CHAPTER 2

LITERATURE REVIEW

2.1 *Staphylococcus aureus*

Kingdom: Eubacteria

Phylum: Firmicutes

Class: Bacilli

Order: Bacillales

Family: *Staphylococcaceae*

Genus: *Staphylococcus*

Species: *aureus*

Staphylococcus aureus is a Gram-positive coccus bacterium, which appears as grape-like clusters under a microscope. The bacterium is a facultative anaerobe that uses aerobic respiration in the presence of O_2 but can grow in the absence of it by anaerobic respiration and fermentation (Fuchs *et al*., 2007). Under anaerobic respiration condition, *S. aureus* uses nitrate (NO_3^-) as the electron accepter in electron transport chain and generates ATP through oxidative phosphoryration (Dworkin *et al*., 2006; Fuchs *et al*., 2007). Under fermentation condition, the bacterium generates ATP by fermentation of glucose producing lactate, formate, and acetate through mixed-acid fermentation and also 2, 3-butanediol by butanediol fermentation (Fuchs *et al*., 2007). It can grow at temperatures ranging from 6.5 to 45^{o}C with optimum temperatures between 30 and 37^{o}C (Prescott *et al*., 2002).

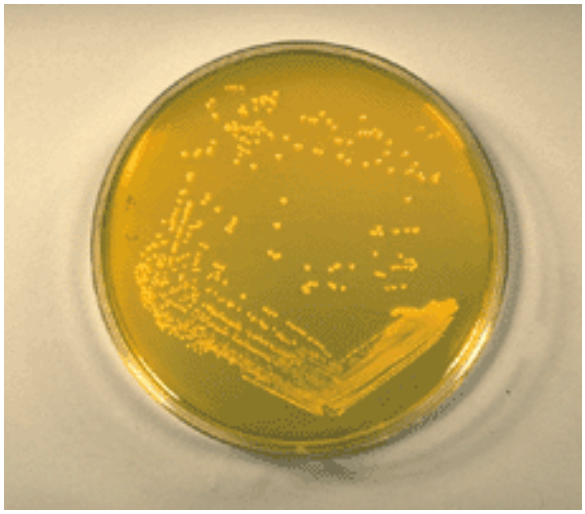
S. aureus is an osmotolerant microorganism that can grow in media containing high concentrations of sodium chloride (more than 3 M). *S. aureus* uses organic compounds such as carbohydrates and lipids as carbon and energy source because oxidation of these compounds releases energy. The bacterium also uses organic compounds as electron donors in oxidation-reduction reaction (Prescott *et al*., 2002).

S. aureus (Fig. 2.1) forms fairly large yellow colonies on rich medium that is the origin of the bacterium's name ("aureus" means "golden" in Latin). The golden color of *S. aureus* results from a carotenoid pigment named staphyloxanthin (Clauditz *et al*.,

2006). Moreover, staphyloxanthin has an antioxidant function that protects *S. aureus* from killing by oxidizing chemical such as hydrogen peroxide, superoxide radical, and hypochloride of host cell (Clauditz *et al*., 2006). *S. aureus* produces coagulase making the bacteria differ from other strains of staphylococci such as *S. epidermidis* and *S. saprophyticus* that do not produce coagulase. Coagulase converts fibrinogen dissolved in blood stream into undissolved fibrin form. Undissolved fibrin causes clot formation leads to depositing on cell surface of *S. aureus*, which protects the bacterium from host defense mechanisms. Coagulase production is therefore the criterion used in the laboratory for isolation of S*. aureus* that is a coagulase poitive causing clot formation while the most staphylococci species are coagulase negaive (Fig. 2.2) (Prescott *et al*., 2002; Fischetti *et al*., 2006).

FIGURE 2.1

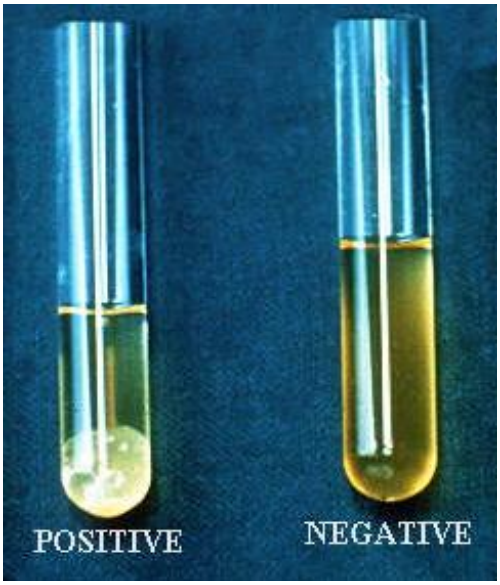
Golden Colonies of *Staphylococcus aureus*



(Retrieved July 13, 2010, from www.biotec.com/images/mansaltagar)

FIGURE 2.2

Coagulase Test of *Staphylococcus aureus*



The positive result shows clot formation in plasma solution (left), in contrast, the negative result has no clot formation (right).

(Retrieved July 13, 2010, from www.chsweb.lr.k12.nj.us/coagu)

2.2 *S. aureus* Cell Walls

Gram-positive bacterial cell wall is the layer of peptidoglycan polymers containing teichoic acids and wall-associated surface proteins and lies outside the plasma membrane. It is important to maintain shape and protect the bacteria from osmotic pressure.

