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Review Article

Significance of MRSA in nosocomial Infections

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ABSTRACT

Staphylococcus aureus is one of the most virulent microbial pathogen amongst gram positive bacteria to cause nosocomial and community acquired infections. An additional concern is the emergence and dissemination of nosocomial organisms with increased resistance to antimicrobial agents. Such microbes include methicillin resistant *S.aureus* (MRSA), *S.epidermidis,* vancomycin resistant *Enterococci* (VRE) and VISA. The development of vaccines and drugs that prevent and cure bacterial infections was one of the twentieth century's major contributions to human longevity and quality of life. Antibacterial agents are amongst the most commonly prescribed drugs of any kind worldwide. Used appropriately, these drugs are lifesaving however, their indiscriminate use drives up the cost of health care leading to a plethora of side effects and drug interactions and fosters the emergence of bacterial resistance rendering previously valuable drugs useless.

INTRODUCTION

Staphylococcus aureus is one of the most common and important pathogen, responsible for the majority of nosocomial infections. *S.aureus* is an opportunistic bacterium, normally, part of the human micro-flora but, attacks immediately when the immune system of the host becomes susceptible. Even though *S.aureus* can be found in different parts of the body but anterior nares are the principal ecological sites in humans[1]. A study had reported nasal carriage as a major risk factor for *S.aureus* infection that differs from person to person. It had been reported that 20% of the healthy individuals carry *S.aureus* persistently, 60% intermittently and 20% never carry *S.aureus*[2].

Staphylococcus aureus is universal in distribution found in pus, boils, abscess, skin, throat, nasophrynx, oral mucosa, soil, sewage, milk and water. They are gram positive cocci arranged singly ranging from 1µm in diameter; pairs, tetrads and short chains but appear predominantly in grape like clusters. The cell wall is composed of peptidoglycan and teichoic acid[3,4].

Mechanism of antibiotic resistance

Penicillin resistance

History and epidemiology

The mortality of patients with *S. aureus* bacteremia in the pre-antibiotic era exceeded

80%, and over 70% developed metastatic infections[5]. The introduction of penicillin in the early 1940s dramatically improved the prognosis of patients with staphylococcal infection. However, as early as 1942, penicillin-resistant staphylococci were recognized, first in hospitals and subsequently in the community[6]. By the late 1960s, more than 80% of both community and hospital-acquired staphylococcal isolates were resistant to penicillin. This pattern of resistance, first emerging in hospitals and then spreading to the community, is now a well-established pattern that recurs with each new wave of antimicrobial resistance[7].

Mechanism of resistance

Staphylococcal resistance to penicillin is mediated by *blaZ*, the gene that encodesβ-lactamase (**Figure 1a**). This predominantly extracellular enzyme, synthesized when staphylococci are exposed to β-lactam antibiotics, hydrolyzes the β-lactam ring, rendering the β-lactam inactive. The gene *blaZ* is under the control of two adjacent regulatory genes, the anti-repressor *blaR1* and the repressor *blaI* [8]. Recent studies have demonstrated that the signaling pathway responsible for β-lactamase synthesis requires sequential cleavage of the regulatory proteins BlaR1 and BlaI. Following exposure to β-lactams, BlaR1, a transmembrane sensor-transducer, cleaves itself[9,10]. A study put forward that the cleaved protein functions as a protease that cleaves the repressor BlaI, directly or indirectly (an additional protein, BlaR2, may be involved in this pathway) and allows *blaZ* to synthesize enzyme[11].

Methicillin resistance

Staphylococcus aureus is a dynamic and adaptable bacterium that has an incredible talent to attain antibiotic resistance. Methicillin resistant *Staphylococcus aureus* (MRSA) strains had rapidly emerged during 1960 and became a major problem in hospitals immediately after the methicillin was introduced in 1959. At that time those nosocomial MRSA strains were highly multidrug resistant (MDR) but, many being susceptible only to glycopeptides[12-14].

The genome of methicillin resistant *staphylococci* contains a 21-67kb heterologous mobile genetic element termed staphylococcal cassette chromosome mec (SCCmec), harboring the mecA gene and other resistance determinants. Methicillin resistance is mediated by production of an altered penicillin binding protein PBP-2a encoded by the mecA gene[12,14,15-17].

