Emergence of Vancomycin-Resistant Staphylococcus Aureus (VRSA)

Corresponding Author:
Dr. Vikneswaran Murugaiyah,
Lecturer, School of Pharmaceutical Sciences, Universiti Sains Malaysia, Minden, Pulau Pinang - Malaysia

Submitting Author:
Ms. Sok Teng Ng,
undergraduate student, School of Pharmaceutical Sciences, USM, Penang - Malaysia

Previous Article Reference: http://www.webmedcentral.com/article_view/2779
Article ID: WMC002787
Article Type: Review articles
Submitted on: 22-Dec-2011, 10:56:35 PM GMT    Published on: 23-Dec-2011, 06:19:14 PM GMT
Article URL: http://www.webmedcentral.com/article_view/2787
Subject Categories: INFECTIOUS DISEASES
Keywords: Staphylococcus Aureus, Vancomycin, VRSA, Resistant

How to cite the article: Ng S T, Lim C Y, Tan C S, Abd Karim A, Haron H, Ahmad N, Murugaiyah V. Emergence of Vancomycin-Resistant Staphylococcus Aureus (VRSA). WebmedCentral INFECTIOUS DISEASES 2011;2(12):WMC002787

Copyright: This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Source(s) of Funding:
None

Competing Interests:
None
Emergence of Vancomycin-Resistant Staphylococcus Aureus (VRSA)

Author(s): Ng S T, Lim C Y, Tan C S, Abd Karim A, Haron H, Ahmad N, Murugaiyah V

Abstract

Background: *Staphylococcus aureus* is one of the bacteria under the genus *Staphylococcus* which is a gram-positive coccus. It is microscopically observed as individual organisms, in pairs and in irregular, grapelike clusters. *S. aureus* is non-motile and non-spore forming bacteria. It is one of the most common community and nosocomial pathogen. It mainly causes skin and soft tissue infections as it is the normal flora on human skin.

Discussion: Infections caused by *S. aureus* are treated with benzylpenicillin, methicillin and others. It is difficult to treat infections caused by *S. aureus* as it destroys neutrophils and shows high antibiotic resistant. MRSA emerged and causes methicillin to be ineffective against them. Thus, vancomycin becomes the drug of choice to be used. Vancomycin acts by inhibiting the biosynthesis of cell wall of *S. aureus*. However, some strains of *S. aureus* developed resistance to vancomycin as well, by increasing the quantities of peptidoglycan and alter the terminal peptide of D-Ala-D-Ala to D-Ala-D-Lac. As a result, hetero-VRSA, VISA and VRSA strains emerged.

Conclusion: Emergence of VRSA is due to the reduced susceptibility of *S. aureus* to vancomycin as *S. aureus* has developed resistant to vancomycin. Normally, antibiotic combination therapy is used to treat infections caused by VRSA. Hygiene precautions should be taken to avoid the transmission of infections caused by *S. aureus*.

Introduction

*Staphylococcus aureus* is also known as *Staph. aureus* or *S. aureus*. It means “golden cluster seed” or “the seed gold”. *S. aureus* was discovered in 1880 by Dr. Alexander Ogston, who was a surgeon in Aberdeen, Scotland.[1] Taxonomy of an organism is according to their natural relationship. Based on “Bergey’s Manual of Systematic bacteriology”, *Staphylococcus aureus* are placed into Kingdom Bacteria; Phylum XIII. Firmicutes; Class I. Bacilli; Order I.Bacillales; Family VIII Staphylococcaceae; Genus I. Staphylococcus; Species *Staphylococcus aureus*. *Staphylococcus aureus* is one of the bacteria under the genus *Staphylococcus*. Bacteria of the genus *Staphylococcus* are gram-positive cocci. It is microscopically observed as individual organisms, in pairs and in irregular, grapelike clusters. *S. aureus* are non-motile, non-spore forming and catalase-positive bacteria.[3] It does not form spores, but can still survive outside the body because it is a facultative anaerobe where it can grow in the presence or absence of oxygen. It is also an opportunistic pathogen where it can be present in vivo without causing any harm but during low immunity or injury, it may cause disease to the person.[2]