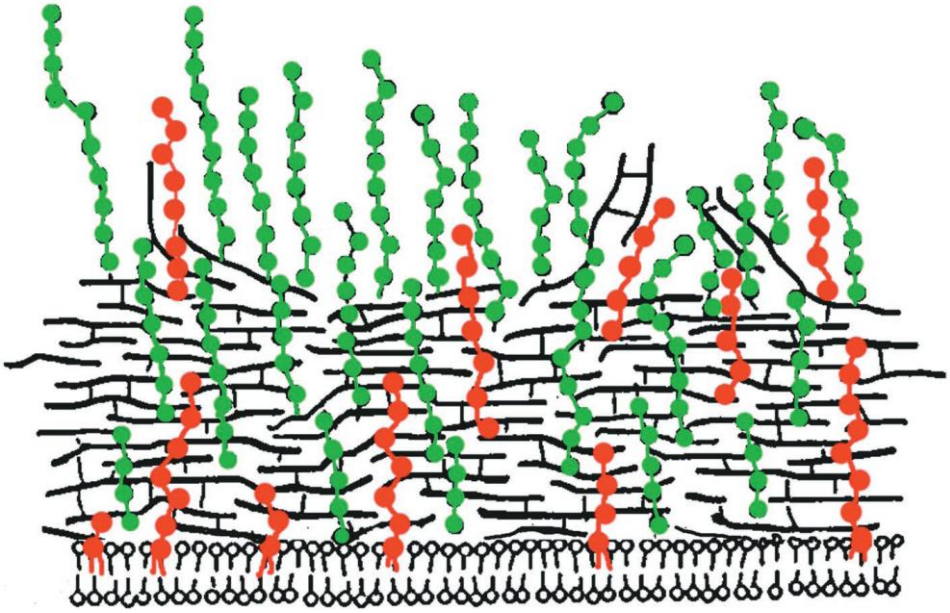
S. aureus cell wall composes of peptidoglycan and teichoic acids that are composed of wall teichoic acid (WTA), covalently linked to *N*-acetylmuramic acid of peptidoglycan, and lipoteichoic acid (LTA), which is attached to plasma membrane (Fig. 2.3) (Navarre and Schneewind, 1999; Neuhaus and Baddiley, 2003). Teichoic acid of *S. aureus* is anionic polymers of ribitol-phosphate, whereas lipoteichoic acid is composed of polymerized glycerol-phosphate (Navarre and Schneewind, 1999). Due to negative charge of teichoic acid, it plays a role in many functions including maintenance of cation homeostasis, defining to electrochemical property of cell wall, biofilm formation, and resistance to cationic antimicrobial peptides of human phagocytes (Gross *et al*., 2001; Neuhaus and Baddiley, 2003). LTA plays a role in autolysin or peptidoglycan hydrolase

modulation. LTA is modified by esterified D-alanine after synthesis. Thus degree of D-alanine influences inhibitory activity of autolysin (Neuhaus and Baddiley, 2003).

Peptidoglycan or murein is a polymer of muropeptides containing two sugar residues, *N*-acetylglucosamine (GlcNAc) linked with *N*-acetylmuramic acid (MurNAc), which attached by a pentapeptide chain, by a β-1,4-glycosidic linkage. The pentapeptide carries L-alanine, D-isoglutamine, L-lysine and an intact D-alanyl-D-alanine. Muropeptides are joined by highly cross-link of pentaglycine oligopeptide units at lysine of one muropetide to D-alanine of neighboring one (Prescott *et al*., 2002; Fischetti *et al*., 2006). Monomeric muropeptides are about 6% of all muropeptides (Fig. 2.4A). About 20% of all muropeptides are dimers of two muropeptides (Fig. 2.4B). About 40% are oligomers (Fig. 2.4C) containing three to nine muropeptides joined by the same cross-linking (Fischetti *et al*., 2006).

FIGURE 2.3

Topography of Cell Wall Structure of *Staphylococcus aureus*

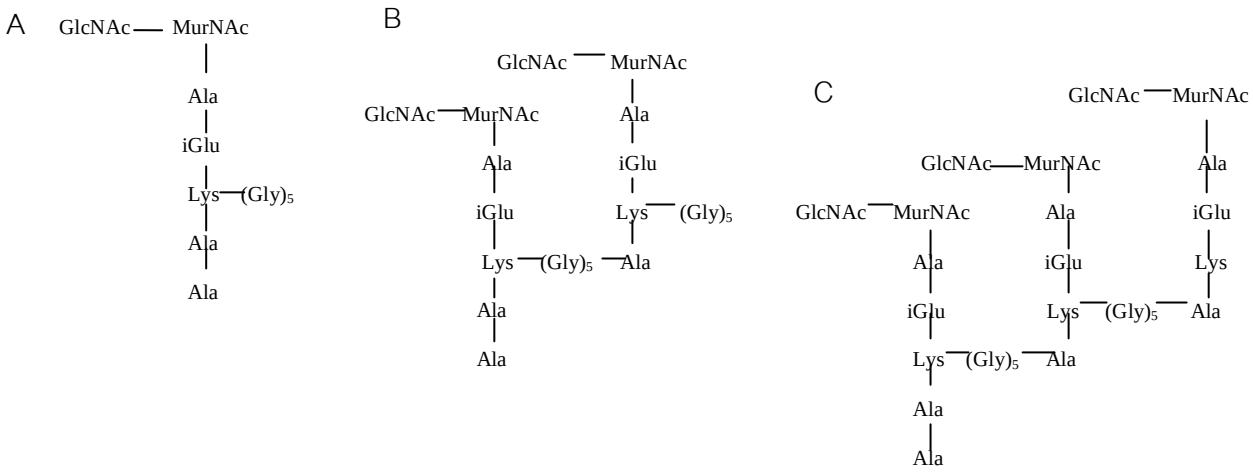


S. aureus cell wall consists of peptidoglycan (black lines), together with WTA (green) and LTA (red).

(Neuhaus and Baddiley, 2003)