Methicillin, like all penicillins exerts its battle by jamming the proteins called penicillin binding protein (PBPs) which are liable for the construction and protection of the bacterial cell wall. *S.aureus* resistant strains acquired a new protein called PBP2a **(Figure 1b**) which was not barren by methicillin and could restore the other PBPs, thus, allowing the continued existence of *S.aureus* in the company of methicillin. PBP2a is encoded by the gene mecA, which is the trademark of MRSA. As different to the penicillinase gene mecA, it does not live on a plasmid but on the chromosome fixed in a large movable genetic element called Staphylococcal chromosome cassette mec or SCCmec [18]. The occurrence of PBP2a means MRSA is not only opposing to methicillin but, moreover to all β-lactam antibiotics together with synthetic penicillins, cephalosporins and carbapenems.

Various methicillin resistant isolates had been reported with alterations in their PBPs[19,20,13]. These isolates had been termed moderately resistant *S.aureus* (MODSA). These isolates are not so ubiquitous in nature with low resistance and without any clinical significance.

It had been reported that isolates which produce large amounts of penicillinase (penicillinase hyper-producers) may express low level resistance under some test conditions[21,22]. These isolates had been referred to as borderline oxacillin resistant *S.aureus* (BORSA). There are no reports of failure of treatment with penicillinase resistant penicillins in infections with such isolates and animal model experiments indicate that their clinical significance is doubtful[23]. Methicillin was the first penicillinase resistant penicillin used in 60s and was recognized at that time as the most reliable agent for routine susceptibility testing, though methicillin is now a day's not used in treatment. That's why; resistant strains were termed methicillin resistant *S.aureus* (MRSA). Later, oxacillin resistant *S.aureus* (ORSA) came into existence after the use of oxacillin as an alternative to methicillin in susceptibility tests.

Vancomycin resistance in *Staphylococcus aureus*

It had been recorded that MRSA infections are difficult to treat as compared to MSSA infections if, they are located at anatomical sites because, at those sites antibiotic penetration is very less[24].

Vancomycin was the first glycopeptide antibiotic that was introduced into clinical practice in 1958 after it was isolated in the mid 1950s[25]. Vancomycin had been the drug of choice for treatment of staphylococcal nosocomial infections especially MRSA throughout the world for the last 20 years. Vancomycin is the second most common antibiotic used in hospitals throughout the world, about 16 tons of the vancomycin is being used every year. Only 8 clinical vancomycin resistant *S.aureus* (VRSA) isolates had been isolated to date, all in the USA and mostly from the state of Michigan[26]. However, recent reports proved some sort of concern regarding vancomycin[27]. The first clinical vancomycin intermediate resistant *S.aureus* (VISA) with a minimum inhibitory concentration (MIC) of 8mg l^{-1} was documented in 1996 while, as the first hetero vancomycin intermediate resistant *S.aureus* (hVISA) with an MIC range of <4 mg l^{-1} but, possess stable sub populations (ca. $1/10^6$) that can grow in the presence of >4 mg l^{-1} of vancomycin was computed by[28].

Since then, vancomycin intermediate resistant *S.aureus* (VISA) strains had been reported in many parts of the different countries around the world where such reports were not recorded earlier[29].

Although, the mechanism of vancomycin resistance is different, e.g., VRSA has acquired vanA gene cluster mediated resistance in contrast glycopeptides intermediate *S.aureus* / hetero glycopeptide intermediate *S.aureus* (GISA/hGISA) have achieved mutation directed resistance[30-32]. Although the genetic mechanism had not been fully understood but, the thickening of the cell wall through accumulation of increased amounts of peptidogly can with reduced levels of cross linking, either by increased synthesis or by reduction of the turn over seems to be common factor to all VISA and hVISA strains. This causes an increase of free D-Ala-D-Ala **(Figure 2)** side chains to which vancomycin can bind which result in trapping of more and more vancomycin molecules in the peptidoglycan layers before they could reach to the cytoplasmic membrane where the synthesis of peptidoglycan takes place[33- 35].

MECHANISM OF PATHOGENESIS AND VIRULENCE FACTORS

Staphylococci are opportunistic organisms that require following steps to start an infection in its host such as inoculation and local colonization of tissue surfaces, invasion, evasion of the host response, and metastatic spread to invade the host and cause infection[36]. A breach in cutaneous or mucosal barriers is essential for initiation of infection.