Rosenbach described two pigmented colony types of staphylococci and proposed appropriate nomenclature: *Staphylococcus aureus* (yellow) and *Staphylococcus albus* (white) in year 1884. The latter species is now named as *Staphylococcus epidermis*. Both of these bacteria are significant in their interactions with humans.[4] *Staphylococcus aureus* has ability to clot plasma – coagulase positive and make it to be the most widely used and generally accepted criteria for its identification.[3] The test was performed by adding 3% hydrogen peroxide to the colony on agar plate or slant. Catalase-positive culture produces oxygen and bubble at once.[4] *Staphylococcus aureus* is resistant to temperature as high as 50°C, to high salt concentrations (10%) and to drying.[2] There are more than 30 different types of *Staphylococci* that can infect human, but most infections are mainly caused by *S. aureus*.[5]

*Staphylococcus aureus* is commonly found in human skin such as nose, skin of healthy adults and the vagina especially during menstruation and when tampon was used. Each year, some 500,000 patients in American hospitals contract a staphylococcal infection. *Staphylococcus aureus* can cause a range of illness from minor skin infections such as pimples to life-threatening diseases such as pneumonia, meningitis and toxic shock syndrome.[1]
wound.[8] The infections caused by *S. aureus* are usually preceded by colonization, which occur in about 30% of healthy people.[7] Some of the common superficial infections include carbuncles, cellulitis, folliculitis, furuncles, and impetigo. The predominant nosocomial infections caused by *S. aureus* are primary bloodstream infections, pneumonia and wound infections. 12% of all nosocomial infections including 20% of all pneumonias, 19% of all surgical site infections and 16% of all bloodstream infections reported to the National Nosocomial Infections Surveillance (NNIS) system hospitals between 1990 and 1992 was caused by *S. aureus*.[9] The mortality rate for *S. aureus* bloodstream infections before the availability of antimicrobial therapy was 82%.[10] A survey was done to evaluate the data from United States, Canada, Latin America, Europe and Western Pacific. It was found that *S. aureus* was the cause of 39.2% of skin and soft tissue infections; 23.2% of lower respiratory tract infections; 22% of bloodstream infections including endocarditis; 15.6% of other infections including infections of the urinary tract, brain and abdominal cavity.[11] Many of the diseases caused by *S. aureus* have been associated with the toxins produced by the bacteria, which includes toxic-shock syndrome toxin (TSST-1), enterotoxins, proteases and hemolysins.[6,12,13,14] Certain populations such as haemodialysis and surgical patients are at high risk of developing serious *S. aureus* disease with a rate of invasive methicillin-resistant *S. aureus* have been estimated to be as high as 45.2 per 1000 populations for dialysis patients.[6]

**Treatment for Staphylococcus aureus**

In the early 1940s, the introduction of benzylpenicillin into chemotherapy has brought the first successes of penicillin therapy to the cure of formerly untreatable staphylococcal diseases. However, by the mid-1950s, the number of *S. aureus* clinical isolates showing high-level resistance to penicillin increased rapidly to such that penicillin ceased to be useful therapeutic agent against staphylococcal infections. In the year 1959, methicillin, a semi-synthetic derivative of penicillin chemically modified to withstand the degradative action of penicillinase was introduced into Europe,[15] but a year later, the first methicillin-resistant *S. aureus* (MRSA) strains were detected.[16] From then on, the preferred antibiotic for treating MRSA infections is the glycopeptide vancomycin. However in 1997, the first vancomycin intermediately susceptible *S. aureus* (VISA) was reported and vancomycin-resistant *S. aureus* caused a widespread alarm among physicians.[17,18] Linezolid, an oxazolidinone antibiotic was found to have activity against MRSA and also *S. aureus* with intermediate resistance to glycopeptides [19,20,21,22] by preventing the formation of 70S initiation complex and thus inhibits the bacterial protein synthesis.[22-27]