FIGURE 2.4

Structure of Muropeptide Components



Various structures of building blocks of peptidoglycan consist of a monomeric muropeptide (A), a dimeric muropeptide (B), and an oligomeric muropeptide (C). (Fischetti *et al*., 2006)

2.2.1 Synthesis of Peptidoglycan

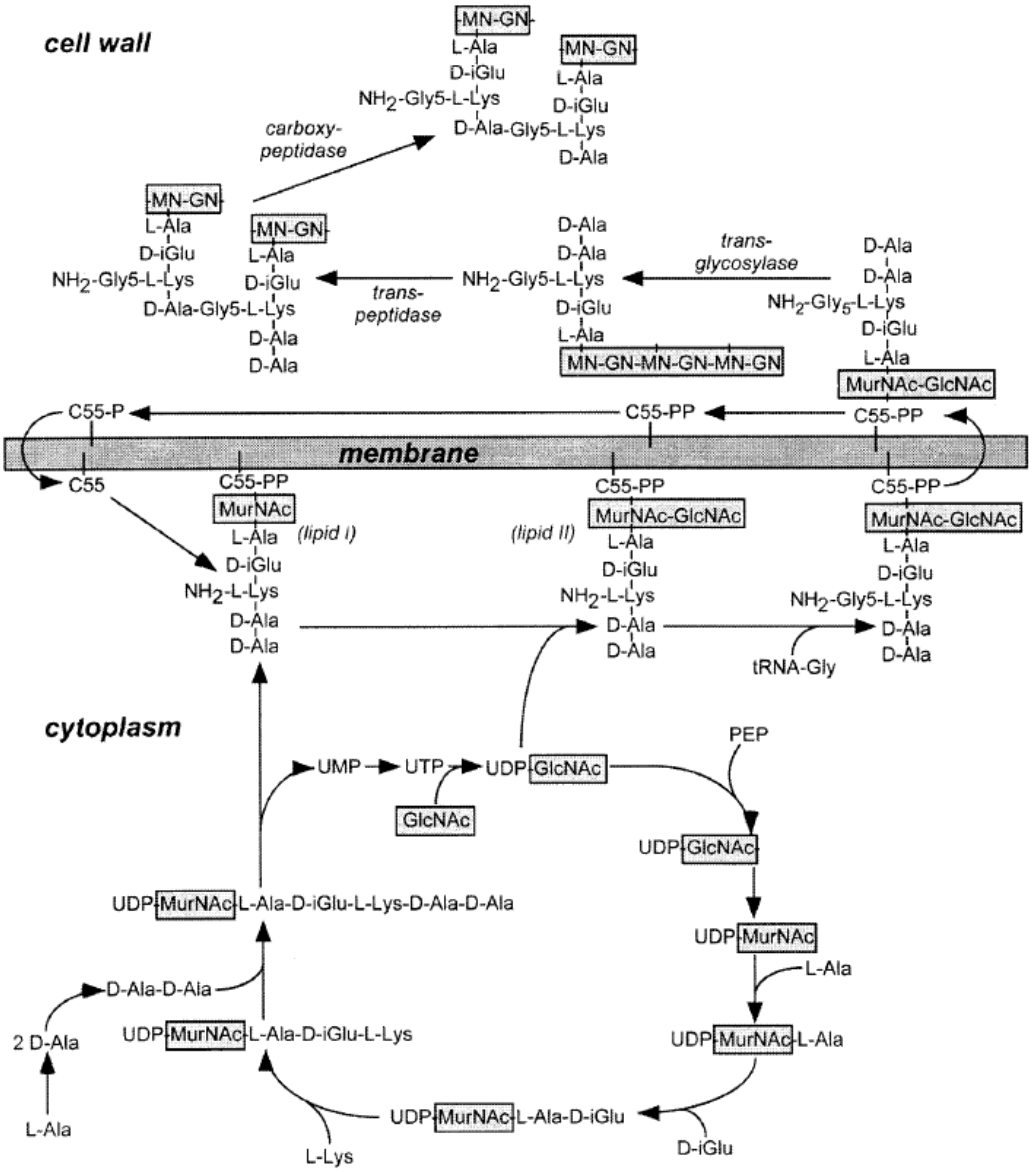
Monomers of peptidoglycan are synthesized in the cytoplasm before transporting to outside the cell membrane for polymerization. Two carriers, uridine diphosphate (UDP) and bactropenol pyrophosphate (C55-PP), transport them through the cell membrane.

The peptidoglycan synthesis begins from synthesis of murein precursors, *N*-acetylglucosamine-UDP and *N*-acetylmuramic acid-UDP, in the cytoplasm. Amino acids are added to UDP-MurNAc to form UDP-MurNAc-peptapeptide. Then UDP is separated and MurNAc-pentapeptide is transferred to bactoprenol pyrophosphate. UDP-GlcNAc is added to NAM-pentapeptide to form the peptidoglycan repeat unit and peptaglycine is then added at the third amino acid, lysine, in the pentapeptide of the peptidolycan repeat unit. The peptidoglycan repeat unit is transported from cytoplasm to outer surface of the membrane by bactoprenol pyrophosphate carrier to form peptidoglycan chains. Finally, cross-link between each glycan chain is formed by peptaglycine interbridge. This step, free amino group of the pentaglycine attacks the D-alanyl-D-

alanine residue of the pentapeptide results in releasing the terminal D-alanine residue (Fig. 2.5) (Prescott *et al*., 2002).

