Vancomycin resistance in *Staphylococcus aureus* may occur faster than expected

**AUTHOR**
Kiran Babu Tiwari  
Research Scientist, Research Laboratory for Biotechnology and Biochemistry (RLABB), Maitidevi, Kathmandu, Nepal  
Faculty, Central Department of Microbiology, Tribhuvan University, Kirtipur, Kathmandu, Nepal  
PBox No.: 13687  
Email: babukiran@hotmail.com  
Tel.: 00977-9841374738

**ABSTRACT**

*Staphylococcus aureus*, one notable example of nosocomial infections, has the characteristic ability to acquire antimicrobial resistance. Methicillin-resistant *S. aureus* have already become endemic worldwide, and vancomycin is the terminal antibiotic of choice for treatment of infections by these strains. Because of selection of vancomycin as the treatment option, now, the emergence of vancomycin resistance in *S. aureus* has been increasing elsewhere. Further, there is no consensus definition of minimum inhibitory concentration to determine the levels of vancomycin resistance in these strains making it difficult in interpretation and management of the resistant strains. As an intervention against cell wall physiology of the bacteria, vancomycin binds with terminal dipeptide of the peptidoglycan monomer. However, vancomycin-resistant strains possess a thickened cell wall with many free monomers capable of binding with the drug. The thickened cell wall not only traps more vancomycin molecules on the immediate cell surface, but also significantly impedes action of the drug towards inner layers of the peptidoglycan network on bacteria. Thus, the normal inner layers of peptidoglycan ensure the structural integrity of cell as a whole. Compounding with the stress selection of vancomycin-resistance in *S. aureus*, the novel mechanism allows the bacteria to reduce susceptibility to the drug easily; hence, emergence of vancomycin resistant strains in the hospital environment may occur faster than expected.

**Key words:** faster, *S. aureus*, vancomycin-resistance

**Introduction:** Staphylococci, a group of facultative anaerobic, catalase positive, Gram-positive clustered cells, include the major pathogen – *Staphylococcus aureus* (Greenwood 1995). *S. aureus* causes both superficial and deep pyogenic infections (Collee 1996; Lowy 1998; Baron 2000) and number of toxin mediated illnesses (Collee 1996) including skin lesions such as furuncles and carbuncles, abscesses, wound infections, pneumonia, osteomyelitis, and others (Baron 2000). *S. aureus* produces a number of enzymes and toxins that contribute substantially to its ability to cause disease. The coagulase-negative staphylococci (CONS) are skin commensals that can cause opportunistic infections associated with prostheses, or foreign bodies, catheters and implants (usually, *Staphylococcus epidermidis*), and urinary tract infection (*Staphylococcus saprophyticus*) (Greenwood 1995). The incidence of *S. epidermidis* is low; however, those which were once recognized only as opportunistic pathogen, are now considered primary pathogens. Infections are harder to treat because of the presence of foreign matter and the antibiotic resistance of the bacteria (Cheesbrough 2000). Acquired antimicrobial resistance is a world-wide problem (Cheesbrough 2000). It is due to extensive use of antimicrobial drugs which have favored the emergence of resistant strains. Clinical microbiologists must be aware of current epidemiological probabilities (Collee 1996). The overuse and misuse of antimicrobials have let to the death of sensitive strains leaving resistant strains to survive, multiply and infect new hosts (Cheesbrough 2000). *S. aureus* strains are notable examples of antibiotic resistance among Gram-positive bacteria (Livermore
Most of the antimicrobial resistance which is now making it difficult to treat some infectious diseases is of genetic origin and transferable between species and genera of bacteria (Cheesbrough 2000). Resistance to antibiotics is achieved by a number of different mechanisms, depending on the class of antibiotics; these include membrane impermeability, alteration of target sites, and enzymatic degradation (Lyon and Skurray 1987). Incidences of penicillin resistance in *S. aureus* emerged during 1940s and that of methicillin resistance during 1960s (Marple and Reith 1992; CDC 2002). Methicillin resistance is a complex property and more than one mechanism is involved (Fung-Tomc et al 1991; Varaldo 1993; Smith et al 1999). Methicillin-resistant *S. aureus* (MRSA) as probably arisen by a succession of mutations and the acquisition of resistance plasmids. The resistance gene *mecA* and regulatory sequences that encode for production of a low-affinity penicillin-binding protein (PBP-2a) are not present in methicillin sensitive strains (Greenwood 1995). Most strains are multi-drug resistant (chloramphenicol, fluoroquinolones, macrolides, clindamycin, rifampin, streptomycin, sulfonamides).