**Why difficult to treat infection caused by Staphylococcus aureus?**

*Staphylococcus aureus* is one of the most common pathogens in our daily life. They are the normal flora on human skin and can cause opportunistic infection in immune-compromised people. Infections caused by *S. aureus* are difficult to be treated. This is because *S. aureus* has the ability to destroy neutrophils.[28] *S. aureus* causes the neutrophils to lyse after phagocytosis, thus killing the neutrophils.[28] This ability is even more enhanced in community-acquired methicillin-resistant *Staphylococcus aureus* infections (CA-MRSA).[28] Despite being able to destroy neutrophils, *S. aureus* also shows antibiotic resistance which makes the infection difficult to be treated. Resistance in *S. aureus* is inducible due to the presence of inducible mecA gene in the resistant (mutant) strains of *S. aureus* which is responsible for the methicillin resistance.[29] This explains the emergence of MRSA shortly after methicillin was introduced for clinical use. mecA gene will be transcribed to synthesize a 78 kDa penicillin-binding protein 2a (PBP2a).[30] The activity of PBP2a is similar to that of serine protease but with low affinity towards β-lactam agents.[30] Thus, enabling *S. aureus* to survive even after penicillins were administered. Since cephalosporins are also β-lactam antibiotics, therefore methicillin resistance also causes the resistance towards cephalosporins.[30] *Staphylococcus aureus* also becomes resistant to quinolones quickly. The quinolones (fluoroquinolones) resistance is caused by spontaneous chromosomal mutation, topoisomerase IV or DNA gyrase or the induction of multidrug efflux pump.[30] Multidrug efflux pumps that can be found on *S. aureus* are QacA/B or NorA.[31] Especially NorA multidrug efflux pump, which is selective towards fluoroquinolones.[31] Increased expression of NorA multidrug efflux pump can result in resistance towards quinolones even at low concentration.[30] Resistance in *S. aureus
towards MLS antibiotics (macrolides, lincosamides, streptogramins) has also been reported. The MLS antibiotics bind to the 50S ribosome of bacteria and inhibit the translocation process in protein synthesis of bacteria. The resistance was caused by the acquisition of erythromycin resistance methylase erm gene.[32] This gene encodes for the enzymes that methylate the 23S rRNA.[32] Hence, the alteration in bacterial ribosome protects the ribosome from being bound by MLS antibiotics.[33] Thus, the bacteria survive since protein synthesis was not inhibited.

In addition, Staphylococcus aureus has also been reported to be resistant to tetracycline antibiotics. This is due to the acquisition of tet(W) gene which encodes for the energy-dependent membrane-associated proteins.[34] This protein will export tetracycline antibiotics out of the bacterial cell.[34] Hence, the concentration of tetracycline within the bacterial cell will not be sufficient to inhibit the protein synthesis. Moreover, some strains of S. aureus acquired the tet(W) gene which encodes for the ribosomal protection proteins.[34] The mode of action of ribosomal protection proteins is thought to be interacting with the base of h34 protein within the ribosome, causing the distortion of binding sites for tetracycline antibiotics and antibiotic molecules are released from the ribosome.[34]

Antibiotics that are still active against MRSA are aminoglycosides and vancomycin. However, many MRSA strains produce aminoglycoside-modifying enzymes, making them resistant to aminoglycosides.[29] Traditionally, vancomycin has been reserved as a drug of “last resort”, used only after other antibiotics treatment had failed.

### Vancomycin

Vancomycin was the first glycopeptides antibiotic discovered in 1956 in United States.[35,36] It is a narrow spectrum bactericidal glycopeptides produced by Streptomyces orientalis.[35,37] Vancomycin is the only glycopeptide antibiotic use clinically in the world.[38] Vancomycin is important in treating infections caused by penicillin-resistant Gram positive organisms such as methicillin-resistant Staphylococcus aureus (MRSA).[35] Due to the large molecule of vancomycin, it is unable to cross the porins in the outer membranes of the Gram negative bacteria.[37] Thus, it is ineffective in treating infection caused by Gram negative bacteria. Therefore, vancomycin and other related glycopeptides are always the last option in treating patients with drug-resistant infection and infections caused by vancomycin-susceptible organisms.[37] Vancomycin has a heptapeptide backbone containing five aromatic residues.[37] The vancomycin family of glycopeptides antibiotics acts by inhibiting the bacterial cell wall biosynthesis.[36,37] It targets the peptidoglycan layer in the cell wall assembly. This is proven through the accumulation of the cytoplasmically located wall precursors which occurred when vancomycin at concentrations near to the minimal inhibition concentration (MIC) is given to treat the intact bacteria.[38] Vancomycin ties up the peptide substrate that is D-alanyl-D-alanine (D-Ala-D-Ala) of N-acetylmuramic acid pentapeptide thus preventing it from reacting with the transpeptidases or transglycosylases.[39] The cup-shaped undersurface of the vancomycin antibiotic forms five hydrogen bonds with the D-Ala-D-Ala dipeptide (Figure 1).[39] As a result, S. aureus bacteria fail to make peptidoglycan crosslinks which cause lysis of the bacterial cell when they multiply. Besides that, vancomycin can also form dimers and bound to the site of biosynthesis with which they interfere (Figure 2).[37, 40] Hence, once one molecule of vancomycin has bound to its binding site, the second molecule is already in place to perform its physiological effect.[40] Furthermore, because the dimer is a large molecule, it caps the tails of the pentapeptide and acts as the steric hindrance, blocking the access of pentapeptide chain to the transpeptidase and transglycosidase enzyme.[40] Both mechanisms inhibits the peptidoglycan synthesis in bacterial cell wall. Although vancomycin was considered as bactericidal, it does not kill the Staphylococcus aureus but prevent the growth of bacteria by the saturation of the available growth points of the peptidoglycans.[38]