FIGURE 2.5

Peptidoglycan Synthetic Parthway in *Staphylococcus aureus*



Syntheis of cell wall precursors begins in the cytoplasm, resulting in UDP-MurNAc–L-Ala–D-iGlu–L-Lys–D-Ala–D-Ala. The peptidoglycan precursor subunit is transferred to a lipid carrier (C55-PP) in the membrane. After further modification, the lipid-anchored peptidoglycan precursor is translocated to the outer surface of the cytoplasmic

membrane. The peptidoglycan precursor is incorporated into the cell wall by transpeptidation and transglycosylation reactions. The terminal D-alanine of the pentapeptide is removed by the action of the free amino group of the pentaglycine. (Navarre and Schneewind, 1999)

2.3 Pathogenesis of *S. aureus*

S. aureus can cause a diversity of diseases ranging from minor skin infections to life-threatening infections in both humans and animals. These bacteria can live freely in environment without host organisms or live within tissues or cells of host organisms. *S. aureus* can infect host organisms when their skins have been injured. When the bacterium enters the host tissues, it will produce extracellular proteins or other important factors that can help the bacteria to survive in host cells. For example, coagulase coagulates fibrinogen in plasma results in clot formation. The clots protect bacterial cells from phagocytosis by host cells. Surface proteins such as fibrinogen-binding protein and fibronectin-binding protein attack to local cellular of diverse tissues promoting widespread invasion in host cells. These processes evoke immune response of host cells. The immune response such as circulating antibodies and generating fibrin created lyse host cells and bacterial cells lead to lesion or pus (Prescott *et al*., 2002; Fischetti *et al*., 2006).

Staphylococcal skin infections are abscesses or boils on the skin. The infections include carbuncle, furuncle, pyoderma and Impetigo. Although staphylococcal infections usually occur as superficial skin infections, deeply penetrating infections can occur depending on healthy individuals. For example, aggression of *S. aureus* to heart valves can cause endocarditis and can spread other tissues. Moreover, there are many life-threatening diseases that associate with *S. aureus* infections such as osteomyelitis, mastitis, and pneumonia (Prescott *et al*., 2002; Fischetti *et al*., 2006).

Several staphylococcal diseases are caused by toxins, such as food poisoning and toxic shock syndrome. Staphylococcal food poisoning is a result of various enterotoxins of *S. aureus* that contaminate in food. Toxic shock syndrome usually occurs in women due to using highly absorbent tampons during menstruation. Toxic shock

syndrome toxin 1 (TSST-1) is a causative agent of this disease (Prescott *et al*., 2002; Fischetti *et al*., 2006).

Spread of staphylococcal infections in community usually occurs through patient to other people. Risk behaviors, such as the use of handkerchief that belongs to patient with carbuncle or the use of nasal ointment containing mupirocin, can aid in increasing spread of community-acquired and also hospital-acquired infections (Fischetti *et al*., 2006).

Hospital-acquired infections are related to many factors. One of the most important factors is the operative procedure such as the degree of microbial contamination of operative devices and the duration of operation. Spread of antimicrobial resistant strains from patients to patients is also common cause of hospital-acquired infections (Fischetti et al., 2006; Hidron *et al*., 2009).

2.4 Treatment and Antibiotic Resistance

Since successful trials of penicillin in animal and human infected with staphylococci were reported in 1940, penicillin was produced and used in staphylococcal infection therapy in the early 1940s (Prescott *et al*., 2002). However, widely use of this drug leads to penicillin resistance in *S. aureus*. In 1950, 40% of *S. aureus* isolates from hospital were penicillin resistance and this occurrence had risen to 80% in 1960 (Chambers, 2001). Staphylococcal resistance to penicillin is mediated by β-lactamase that is produced from penicillin-resistant *S. aureus*. The enzyme is able to cleave β-lactam ring of penicillin molecules resulting the antibiotic ineffective (Fischetti *et al*., 2006).

2.4.1 Methicillin Resistance

To resolve the problem occurred from penicillin resistance, semisynthetic penicillins, such as methicillin, were developed. Methicillin was able to resist degradation by staphylococcal β-lactamase and used to treat penicillin-resistant *S. aureus*. However, immediately after methicillin was introduced, methicillin-resistant *S. aureus* (MRSA) was first reported in England in 1961 (Jevons, 1961). Since 1980s, MRSA has become common infections in hospitals worldwide (Bozdogan *et al*., 2004).

Not only the number of hospital-acquired infections caused by MRSA (HA-MRSA) is increasingly reported, the risk in mortality for patients infected with MRSA also increases. The report from Concord hospital in 1993 revealed mortality of patients infected with MRSA was higher than patients infected with methicillin-susceptible *S. aureus* (Fischetti *et al*., 2006). Despite many efforts to inhibit their spread, they remain the most common causes of hospital- and community-acquired infections.

In Norway, there were 216 case reports of community-acquired MRSA (CA-MRSA) in 2003 compared to 67 case reports three years ago (Hansen *et al*., 2005). Moreover, with the emergence of CA-MRSA, reports of community-acquired necrotising pneumonia in young patients and others without the hospital-acquired risk factors were increasing (Hidron *et al*., 2009). Emergence of CA-MRSA also rises in Southeast Asia including report of patients with serious infections of MRSA in a range of 2001 to 2004 in Singapore (Hsu *et al*., 2005) and the first report of MRSA in Malaysia (Shamsudin *et al*., 2008). In Thailand, MRSA highly spreads in tertiary government hospital and risk factors of epidemiology were such as long admission and history of MRSA infection (Jariyasethpong *et al*., 2010).