S.aureus expresses many potential virulence factors: (1) Surface proteins that promote colonization of host tissues (2) Invasins that promote bacterial spread in tissues (leukocidin, kinases, hyaluronidase) (3) Surface factors that inhibit phagocytic engulfment (capsule, Protein-A) (4) Biochemical properties that enhance their survival in phagocytes (carotenoids, catalase production); (5) Immunological disguises (Protein A, coagulase, clotting factor) and (6) Membrane-damaging toxins that lyse eukaryotic cell membranes (hemolysins, leukotoxin, leukocidin (7) Exotoxins that damage host tissues or otherwise provoke symptoms of disease (enterotoxin B, TSST-1, α-toxin (8) Inherent and acquired resistance to antimicrobial agents[37].

Nasal Colonization

In human, the principal site of staphylococcal colonization is anterior nares[38]. Little is known about the biology of this colonization process in *S.aureus*. Nasal mucin and keratinized epithelial cells of the anterior nares are involved in the attachment of *S.aureus*. Other factors like influence of other resident nasal flora and their bacterial density, nasal mucosal damage (e.g., that resulting from inhalational drug use), the antimicrobial properties of nasal secretions, and host genetic factors e.g., human leukocyte antigen (HLA) type, may contribute to colonization rate[37].

Inoculation and Colonization of Tissue Surfaces

Staphylococci may get introduce into tissue as a result of minor abrasions, administration of medication such as insulin, or establishment of intravenous access with catheters. A bacterium replicates and colonizes the host tissue surface after their introduction into a tissue site. A family of structurally related *S.aureus* surface proteins referred as microbial surface components recognizing adhesive matrix molecules plays a major role as a mediator of adherence to these sites[38].

Various MSCRAMMs such as fibronectin binding protein, clumping factor and collagen binding protein enable the bacteria to colonize different tissue surfaces. They contribute to the pathogenesis of invasive infections such as endocarditis and arthritis by facilitating the adherence of *S.aureus* to surfaces with exposed fibronectin, fibrinogen or collagen[39].

Invasion

After colonization, *S.aureus* replicates at the initial site of infection produces enzymes like serine, proteases, hyaluronidases, thermonucleases and lipase[36]. These enzymes facilitate local spread across tissue surfaces and bacterial survivals although, their precise role in infections is still not clear. The lipases help the organism to survive in lipid rich areas such as the hair follicles where *S.aureus* infections are often initiated. The *S.aureus* produces a toxin panton valentine leukocidin which is cytolytic to polymorphonuclear leukocytes (PMN), macrophages and monocytes. The cell wall of *S.aureus* contains alternating N-acetyl muramic acid and N-acetyl glucosamine units in combination with an additional cell wall component lipoteichoic acid that can initiate an inflammatory response that includes the sepsis syndrome[1].

Evasion of Host Defense Mechanisms

Evasion of host defense mechanisms is critical to invasion. *Staphylococci* possess an anti-phagocytic polysaccharide microcapsule. Most human *S.aureus* infections are due to capsular types 5 and 8. The *S.aureus* capsule also appears to play an important role in the induction of abscess formation[36]. The capsular polysaccharides are characterized by a zwitter ionic charge pattern (the presence of both negatively and positively charged molecules) that is critical to abscess formation. Protein-A an MSCRAMM unique to *S.aureus* acts as an Fc receptor. This protein can bind the Fc portion of IgG subclasses 1, 2 and 4 preventing opsono-phagocytosis by polymorphonuclear leukocytes (PMNs)[39].

An additional mechanism of *S.aureus* evasion of the host response is its capacity for intracellular survival. Both professional and nonprofessional phagocytes are capable of internalizing *staphylococci*. Staphylococcal internalization by endothelial cells provides a sanctuary that protects bacteria against the host's defenses. It also results in cellular changes such as the expression of integrins and Fc receptors and the release of cytokines. These cellular changes may contribute to systemic manifestations of disease including sepsis and vasculitis.

Host Response to *Staphylococcus aureus* Infection

The primary host response to *S.aureus* infection is the polymorpho-nuclear leukocytes. Bacterial components such as formylated peptides or peptidoglycan attract the PMNs to the site of infection[36]. These cells are also attracted by the cytokines tumor necrosis factor (TNF) and interleukins (ILs) 1 and 6, which are released by activated macrophages and endothelial cells.