**Vancomycin is the terminal antibiotic for MRSA:** *S. aureus*, one of the most common causes of nosocomial infections (Tiwari and Sen 2006), has the ability to rapidly acquire antimicrobial resistance (Walsh and Bowe 2002). Most *S. aureus* strains (>90%) are resistant to penicillin, and since the 1980s, MRSA strains have become endemic in hospitals worldwide (Coia et al 1988; Hiramatsu et al 1997a; Hiramatsu et al 1997b; Tiwari and Sen 2006). During 1990s, *S. aureus* isolates with diminished susceptibility to vancomycin (vancomycin intermediate resistant *S. aureus*, VISA) were reported (CDC 1997; Hiramatsu et al 1997a; Ploy et al 1998; Tenover et al 2001; Wootton et al 2001; Avison et al 2002; Walsh and Bowe 2002; Assadullah et al 2003; Bozdogan et al 2004; Song et al 2004; Bhateja et al 2005). The glycopeptides, viz. vancomycin and teicoplanin, are frequently the antibiotics of choice for treatment of infections caused by the now common MRSA (Fung-Tomc et al 1991; Ariza et al 1999; Tiwari and Sen 2006). The vancomycin was introduced clinically in 1958 for the treatment of gram-positive bacteria (Srinivasan et al 2002). Use of this agent has increased dramatically in the last 20 years, in large part because of the increasing prevalence of methicillin resistance in both CONS and *S. aureus* (Ena et al 1993). Vancomycin resistance has been reported in clinical isolates of both CONS and *S. aureus* as well (T Srinivasan et al 2002; Tiwari and Sen 2006). There may be species differences in the CONS with respect to vancomycin susceptibility (Srinivasan et al 2002). Despite a few case reports of intermediate susceptibility, almost all *S. epidermidis* isolates, which represent 60 to 90% of clinical isolates, remain sensitive to vancomycin (Srinivasan et al 2002). Some investigators have reported that vancomycin resistance in *S. epidermidis* has been difficult to induce (Archer 1978; Schwalbe et al 1987). VISA isolates were first found in nature more than 15 years ago while investigators were screening isolates for vancomycin susceptibility; however, it was not until 1995 that the first clinical isolate was reported from a French child who had been receiving vancomycin for an MRSA infection (Assadullah et al 2003). In 1996, a wound infection caused by VISA was reported in Japan in a child receiving vancomycin for an MRSA wound infection (Hiramatsu et al 1997a; Hiramatsu et al 1997b). Strains of VISA have been reported from Japan, United States, France, United Kingdom and Germany (Tiwari and Sen 2006). Most of these isolates appear to have developed from preexisting MRSA infections. In the laboratory, the combination of nafcillin and vancomycin was synergistic in the treatment of VISA endocarditis in rabbits (Climo et al 1999). One study showed that the recently approved antibiotics quinupristin-dalfopristin and linezolid had a good activity against three separate VISA strains (Rybak et al 2000; Tsiodras and Gold 2001). Since, the first clinical vancomycin resistant *S. aureus* (VRSA), Mu50, was isolated in Japan in 1997 (Hiramatsu et al 1997a; Hiramatsu et al 1997b; Ploy et al 1998), the incidences have been known increasing worldwide (Ploy et al 1998; Avison et al 2002). Shortly after, two additional cases were reported from United States (CDC 1997). However, first clinical isolate of VRSA was reported from United States in 2002 (CDC 2002). Now, VRSA from Brazil (Palazzo et al 2005) and Jordan (Bataineh 2006) are also reported. Recently, Tiwari and Sen (2006) found two VRSA and six VISA strains, and one vancomycin resistant CONS
strain. However, no clinical isolate of VISA (and/or VRSA) have been reported in Nepal yet. Failures of vancomycin therapy, due to the emergence of significantly less susceptible strains, are now well established (Avison et al 2002; Walsh and Bowe 2002). Given the well-known virulence of *S. aureus*, the isolation of vancomycin resistant organisms generated enormous concern in the medical community and has prompted a flurry of activity aimed at limiting their emergence (Srinivasan et al 2002).

**Early detection of the pathogen is of utmost importance:** The emergence of *S. aureus* resistant to vancomycin has caused considerable concern (Daum et al 1992; Ploy et al 1998). Such strains are currently rare, although they have been isolated from many parts from the world (Walsh and Bowe 2002). Emergence of a truly VRSA seriously threatens the most important treatment option available to clinicians for infections resulting from MRSA (Hiramatsu et al 1997a; Saderi et al 2005). In the absence of vancomycin pressure, vancomycin resistance was found to be unstable and expressed at a low level. This low-level expression of vancomycin resistance in *S. aureus* may be the reason why these strains are hard to detect clinically (Bozdogan et al 2004). So, early detection of the pathogen is of utmost importance. Vancomycin resistance in staphylococcal species is only beginning to emerge as a clinical issue, yet the attention it has already achieved underscores the seriousness of the problem (Srinivasan et al 2002).