Community-acquired infection has different characteristics from those of hospital-acquired infection (Huang *et al*., 2006; Millar *et al*., 2008). Patients with CA-MRSA usually are young healthy individuals in the community and lack risk factors of HA-MRSA including prolonged-antibiotic treatment, indwelling devices, long-term hospitalization, and co-morbid conditions such as diabetes and chronic renal failure. Most of CA-MRSA infections are skin and soft tissue infections; however, there are cases of septic shock and pneumonia. CA-MRSA contains *SCCmec*, a staphylococcal chromosome cassette *mec* that introduces *mecA* into *S. aureus*, type IV whereas HA-MRSA is referred to *SCCmec* type I, II, and III (Huang *et al*., 2006; Millar *et al*., 2008).

MRSA can often be resistant to all clinically available β-lactam antibiotics. The genetic basis of this resistance is the *mec*A gene that encodes an altered penicillin-binding protein (PBP2a) that has low affinity for binding β-lactam results in resistance to all β-lactam antibiotics (Fischetti *et al*., 2006)

2.5 Vancomycin and Resistance

The resistance of MRSA strains to β-lactam antibiotics leads to the development of new board-spectrum antibiotics. Vancomycin (Fig. 2.6) is a glycopeptide antibiotic used in treatment of infections caused by Gram-positive bacteria. It becomes important in clinical use after the spread of MRSA.

2.5.1 History

Vancomycin was discovered more than fifty years at Eli Lilly Company. In 1952, Dr. Edmund Kornfield, an organic chemist at Eli Lilly, isolated *Streptomyces orientalis* from a sample of dirt. This organism produced a substance named “compound 05865” that was active against Gram-positive organisms including penicillin-resistant staphylococci. The compound was tested with an animal experiment and result was that it might be safe and effective in humans. The compound was then improved by passaging over an ion-exchange resin and resulted in drug that was named “vancomycin”, from the word “vanquish” (Levine, 2006).

Vancomycin was tested with volunteers and resulted in successful treatment in 8 of 9 patients with staphylococcal infection. As information about this success, vancomycin was requested for cases which other drugs were failure. Several reports of successful treatment were submitted to the US Food and Drug Administration. Vancomycin was immediately approved and confirmed efficacy of the drug in 1958 (Levine, 2006).

2.5.2 Toxicity

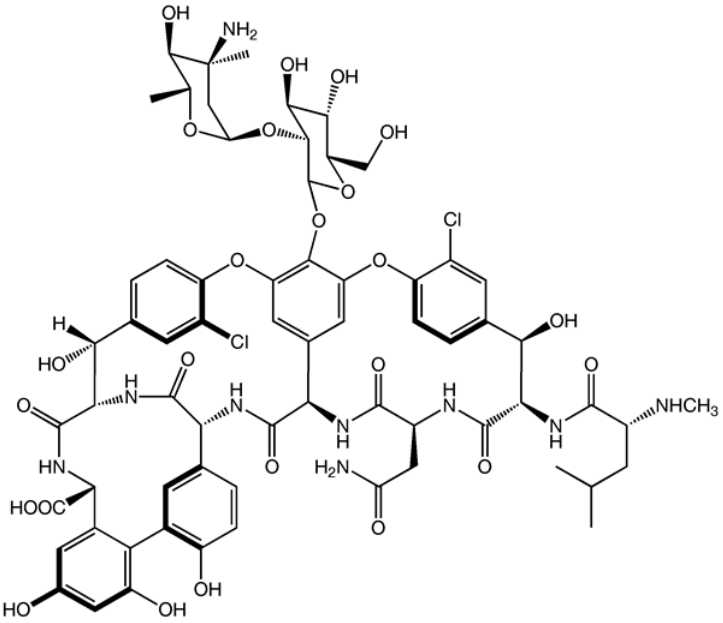
In the initial trails, vancomycin was considered to be less toxic. Ototoxicity, especially presenting as tinnitus, was observed by investigation of elevated serum concentrations found in patients with renal failure. This problem occurred in association with the use of early preparation of vancomycin and was attributed to the removal of impurities in the drug. However, subsequent studies indicated that ototoxicity by using vancomycin was an infrequent occurrence. Using purer forms of vancomycin found nephrotoxicity was a side effect, but it was possibly potentiated by concomitant aminoglycoside therapy. Moreover, the combination of vancomycin and aminiglycoside

was reported to cause renal failure, especially in adults who received prolonged therapy and vancomycin concentrations over 10 µg/ml (Levine, 2006).

Rapid infusion of vancomycin in a dilute solution has been associated with red man syndrome or red neck syndrome. This syndrome characterized by flushing and an erythematous rash that affect to a face and neck. Sometimes, hypotension and angioedema may occur. This syndrome usually appearing within 4 to 10 minutes after completion of an infusion (Rybak *et al*.,1992; Levine, 2006).