### Emergence of vancomycin-resistant Staphylococcus aureus

Over the last decade, methicillin resistant Staphylococcus aureus (MRSA) strains have become endemic in hospitals worldwide. In 1980’s, because of the extensive occurrence of MRSA, methicillin was replaced by vancomycin to be the therapy of Staphylococcal infections.[41] Uses of vancomycin increased in the healthcare institutions of United States due to the increasing number of infections with Clostridium difficile and Coagulase negative Staphylococci (CoNS).[41] In early 1990’s, the usage of vancomycin was increasing drastically. This situation causes selective
pressure to be established that eventually led to the emergence of strain of *Staphylococcus aureus* and other species of Staphylococci with decrease susceptibility to vancomycin and other glycopeptides.[41] The National Committee for Clinical Laboratory Standards (NCCLS) defines Staphylococci in the way where the staphylococci that need vancomycin’s concentration of less than 4 µg/mL to inhibit growth as "susceptible", those need 8–16 µg/mL for inhibition are considered "intermediate" and if the vancomycin’s concentration needed for growth inhibition is more than 32 µg/mL, then the staphylococci are considered "resistant".[42] Besides that, heteroresistant VRSA (Hetero-VRSA) strains are strains of *S. aureus* that have subpopulations of vancomycin-resistant daughter cells.[42]

In 1997, first strain of *Staphylococcus aureus* with reduced susceptibility to vancomycin was reported from Japan.[17,18,41,42] After that, two more cases were reported from United State. In 2002, the first clinical isolate of vancomycin resistant *Staphylococcus aureus* (VRSA) was reported in United States. Further cases were reported by workers from Brazil and Jordan.[41] Strain of Vancomycin Intermediate *Staphylococcus aureus* (VISA) with vancomycin’s MIC of 8µg/mL had been reported from Japan, United States, France, United Kingdom and Germany.[41] Most of these isolate developed from pre-existing MRSA infections.

**Resistance of vancomycin in Staphylococcus aureus**

There are two forms of *Staphylococcus aureus* becoming resistance to vancomycin. One form has been identified in the VISA strains, which have MICs to vancomycin of 8-16µg/mL. Pre-VISA stage of resistance (heterogeneously resistance) have been identified. Only subpopulation remains susceptible to vancomycin for heteroresistance.[30] The VISA isolated was selected from the vancomycin resistance subpopulation. Result shows that the reduced in the susceptible to the vancomycin appeared due to the changes in the peptidoglycan synthesized. The VISA strains were detected for the additional quantities of synthesized peptidoglycan that results in irregularly shaped, thickened cell wall. This also decreases cross-linkage of peptidoglycan strand, which leads to the exposure of more D-ala-D-ala residue. The changes causes reducing of L-glutamine that was available for amidation of D-glutamate in the peptide bridge.[30]