**Definition of vancomycin resistance is controversial:** Unfortunately, confusion over the definitions of vancomycin resistance has been generated by recent literature (Srinivasan et al 2002). The source of this confusion seems to be the different breakpoints in vancomycin susceptibilities used in the various countries where VRSA have been reported (Srinivasan et al 2002). Vancomycin resistance can be difficult to detect in clinical microbiology laboratory (Tenover et al 1998; Marchese et al 2000; Tenover et al 2001). Disk diffusion sensitivity testing by standard 30µg vancomycin frequently misclassifies intermediately susceptible isolates as fully susceptible. Considerable controversy surrounds strains of *S. aureus* displaying heterogeneous resistance to vancomycin regarding their definition and methods for detection (Walsh and Bowe 2002). No simple correlation between glycopeptide and beta-lactam MICs was seen, while significant correlations between MICs of vancomycin and teicoplanin (r = 0.679; P < 0.001) and between MICs of imipenem and oxacillin (r = 0.787; P < 0.001) were recognized (Cui et al 2000; Cui et al 2003). The term VRSA is based on the vancomycin breakpoint of the British Society for Chemotherapy, where a strain for which the MIC is 8 mg/liter is defined as resistant (Cui et al 2000; Cui et al 2003). As per National Committee for Clinical Laboratory Standards (NCCLS) staphylococci with MIC of vancomycin < 4 mg/liter is susceptible, while for which the MIC is 8-16 mg/liter are intermediate and those with MIC > 32 mg/liter are resistant (Walsh and Bowe 2002). The clinical significance of heteroresistance is thus difficult to understand. There is an obvious need for simple and accurate phenotypic screening and confirmatory tests for glycopeptide resistance in staphylococci (Srinivasan et al 2002).

**Mechanism of vancomycin resistance in *S. aureus* is novel** (Hiramatsu et al 1997a): Unusually, a thickened cell wall is responsible for the vancomycin resistance in clinical VRSA strains (Cui et al 2000; Walsh and Bowe 2002). Mu50 produces excessive amounts of peptidoglycan to make the thickened cell wall which becomes thinner with the loss of vancomycin resistance during drug-free passages (Cui et al 2003). The differences in the cell wall thickness between VRSA and passage-derived strains and control strains were statistically significant (P < 0.001) (Cui et al 2003). Glycopeptides form a stoichiometric 1:1 complex with the D-alanyl-D-alanine residues of the stem peptide of the membrane-anchored murein monomer and inhibits transglycosylation and transpeptidation reactions thereby preventing incorporation of the precursors into the bacterial cell wall (Chatterjee and Perkins 1966; Watanakunakorn 1984; Daum et al 1992; Walsh and Bowe 2002; Périchon et al 2004). Associated with it, the amidation of D-glutamate into D-glutamine in the stem peptide is hypothesized as the important regulation step, rendering non-amidated muropeptides as
poorer substrates for transpeptidases (Hanaki et al 1998; Cui et al 2000; Cui et al 2003). Cell wall thickness and reduced cross-linking among murein monomers increase the amount of free terminal D-alanyl-D-alanine dipeptide (Avison et al 2002; Walsh and Bowe 2002). Thus, the thickened cell wall not only traps a greater number of vancomycin molecules (Cui et al 2003) but also significantly impedes other glycopeptide molecules from reaching the sites of cell wall biosynthesis at the plasma membrane (Sieradzki et al 1999). The fine structure of the Mu50 cell wall is similar to that of an MRSA strain such as N315, except that the Mu50 peptidoglycan chains show significantly less cross-linking, and an increased content of pentapeptide chains (Avison et al 2002; Walsh and Bowe 2002). In the study by Tiwari and Sen (2006), all VRSA, VISA and vancomycin resistant CONS were mecA PCR positive, however, none of these isolates have demonstrated vanA/vanB gene by PCR. Despite the efforts made so far, the genetic basis of these changes has not been determined yet (Avison et al 2002; Kuroda et al 2000; Marchese et al 2000; Walsh and Bowe 2002; Clark et al 2005). However, the resistance in enterococci (VRE) is due to synthesis of plasmid encoded proteins (VanR, VanS) and enzymes (VanA, VanX and VanH), ending in D-alanyl-D-lactate in place of D-alanyl-D-alanine that results in 1,000-fold-lower affinity for glycopeptides (Périchon et al 2004). Vancomycin-resistant Enterococcus faecalis emits a sex pheromone that promotes plasmid transfer, and it has been recently demonstrated that this same pheromone is produced by S. aureus. Emission of this pheromone by S. aureus organisms that are in proximity to VRE that contain plasmids encoding van genes could result in transfer of these resistance genes (Showsh et al 2001). The possibility of transfer of vancomycin resistance genes to other gram-positive organisms raises significant concerns about the emergence of VRSA (Cetinkaya 2000).

