

6.4. Mechanism of methicillin resistance of *Staphylococcus aureus*

Resistance to methicillin, oxacillin, and nafcillin that are semisynthetic β-lactamase-insensitive β-lactams has developed by acquiring of the *mecA* gene [5]. MRSA is not only resistant to methicillin, but also resistant to all β-lactams [5, 36].

*mec*A gene expression is regulated by *mec*I and *mec*RI that are own regulators. In the lack of β-lactam antibiotic, MecI that bound to promoter-operator region repress *mecA*, and *mec*I-*mec*RI operon, so transcription of *mec*A is not happen. In the usage or supplementation of β-lactam antibiotic to growth media, β-lactam binds to MecRI that is a β-lactam-sensing signal transducer, and then, metallo-protease domain of MecRI that is placed in cytoplasmic site is

separated and cleaves MecI that is already bound to operator. By this way, *mecA* is transcribed to PBP2a of which affinity is low to β-lactams [49]. Low affinity of PBP2a to β-lactams permits MRSA to grow as a result of peptidoglycan synthesis in the presence of β-lactams concentrations that can inactivate transpeptidase activity of PBPs. PBP2a that belongs to PBPs contains transpeptidase domain and non-penicillin-binding protein (**Figure 2**) [5].

Structure, function, mechanism, and molecular organization of *mec*I and *mec*RI are similar to *bla*I and *bla*RI, respectively [36]. Expression of *mecA* is regulated by both MecI and BlaI. When MecI and BlaI are both present at the same time, *mec*A is repressed even stronger; the *mec*I gene of most of the clinical MRSA isolates has deletions; due to this, expression of *mec*A is regulated by BlaI [5].

6.5. Multidrug resistance

There are eight types of *SCCmec* (I-VIII). *SCCmec* type II and III that demonstrate multi-resistance also contain additional antibiotic resistance genes such as erythromycin and tetracycline as well as methicillin. *SCCmec* type IV that is essential for community-acquired MRSA strains (CA-MRSA) that is one of the virulent strains and infect healthy person in community rather than hospital arised. The other types of *SCCmec* are rare [7].

IS*431* is mainly found in chromosome and plasmids of *Staphylococcus* and is also related to encoding various resistance factors such as tetracycline, mercury, and cadmium resistance. If other additional resistance genes such as *aadD* encoding an enzyme for tobramycin resistance are integrated within *SCCmec* cassette (IS*431mec*), multiple drug resistance is developed in methicillin-resistant *Staphylococcus* [36]. Plasmids pUB110, pI258, and pT181 integrated in *SCCmec* have additional resistance genes encoding kanamycin, tobramycin and bleomycin resistance (*ant(4′)*), penicillin and heavy metal resistance, and tetracyclin resistance, respectively. Tn554 integrated in *SCCmec* have additional resistance gene *erm*A encoding inducible macrolide, lincosamide, and streptogramin resistance [49].

6.6. Homogeneous and heterogeneous resistance of MRSA

Heterogeneity is a characteristic of MRSA of which resistance level varies according to contents and ingredients of culture medium in which MRSA is grown and β-lactam antibiotic used. Most of the cells of heterogeneous methicillin resistance (HeR) strains (~99.9% or above) are susceptible to β-lactam of which concentration is low that is about 1–5 μg/mL of methicillin, whereas just a few subpopulations (such as 1 in 10^6 cfu/mL) grow in 50 μg/mL or above of methicillin by expressing high-level resistance. Homogeneous strains (HoR) are resistant to low concentration of β-lactam and can grow in higher concentrations of methicillin that is about 5 μg/mL or above [36].

Heterogeneity of MRSA is unstable and changeable according to growth conditions. HeR strains become homogeneous strains (HoR) by growth media supplemented with NaCI or sucrose for providing hypertonicity of media, or supplemented with higher concentrations of β-lactam antibiotic, or incubated at 30°C in incubator. Supplementation of growth media with EDTA or incubation at 37–43°C leads to conversion of HoR strains to HeR [36]. This conversion

of HeR and HoR in distinct culture conditions is due to the regulation of gene expression by Agr regulator system [42]. These conversions of MRSA can be repeated by repeated culturing in changed media that have different supplementations.

Most clinical isolates of MRSA grow as HeR in routine growth conditions, and most of them show low or moderate level of resistance, whereas a few subpopulations show high-level resistance [36].

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