FIGURE 2.6

Structure of Vancomycin Molecule



(Retrieved July 13, 2010, from http://en.wikipedia.org/wiki/File:Vancomycin)

2.5.3 Mechanism of Action

Vancomycin acts by inhibiting cell wall biosynthesis of Gram-positive bacteria. It prevents incorporation of GlcNAc and MurNAc peptide subunits to form peptidoglycan. The drug binds to the terminal D-alanyl-D-alanine residue of pentapeptide forming hydrogen bond interaction (Schafer *et al*., 1996; Prescott *et al*., 2002; Fischetti *et al*., 2006; Levine, 2006). When the D-alanyl-D-alanine molecule is captured, peptidoglycan subunits will not connect together (Prescott *et al*., 2002; Fischetti *et al*., 2006). Thus, vancomycin halts formation of new cell walls during cell division and stops bacteria from

dividing. This effectively prevents bacteria from growing but does not kill bacteria (Reynolds, 1989).

2.5.4 Vancomycin Resistance

Vancomycin, a glycopeptide antibiotic, is the first-line treatment for MRSA infections. Until the late 1990s, *S. aureus* was uniformly susceptible to vancomycin. Unfortunately, with an increased use of the drug, *S. aureus* strains became reduced susceptibility to vancomycin. These strains were isolated from patients who were received long-term chemotherapy with vancomycin (Fischetti *et al*., 2006).

Vancomycin susceptibility levels are used to define vancomycin resistance in *S. aureus*. According to Clinical and Laboratory Standards Institute (CLSI), *S. aureus* strains for which MIC of vancomycin is $\leq$ 2 µg/ml are vancomycin-susceptible *S. aureus* (VSSA), strains with MIC of vancomycin is 4 to 8 µg/ml are defined as vancomycin-intermediate *S. aureus* (VISA), and strains for which MIC of vancomycin is $\geq$ 16 µg/ml are vancomycin-resistant *S. aureus* (VRSA) (CLSI, 2010).

The report of VISA from Japan in 1997 was the first case of a reduced susceptibility strain of vancomycin (Hiramatsu *et al*., 1997). Since then, VISA strains have been increasingly reported worldwide including Asia (Bierbaum *et al*., 1999; Srinivasan *et al*., 2002; Bozdogan *et al*., 2004; Song *et al*., 2004). Furthermore, there were reports of VISA strains showing heterogeneous resistance to vancomycin (hVISA) from many Asian countries (Song *et al*., 2004). Heterogeneous VISA strains were found in India, South Korea, Japan, Philippines, Singapore, Thailand, and Vietnam during the period from 1997 to 2000 (Song *et al*., 2004). The hVISA is defined as the strain that contains subpopulation of vancomycin-intermediate daughter cells which the vancomycin MICs for the parental strain of these daughter cells fall in the range of 1 to 4 µg/ml (Liu and Chambers, 2003; Song *et al*., 2004). It has been suggested that hVISA associated with treatment failure with vancomycin (Srinivasan *et al*., 2002; Liu and Chambers, 2003; Loomba *et al*., 2010). The clinical treatment failure was due to the character of heterogeneous resistance of hVISA resulting in survival of resistant subpopulations which were able to grow in higher concentrations of vancomycin than the MICs of their strains (Liu and Chambers, 2003). This contributed to increasing

number of patients who infected with hVISA (Srinivasan *et al*., 2002; Song *et al*., 2004; Maor *et al*., 2007). Moreover, long-term exposure of hVISA to vancomycin favors outgrowth of resistant subpopulations of the hVISA, therefore it precedes the development stage of VISA (Liu and Chambers, 2003; Maor *et al*., 2007). Thus, the occurring of hVISA is warning that *S. aureus* strains with high resistance level might emerge.

Not only occurring of VISA, long-term therapy with vancomycin also leads to high-level of vancomycin resistance (Fischetti *et al*., 2006). In the period ranging from 2002 to 2004, VRSA strains were reported from Michigan, Pennsylvania, and New York (Song *et al*., 2004; Fischetti *et al*., 2006). Since then, VRSA strains were reported from many countries such as Japan (Cui *et al*., 2003), Germany (Bozdogan *et al*., 2004), and Brazil (Palazzo *et al*., 2005). Recently, the Centers for Disease Control and Prevention (CDC) reported latest case of VRSA infection in USA in 2010 (CDC, 2010). Moreover, hospitalized patients infected with VRSA were increasingly found (Loomba *et al*., 2010).

Several years after the first VISA report, there have been investigated VISA and VRSA strains to identify mechanism of vancomycin resistance. Although the true mechanism of vancomycin resistance in *S. aureus* is not known, there is described about common features of *S. aureus* strains with reduced susceptibility to vancomycin that differ from susceptible strains. Those features of VISA and VRSA such as reduced peptidoglycan cross-linking, increased cell wall thickness, and reduced autolyic activity are well described (Pfeltz *et al*., 2000; Cui *et al*., 2003; Koehl *et al*., 2004; Fischetti *et al*., 2006; Utaida *et al*., 2006).

In the case of VRSA, it is possible that due to *vanA* gene of *Enterococcus faecalis* that was successfully transferred to *S. aureus* through plasmid containing *van* gene in the laboratory (Srinivasan *et al*., 2002; Fischetti *et al*., 2006). However, *vanA* has not been found in VISA isolates (Srinivasan *et al*., 2002; Cui *et al*., 2006). VanA-type resistance is mediated by transposon Tn*1546* which encodes VanH, a dehydrogenase. VanH reduces pyruvate to D-lactate and resulting in formation of D-alanyl-D-lactate replaces D-alanyl-D-alanine in stage of peptidoglycan precursor synthesis. D-alanyl-D-lactate reduces affinity of vancomycin binding to peptidoglycan precursors (Courvalin *et al*., 2006; Fischetti *et al*., 2006). Moreover, the other resistance phenotypes that modify

vancomycin-binding targets are such as *vanB* and *vanC* that possess D-alanyl-D-lactate and D-alanyl-D-serine residues, respectively (Courvalin, 2006; Fischetti *et al*., 2006).