Staphylococcus aureus Infections

S.aureus has the ability to cause a broad range of infections that had been divided in to three general types: (i) Superficial lesions such as wound infections (ii) Systemic and life-threatening infections such as endocarditis, osteomyelitis, pneumonia, brain abscesses, meningitis and bacteremia and (iii) Toxinoses such as food poisoning, scalded skin syndrome and toxic shock syndrome[40].

INCREASED COSTS AND MORTALITY

As per the National Nosocomial Infections Surveillance System (NNIS) estimation around 80,000 patients get an MRSA

infection after entering in to the hospitals per year. Reports indicate that infection with MRSA increases the cost and the risk of mortality[41]. The higher cost of treating MRSA infections is due to a variety of factors such as patients with MRSA infection resides for the longer periods in the hospitals, preventive measures are taken to isolate the patients suffering with MRSA and keep them away from infecting other patients, vancomycin has become the drug of choice for MRSA infections, but is more expensive than the drugs normally used to treat *S.aureus* infections[42]. It had been determined that the death rate of patients with MRSA bacteremia is about two times higher than the death rate due to bacteremia caused by MSSA[43].

A 1.9 fold increase in hospital charges and a 3.4 fold increase in mortality rate during the 90 day post operative period had been described when compared patients with MSSA surgical site infections (SSI) with patients of MRSA (SSI) because, the patients with MRSA SSI had to stay five additional days in hospital[44]. In a study it was expected that an amount of US$250,000 is required to bring an outbreak of MRSA (in which three to five patients are infected) under control in the Utrecht University Hospital, The Netherlands[45]. This would involve closure of the intensive care unit (ICU) or ward, postponing operating programs, surveillance cultures etc. Mortality rate and societal costs of *S.aureus* infections can be decreased by reducing the incidence of methicillin resistant and sensitive nosocomial infections[46].

SPECIFIC MEASURES TO CONTROL AND PREVENT MRSA

Surveillance and screening of patients

According to the (SARI, 1999) effective control strategies are dependent on good surveillance data and early detection and following patients should be screened for MRSA:

- Patients known to be previously positive and who are being re-admitted to hospital.
- Patients admitted from another hospital or health-care facility unless, that hospital or facility is known to be free of MRSA.
- During an outbreak as determined by the infection control team.
- Patients with non-intact skin including wounds and ulcers.
- Patients due to undergo elective high-risk surgery (e.g. cardiothoracic surgery, orthopedic implant surgery). On admission to ICU/high-risk areas with weekly screening thereafter other patients as, determined by local risk assessment
- There is no indication for the routine screening of patients prior to discharge i.e. discharge screening.
- When screening patient's swabs from the anterior nares, perineum or groin, any skin lesions (e.g. surgical site) and any medical device sites (e.g. urinary catheter, central venous catheter) should be obtained from the patient. Other samples may be taken e.g. throat swab if, MRSA is persistent following attempts at decolonization.
- Periodic e.g. weekly surveillance cultures should continue to be taken from patients remaining in high-risk areas of the hospital e.g. ICU, special baby care unit, orthopedic unit, and solid organ or bone marrow transplant unit and especially where MRSA is epidemic or where it has been endemic in the past.
- Patients with MRSA who have had three consecutive negative sets of screening samples at least 72 hours apart after decolonization regimens can be removed from isolation. However, such patients should continue to be screened at weekly intervals whilst in hospital.
- Patients with MRSA who have wounds or large areas of non-intact skin (e.g. decubitus ulcers), are not likely to lose MRSA and generally require isolation until the wound is healed. When re-admitted to hospital in the future these patients should be placed in isolation pending the results of screening samples.

Infection Control Measures in Hospitals

- Hand hygiene must be carried out before and after each patient contact, before and after handling or manipulation of any invasive device, before entering and upon leaving critical care areas, isolation rooms

and areas used for cohorting of MRSA cases.