**Development of resistance in S. aureus in the hospital ecological environment may, hence, occur faster than other antibiotics:** At present, the proportion of MRSA with reduced susceptibility to vancomycin is well known (Hiramatsu et al 1997a). Only 21 strains have so far been reported in literature since the first VRSA (Mu50) and VISA (Mu3) reported from Japan (Bhateja et al 2005). VISA is the precedent strain of VRSA (Hiramatsu et al 1997a; Howe et al 1999) and the emergence of VRSA would be the result of vancomycin selection exerted upon a VISA strain in the hospital environment (Wootten et al 2001; Cui et al 2003). The conversion of vancomycin-sensitive S. aureus (VSSA) to VISA is supposed to be achieved in association with methicillin resistance caused by beta-lactam selection (Assadullah et al 2003; Cui et al 2003). This suggests that the use of beta-lactam antibiotics for MRSA infection is a risk factor for the emergence of VISA, although the precise genetic mechanism remains to be clarified (Cui et al 2000; Cui et al 2003). The unusual feature of cell wall physiology of the vancomycin stressed S. aureus makes the culprit to respond against the drug quite easily. As the thickness of the cell wall increases, clearly, more vancomycin molecules can be trapped outside the bacterial cell. Thus, the vancomycin layer on the bacterial surface hinders antibacterial action of the very same antibiotic. During prolonged vancomycin exposure, further, the bacterium can shed out peptidoglycan monomers from the peptidoglycan layers (Walsh et al 2001; Avison et al 2002) which can sequester more vancomycin molecules before encountering the bacterium. On the other hand, *in vitro* transformation of S. aureus with VRE-plasmid significantly warns against the emerging threat of VRSA (Noble et al 1992; Périchon et al 2004). Because of the limited knowledge about the mechanism and corresponding components in S. aureus, it will take longer time to explore counter-drug against the increment of vancomycin resistance. Moreover, the so seemed simple mechanism of resistance in vancomycin stressed S. aureus isolates allows them to reduce susceptibility to the drug easily. This may challenge the clinical microbiologist more than any other antibiotic practices in the modern chemotherapy (Hiramatsu et al 1997a; Saderi et al 2005).

**Hospital Infection Control Practices Advisory Committee (HICPAC) Recommendations:** In an effort to bring about more prudent use of antibiotics, HICPAC (Hospital Infection Control Practices Advisory Committee) emphasizes the importance of education of medical staff and students about the situations in which the use of vancomycin is considered appropriate. According to HICPAC
recommendations, situations in which the use of vancomycin is appropriate or acceptable are as follows (CDC 1995): (i) for treatment of serious infections due to beta-lactam-resistant gram-positive microorganisms; (ii) for treatment of infections due to gram-positive microorganisms in patients with serious allergy to beta-lactam antimicrobials; (iii) when antibiotic-associated colitis fails to respond to metronidazole therapy or is severe and potentially life-threatening; (iv) prophylaxis, as recommended by the American Heart Association, for endocarditis following certain procedures in patients at high risk for endocarditis; and (v) prophylaxis of major surgical procedures involving the implantation of prosthetic materials or devices, e.g., cardiac and vascular procedures and total hip placement, at institutions with a high rate of infections due to MRSA or methicillin-resistant S. epidermidis (MRSE). A single dose administered immediately before surgery is sufficient unless the procedure lasts more than 6 h, in which case the dose should be repeated. Prophylaxis should be discontinued after a maximum of two doses.

**Conclusion:** The emergence of VRSA/VISA might also be prevalent in every corner of the world where antibiotic misuse is equally common. Hence, there should be an immediate response from the concerned authorities to check further emergence and spreading of these notorious VRSA strains. A strict regulation on irrational antibiotic usage might be an appropriate and effective approach in this direction. It is recommended that every country should carry out nationwide surveillance program to map the vancomycin susceptibility pattern. For this, all strains with vancomycin MIC 4 μg/ml should be earmarked and sent to the reference laboratory for further characterization (Tiwari and Sen 2006). This will help to identify the potential areas which are already under the major threat of VISA/VRSA emergence and therefore draw more focused attention of Government for prompt tackling of this problem.

**References:**


Tiwari HK, Sen MR. 2006. Emergence of vancomycin resistant *Staphylococcus aureus* (VRSA) from a tertiary care hospital from northern part of India. BMC Infect Dis URL: http://www.biomedcentral.com/1471-2334/6/156