2.5.5 Cell Wall Thickening

Cui *et al*. (2003) reported that VRSA strains had significantly thickened cell walls comparing with vancomycin-susceptible control strains (Fig. 2.7). Moreover, when the VRSA strains were subjected to drug-free medium passages, passage-derived strains with decreased MICs of vancomycin were obtained and their cell walls became thinner. These results showed that the thickness had correlation with the MICs of vancomycin and the thickened cell wall is responsible for vancomycin resistance in VRSA strains (Cui *et al*., 2003).

Hanaki *et al*. (2006) investigated cell wall synthesis in VRSA strains and found increasing in amounts of intracellular murein monomer in these strains comparing with their vancomycin-susceptible control strains. Transmission microcopy electron showed the thickened cell wall in VRSA strains comparing with their control strains (Hanaki *et al*., 1998). Similarly, VISA strains also have the thickened cell wall (Pfeltz *et al*., 2000; Koehl *et al*., 2004; Fischetti *et al*., 2006).

The thickened cell wall may contribute to vancomycin resistance in *S. aureus* strains (Pfeltz *et al*., 2000; Srinivasan *et al*., 2002; Cui *et al*., 2003; Cui *et al*., 2006; Fischetti *et al*., 2006). The studies of the resistance strains possessing thickened cell wall show that they have overproduction and accumulation of proportion of monomeric muropeptides containing intact terminal D-alanyl-D-alanine more than dimeric and oligomeric muropeptides at the outer surface of cell membrane (Pfeltz *et al*., 2000; Cui *et al*., 2003; Koehl *et al*., 2004; Fischetti *et al*., 2006). Monomeric muropeptides terminating D-alanyl-D-alanine residues are known to be the binding sites of vancomycin. The mechanism of resistance may be correlated to trapping of vancomycin at the binding sites that disorganized the pepidoglycan biosynthesis that accumulate at the outer surface, thus sequestering vancomycin molecules from reaching true target at plasma membrane (Sieradzki *et al*., 1997; Pfeltz *et al*., 2000; Cui *et al*., 2006; Fischetti *et al*., 2006). This similar to “false-target hypothesis”, which proposed by Hiramatsu (1998)

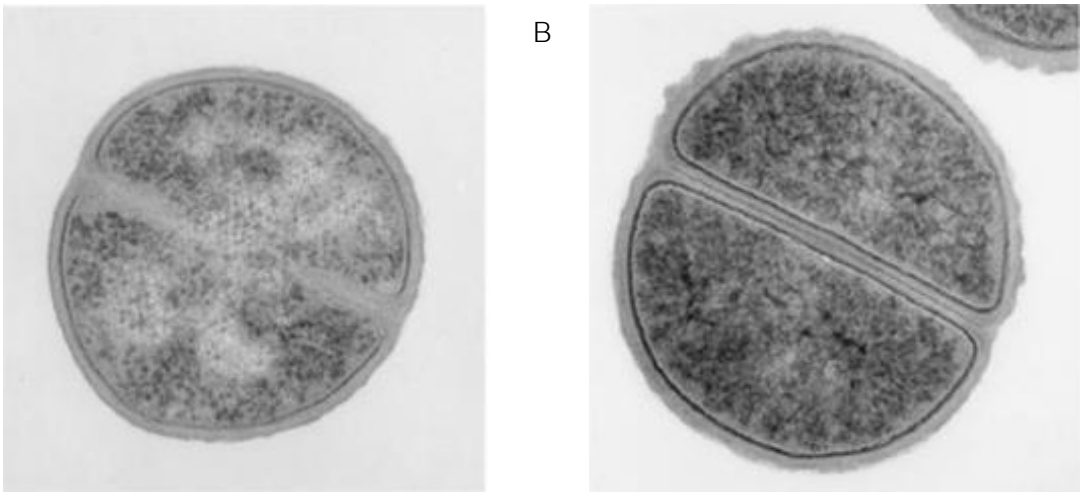
in that free D-alanyl-D-alannine binding sites shield the true targets of glycopeptide antibiotics (Hiramatsu, 1998).

Moreover, there were reports suggested that the reduced vancomycin-susceptible *S. aureus* had uneven (roughened) cell wall surface (Sieraszki and Tomasz, 1997; Pfeltz *et al*., 2000). The authors suggested that VISA had uneven walls when the bacteria grown in the medium containing vancomycin. They demonstrated that vancomycin may halt cell wall turnover leading to retention of cell wall materials at the outer surface of plasma membrane and resulting in abnormal cell wall morphologies (Sieraszki and Tomasz, 1997; Pfeltz *et al*., 2000).

FIGURE 2.7

Transmission Electron Microscopy of Vancomycin Susceptible *Staphylococcus aureus* and Vancomycin Resistant Strain

A B



Cell wall morphology of susceptible strain (A) and resistant strain of *S. aureus* (B). The cell wall surface of the resistant strain is more thickened and roughened than the susceptible strain.

(Cui *et al*., 2003)

2.5.6 Autolysins in Staphylococci

Autolysis is the mechanism of cell wall degradation leading to cell lysis by itself that occurs through activity of autolysins or peptidoglacan hydrolases. Autolysin plays an import role in cell growth, cell separation, and peptidoglycan remodeling (Sugai *et al*., 1997; Prescott *et al*., 2002; Boyle-Vavra *et al*., 2003). When there is high osmotic pressure in a bacterial cell, it needs to add new peptidoglycan instead weak peptidoglycan for cell growth. To reorganize the peptidoglycan structure, the old peptidoglycan is lysed by autolysins and then the new peptidoglycan is able to form the new structure (Prescott *et al*., 2002; Fischetti *et al*., 2006).