Vancomycin binds to two classes of targets in *Staphylococcus aureus* cells, they are D-alanyl-D-alanine residues in the completed peptidoglycan chain and the murein monomers found in the cytoplasmic membrane that serves as the precursor for peptidoglycan synthesis.[43] Binding of vancomycin to the D-alanyl-D-alanine residues in the completed peptidoglycan layer does not inhibit the peptidoglycan synthesis but binding to the murein monomers in the cytoplasmic membrane completely stops the peptidoglycan synthesis and the cells die.[43]

However, cell-wall thickness is a major contributor to vancomycin resistance. It is suggested that the first clinical VRSA strain, Mu50 (VRSA) produces increased amount of peptidoglycan, meaning more peptidoglycan layers and murein monomers are found in the cell wall.[43] Hence, more vancomycin molecules are trapped in the peptidoglycan layers before reaching the site of peptidoglycan synthesis on the cytoplasmic membrane.[43] In addition, a higher concentration of vancomycin was needed to saturate all the murein monomers that are being produced at an increased rate in Mu50.[43] According to the experiments done by researchers, it was suggested that the mesh structure of the outer layers of thickened peptidoglycan was destroyed by the trapped vancomycin molecules themselves.[43] Since the mesh structure of the outer layers of thickened peptidoglycan was destroyed, thus vancomycin molecules can no longer recognize the structure and bind to it, hence preventing the trapped vancomycin molecules of having the chance to penetrate further into the inner part of cell-wall layers.[43]

The second form of the vancomycin resistant *S. aureus* is mediated by vanA operon (encoded on a conjugative plasmid).[44] The MICs for complete vancomycin resistance is more than or equal to 128µg/mL. Resistance of these isolates caused by alteration of the terminal peptide to D-Ala-D-Lac instead of D-Ala-D-Ala. D-glutamic acid replaces the D-alanine in the pentapeptide chain.[37] Thus, terminal ester link
was formed instead of amide bond which is needed for hydrogen bonding with vancomycin.[37] As a result, the binding affinity of vancomycin to the pentapeptide chain decreases, thus making the antibiotic ineffective. Furthermore, lactate acts as leaving group compared to D-alanine.[37] Synthesis of D-Ala-D-Lac occurs only with exposure to low concentrations of vancomycin. As a result, the additional biosynthetic demands are limited and the VRSA strain is ecologically fit.[30]

Treatment for VISA, VRSA and Hetero-VRSA

New agents are being developed against MRSA. Some of them are expected to have considerable activity against hetero-VRSA and VRSA strains as well. A new quinolone antibiotic, DU-6859a, has MICs of 0.5 and 1 mg/L against Mu3 (Hetero-VRSA) and Mu50 (VRSA) which are resistant to other quinolones such as levofloxacin, ciprofloxacin, sparfloxacin, and tosufloxacin.[43] Normally, antibiotic combination therapies were used against VISA and VRSA. Quinupristin-dalfopristin (trade name Synercid) which is a combination of two antibiotics has potent activity against hetero-VRSA and VRSA strains.[43] ß-lactam agents work synergistically with vancomycin against VISA. The isolates of VISA can be inhibited at lower vancomycin concentration when they are exposed to nafcillin or cefazolin.[42] Moreover, ampicillin or sulbactam would be a good partner for vancomycin; even the agent alone has good anti-microbial activity against VRSA in an experimental infection model.[43] Linezolid has either a synergistic or additive effect on hetero-VRSA and VRSA strains when combined with ampicillin or sulbactam. When vancomycin was used, it can be seen from its mechanism of action and mechanism of resistance that efforts have to be put to reduce the bacterial burden from the patient’s body with procedures such as surgical drainage, debridement, and removal of contaminated lines, foreign bodies, or prosthetic materials. When vancomycin therapy is still unsuccessful with these procedures, triple therapy is used which includes vancomycin, rifampicin (oral) and co-trimoxazole (TMP-SMZ).[43] A report from Nevada found that a patient treated with surgery together with linezolid, TMP-SMZ, and doxycycline therapy was successful in treating VISA.[42] Even though there are some antibiotics that can be given to treat infections caused by VISA and VRSA, prevention is the primary infection control issue. This precautions need to be taken in order to prevent the transmission of the infections among the patients in the hospitals. The VRSA infection can be prevented by hygienic precaution such as:[45]

• Prevent contact with other patient’s wounds. Cover the wound infections with hygienic, dry bandages until they are healed. Put the used bandages in a sealed plastic bag before disposing.
• Avoid sharing personal items with other people such as plate, cup, toothbrush and others.
• Limit the use of antibiotics.
• Place contaminated laundry in a plastic bag and wash with hot water and detergents. Dry them in clothes dryer on the hot situation.
• Use alcohol or chlorine-based disinfectants and wear gloves, mask and gown when cleaning.
• Wash hands frequently with soap and warm water. If there is no water available, germ-killing hand lotion can be used to clean hands.