- Cuts or breaks in the skin of careers should be covered with impermeable dressings.
- The hospital environment must be visibly clean, free of dust, soil-age and acceptable to patients, visitors and staff and all hospital surfaces should be intact and made of a durable, washable material. This is fundamental to the control of all healthcare associated infections including MRSA.
- Hospital management should ensure that all hospital staff (including supervisory staff) involved in cleaning processes must be trained and certified as competent in such processes. Training should commence within the first week of employment.
- The Chief Executive Officer or equivalent of every healthcare facility must take corporate responsibility for ensuring cleanliness standards are maintained and for providing adequate resources for both cleaning and training. National recommendations on hand hygiene should be followed.
- All healthcare staff should comply with best practice for insertion and care of invasive medical devices such, as intravascular catheters, urinary catheters etc. Additional cleaning and disinfection measures are necessary on discharge of MRSA patients and in outbreak situations

Antibiotic Stewardship

- Inappropriate or excessive antibiotic therapy and prophylaxis should be avoided in all healthcare settings. Particular attention should be given to obtaining an accurate diagnosis when considering antibiotic therapy and ensuring that antibiotic therapy if required is appropriate to the diagnosis.
- Antibiotics should be given at the correct dosage, correct timing and for an appropriate duration. Excessive duration of antibiotic therapy is particularly associated with selection of resistance and should be avoided.
- The use of glycopeptides antibiotic should be limited to situations where their use has been shown to be appropriate. Prolonged courses of glycopeptide therapy should be avoided if possible, as this is strongly associated with the selection of glycopeptides resistance[47].

Treatment Options

Daptomycin is an acidic lipopeptide with a mode of action requiring calcium. Daptomycin has recently demonstrated significantly better bactericidal activity than vancomycin against *S.aureus* and enterococci and has activity against a small number of glycopeptide intermediate *S.aureus* strains and vancomycin resistant enterococcus.

Mupirocin is a bacteriostatic antibiotic used exclusively as a topical agent. It exerts its antimicrobial effect by specifically and irreversibly binding to bacterial isoleucyl tRNA synthetase, thus preventing protein synthesis. It has been used widely for the clearance of nasal methicillin-resistant *Staphylococcus aureus* (MRSA) carriage during outbreaks and has been recommended for the decolonization of methicillin sensitive *S.aureus* (MSSA) in healthcare personnel. Intranasal application of mupirocin ointment is effective in reducing surgical site infections and the likelihood of broncho pulmonary infection.

Few other drugs including linezolid (a synthetic oxazolidinone), tigecycline (a derivative of minocycline) and daptomycin (a cyclic lipopeptide) appear promising in treatment.

Other alternatives include minocycline, clindamycin, or a macrolides antibiotic depending on local susceptibility patterns.

CONCLUSION

The increasing prevalence of MRSA infections in the hospitals, day care centers and in the community has become a worldwide incident. The wide spread distribution of multiple drug resistant strains and antibiotic clones of the bacterium facilitated by inherent or acquired molecular element is perturbing as it complicates diagnosis and chemotherapy. More so, the presence of wide array of virulence and potential risk and spreading factors compounds morbidity and control measures. There is need for adequate policy framework on infection control that will reflect the current realities on the epidemiologic

characters of MRSA as well as strict implementation of such control program to prevent the spread of MRSA infections.