The *atl* gene in *S. aureus* encodes an autolysin, a bifunctional protein of molecular size of 138-kDa. The protein contain an amidase domain and glucosaminidase domain that undergoes proteolytic processing to generate a 51-kDa endo-*β*-*N*-acetylglucosminidase (GL) and a 62-kDa *N*-acetylmuramyl-L-alanine amidase (AM) at the cell wall (Sugai *et al*., 1997; Fischetti *et al*., 2006; Ledala *et al*., 2006; Antignac *et al*., 2007). Not only *atl*, several autolysin genes are also present in *S. aureus* including *lytM*, which encodes glycyl-glycine endopeptidase and *lytN*, which encodes cell wall hydrolase (Ramadurai and Jayaswal, 1997; Ingavale *et al*., 2003; Singh *et al*., 2010). These autolysins have hydrolytic activities which are specific for different structural components of peptidoglycan (Ingavale *et al*., 2003).

LytM, molecular mass is approximately 34 kDa, was identified in *S. aureus* strain with autolysis-deficient mutant (Lyt^{-}) (Ramadurai and Jayaswal, 1997; Singh *et al*., 2010). There have been studies for properties of LytM in several years and found that LytM was elevated in vancomycin-resistant *S. aureus* comparing to susceptible stains (Mongodin *et al*., 2003). The expression of LytM was highest in early exponential phase of growth (Singh *et al*., 2010). However, role as the autolysin should be further investigated (Singh *et al*., 2010).

The regulation of autolysins in *S. aureus* is complex. Ingavale *et al*. (2003) reported that two-component regulatory systems of autolytic activities, LytSR and ArlSR, were decreased expression by *rat* gene resulting in negative regulation of *lytM* and *lytN* expression but not for *atl* (Ingavale *et al*., 2003). Moreover, the change in cell wall teichoic acid affected autolytic activities (Koehl *et al*., 2004). This due to *atl* gene

products are located on teichoic acid (Neuhaus and Baddiley, 2003; Fischetti *et al*., 2006).

Cell wall-active antibiotics such as vancomycin, oxacillin, and penicillin affect to block peptidoglycan biosynthesis and often results in induction of autolytic enzymes leading to lysis of the bacterial cells (Sugai *et al*., 1997; Prescott *et al*., 2002; Ledala *et al*., 2006; Antignac *et al*., 2007). On the other hand, the vancomycin resistant strains of *S. aureus* have reduced autolytic activity (Pfeltz *et al*., 2000; Boyle-Vavra *et al*., 2003 Utaida *et al*., 2006). Sieradzki and Tomasz (2006) reported the effect of vancomycin on autolysins of VISA. The authors revealed that vancomycin attached to D-alanyl-D-alanine residues at the outer surface of cell membrane blocking the access of autolysins to cell wall substrates resulting in inhibition of cell wall degradation. This result contributed to vancomycin tolerance in VISA and allowed them to survive in high concentrations of the drug.

Pfeltz *et al.* (2000) reported that VISA strains had reduced autolytic activity comparing to parental strains. The authors suggested that the reduced autolytic activity may contribute to the accumulation of cell wall materials in the vancomycin resistance strains possessing the thickened cell walls that associated with increasing binding sites of vancomycin. Thus the reduced autolytic activity correlates to preventing peptidoglycan from degradation of vancomycin and leading to tolerance of vancomycin in the vancomycin resistance strains (Pfeltz *et al*., 2000).

Similarly, Koehl *et al.* (2004) studied autolytic enzyme properties of VISA and their parental strains. VISA had reduced autolytic activity compared to that of parental strains. Northern blot analysis results indicated that *atl*, the major autolysin gene, was significantly down-regulated in VISA strains comparing to parental strains. The authors attributed that decreased autolytic activity was the common feature of VISA strains and could be involved in tolerance of vancomycin (Kohel *et al*., 2004).

2.5.7 New Antibiotic Against Vancomycin Resistance

Due to increasing in problem of vancomycin resistance, the development of new drugs to replace vancomycin has been occurred. Currently, available glycopeptides antibiotics are not always adequate against organisms. The search for new antibiotic active against antibiotic-resistant gram-positive cocci has revealed such as oxazolidinones, streptogramins, everninomycin, and new glycopeptides. Oxazolidinones and streptogramins are currently available in some countries to overcome the problem of glycopeptides-resistant pathogens. Linezolid, a member of the oxazolidinones class, has been proved to be an important antibiotic for treating infections caused by multi-drug resistant gram-positive cocci. Oritavancin, a lipoglycopeptide antbiotic was developed in the 1990s (Bouza and Burillo, 2010). Mode of action of oritavancin is inhibition of cell wall biosynthesis by binding both to D-alanyl-D-alanine and D-alanyl-D-lactate. Thus, the drug can against VISA, VRSA, and vancomycin-resistant enterococci, and also other Gram-positive bacteria such as streptococci (Bouza and Burillo, 2010).

Oritavancin has shorter duration therapy and higher bactericidal activity than vancomycin (McKay *et al*., 2009). Another advantage of oritavancin is activity against stationary phase of bacterial growth and bacteria in biofilm leading to the choice for treating prosthetic device infections (Bouza and Burillo, 2010).