Although recommended measures to control the spread of VRSA have been promoted for several years, it is still not appreciably slow the increasing rate of infection or colonization of the Staphylococcus aureus especially at the country like United States. Preventing the emergence of multidrug resistant organism require a systematic and comprehensive approach that build up the health care and public health system. The encouragement to the public health care system are very important to recommend prevention and control guidelines, conduct active surveillance and ensure vigorous antibiotic stewardship by health care provider.[46]

Conclusion

Staphylococcus aureus is one of the most common community and nosocomial pathogen and it mainly causes skin and soft tissue infections. The strains of Staphylococcus aureus are always changing over the years, thus it becomes resistant to many drugs. The usage of vancomycin, which has been one of the most popular drugs in treating infections caused by S. aureus, was reduced due to the decrease susceptibility of S. aureus to this drug as vancomycin-resistant Staphylococcus aureus (VRSA) strains are emerging. However until today, there is very little single drug which can be used to treat infections caused by VRSA. Therefore, normally an antibiotic combination therapy is used against VISA and VRSA. The antibiotic combination therapy can be a double therapy or triple therapy. One of the common antibiotics used is Synercid which is the combination of quinupristin-dalfopristin. Besides that, in order to prevent the transmission of the infections caused by VRSA, hygiene precaution should be practiced by
Acknowledgement

At the end of this review article which took almost two months time to complete, we would like to appreciate all the individuals in helping us directly or indirectly to complete this review article. Firstly, we would like to show our appreciation to our research project supervisor, Dr. Vikneswaran Murugaiyah for his guidance. Without his patient guidance, this project would not be a success. His kindness in leading us to the correct ways in writing review article enabled us to complete this project work. Furthermore, we would also like to extend our gratitude to the course coordinator of FAR 241 Antimicrobial Therapy, Dr. Amin Malik Shah bin Abdul Majid who was willing to provide useful guidelines and websites to us. Lastly, we would like to praise all the group members who showed their participation and passion in doing this review article.

References


40. Williams DH. The mode of action of vancomycin group antibiotics and their enhanced binding to a bacterial cell wall surfaces. Cambridge Centre for Molecular Recognition, Department of Chemistry.

41. Tiwari HK And Sen MR. Emergence of vancomycin resistant Staphylococcus aureus (VRSA) from a tertiary care hospital from northern part of India. BMC Infectious Diseases. 2006;6(156):no page.


45. Vancomycin Resistant Staphylococcus aureus [Internet]. Drugs.com; 2011 [cited 2011 Nov 8]. Available from:
Illustrations

Illustration 1

Figure 1: Complexation of the D-Ala-D-Ala termini of peptidoglycans by vancomycin in a network of five hydrogen bonds [37] (------- represent hydrogen bonds)

Illustration 2

Figure 2: Dimerization of vancomycin [37] (------- represent hydrogen bonds)
Disclaimer

This article has been downloaded from WebmedCentral. With our unique author driven post publication peer review, contents posted on this web portal do not undergo any prepublishation peer or editorial review. It is completely the responsibility of the authors to ensure not only scientific and ethical standards of the manuscript but also its grammatical accuracy. Authors must ensure that they obtain all the necessary permissions before submitting any information that requires obtaining a consent or approval from a third party. Authors should also ensure not to submit any information which they do not have the copyright of or of which they have transferred the copyrights to a third party.

Contents on WebmedCentral are purely for biomedical researchers and scientists. They are not meant to cater to the needs of an individual patient. The web portal or any content(s) therein is neither designed to support, nor replace, the relationship that exists between a patient/site visitor and his/her physician. Your use of the WebmedCentral site and its contents is entirely at your own risk. We do not take any responsibility for any harm that you may suffer or inflict on a third person by following the contents of this website.