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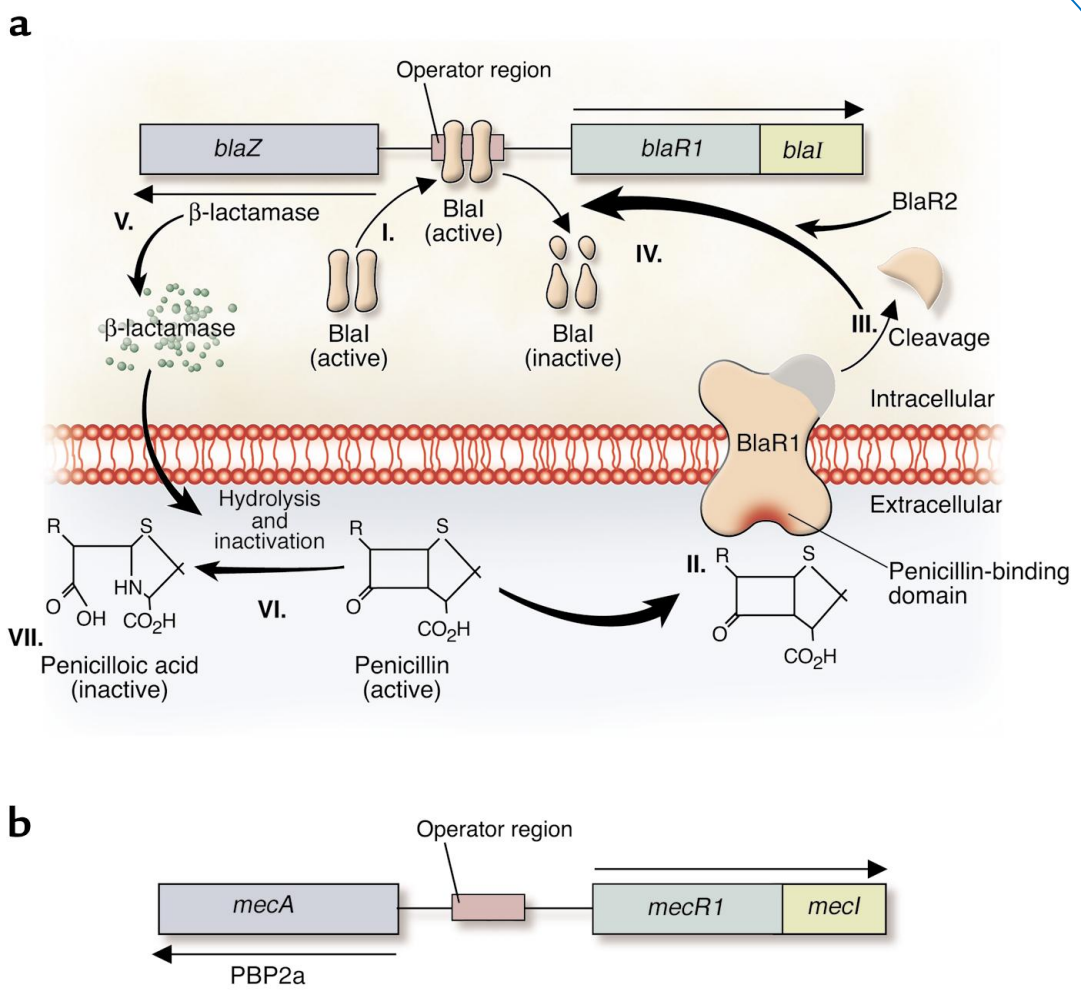

Figure 1. (a) Induction of staphylococcal β-lactamase synthesis in the presence of the β-lactam antibiotic penicillin. I. The DNA-binding protein BlaI binds to the operator region, thus repressing RNA transcription from both *blaZ* and *blaR1- blaI* (b) Mechanism of *S. aureus* resistance to methicillin[49].

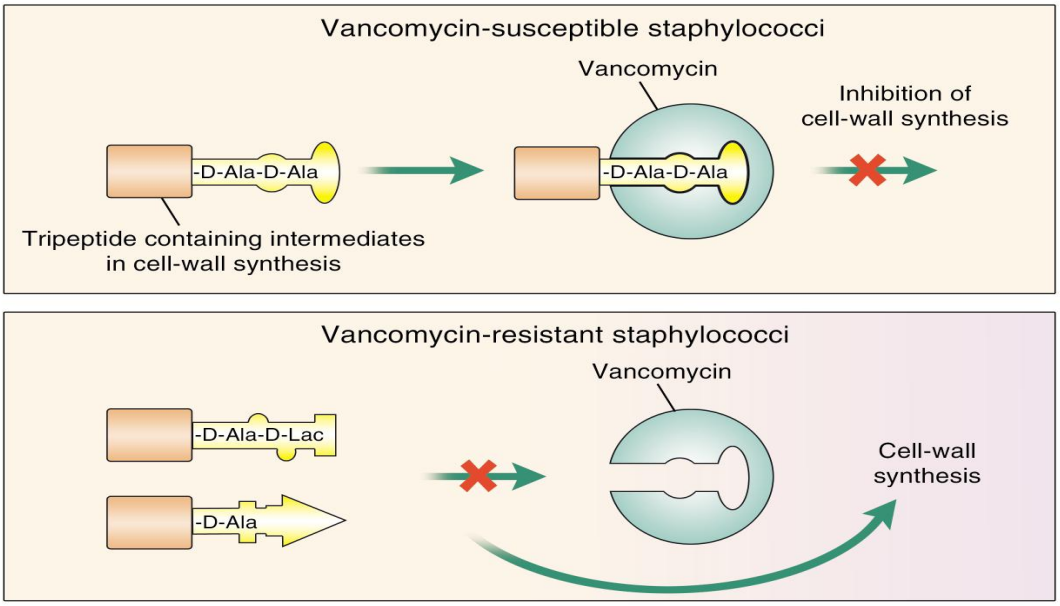

Figure 2. Mechanism of Vancomycin resistance in *S.aureus* [49]