Vancomycin-resistant Staphylococcus aureus: a new model of antibiotic resistance

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Vancomycin has been the most reliable therapeutic agent against infections caused by meticillin-resistant Staphylococcus aureus (MRSA). However, in 1996 the first MRSA to acquire resistance to vancomycin, was isolated from a Japanese patient. The patient had contracted a post-operative wound infection that was refractory to long-term vancomycin therapy. Subsequent isolation of several vancomycin resistant Staphylococcus aureus (VRSA) strains from USA, France, Korea, South Africa, and Brazil has confirmed that emergence of vancomycin resistance in S aureus is a global issue. A certain group of S aureus, designated hetero-VRSA, frequently generate VRSA upon exposure to vancomycin, and are associated with infections that are potentially refractory to vancomycin therapy. Presence of hetero-VRSA may be an important indicator of the insidious decline of the clinical effectiveness of vancomycin in the hospitals. Vancomycin resistance is acquired by mutation and thickening of cell wall due to accumulation of excess amounts of peptidoglycan. This seems to be a common resistance mechanism for all VRSA strains isolated in the world so far.

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Meticillin-resistant Staphylococcus aureus (MRSA) has occurred in many countries since its discovery in 1961. However, in recent years, clinicians have been concerned by the increased frequency of MRSA infections. This resurging MRSA problem seems to be based on the lack of potent therapeutic agents having an unequivocal cell-killing effect, and thus capable of eliminating MRSA from the patient’s body. Increased use of vancomycin—a drug with rather weak cell-killing potency against prevailing MRSA—seems to have set a basis for the selection of vancomycin resistance in MRSA. In 1997, we reported the first MRSA strains with reduced susceptibility to vancomycin, which were isolated from patients in whom vancomycin therapy was ineffective.

We reported two classes of vancomycin-resistant strains: vancomycin-resistant S aureus (VRSA) that has a vancomycin minimum inhibitory concentration (MIC) of 8 mg/L, and hetero-VRSA that spontaneuously generates VRSA within the cell population. The nomenclature is based on the MIC breakpoints of the British Society for Antimicrobial Chemotherapy who define the MIC of 8 mg/L as “resistant.” However according to the National Committee for Clinical Laboratory Standards (NCCLS) breakpoint, these strains are called vancomycin-intermediate S aureus (VISA) or glycopeptide-intermediate S aureus (GISA) in the USA. Although hetero-VRSA is categorised as “susceptible” to vancomycin based on current MIC breakpoints, it generates VRSA cells at a high frequency within its cell population.

To date, as well as Japan, VRSA strains have been isolated from USA, France, Korea, South Africa, Brazil, and Scotland. In addition hetero-VRSA strains have been reported from many more countries, indicating that the problem is a global one. In this review, the mechanism of glycopeptide resistance in S aureus primarily based on the analyses of clinical strains will be summarised. The viewpoint that hetero-VRSA constitutes a precursor stage to vancomycin resistance, and that the emergence of VRSA is an outcome of the prevalence of hetero-VRSA will be explained.

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The mechanism of vancomycin resistance

Cell-wall peptidoglycan synthesis

Both beta-lactam and glycopeptide (including vancomycin and teicoplanin) antibiotics exert their antimicrobial effects by inhibiting the cell-wall synthesis of *S aureus*. The cell has a high osmotic pressure (10–13–20–26 × 10⁻¹³ Pa). For *S aureus* cells to multiply in an environment with a lower external pressure, they must keep synthesising a strong extracellular structure called peptidoglycan (or murein) to prevent the cells from rupturing. To produce peptidoglycan, its monomeric component (murein monomer) must be synthesised inside the cell, and transferred to the outside by lipid carriers present in the cytoplasmic membrane (figure 1).

Two enzymes located in the cytoplasmic membrane, glycosyltransferase and transpeptidase, assemble the murein monomer into a gigantic structure of peptidoglycan (figure 2). Glycosyltransferase polymerises murein monomers between their amino-sugar moieties to produce nascent peptidoglycan chains. Then, transpeptidase, also known as penicillin-binding protein (PBP), links the newly formed nascent peptidoglycan chains to pre-existing peptidoglycan layers of the *S aureus* cells. In this step, PBP recognises D-alanyl-D-alanine residues of murein monomer, and cuts in between the two D-alanines and ligates penultimate D-alanine to the pentaglycine of the neighbouring murein monomer (figure 2). When the inter-peptide bridge is formed, the terminal D-alanine of the murein monomer is lost from the completed peptidoglycan. However, it is known that about 20% of D-alanyl-D-alanine residues remain unprocessed by PBPs. As a result, as many as 6 × 10⁰ unprocessed D-alanyl-D-alanine residues remain in the cell wall of a single *S aureus* cell.¹⁷

PBP is the target of beta-lactam antibiotics such as penicillin. Beta-lactam is a structural analogue of D-alanyl-D-alanine, and it covalently binds to the *S aureus* PBP (depicted in red in figure 3) at its D-alanyl-D-alanine-binding pocket. This inactivates the PBP and inhibits the cross-bridge formation step of peptidoglycan synthesis, causing the cell to rupture from the peptidoglycan mesh. However, MRSA produces a unique PBP, designated PBP2’ (or PBP2A; in green in figure 3), which has an extremely low binding affinity to beta-lactam antibiotics.¹⁸ As a result, the PBP2’ can keep on synthesising the peptidoglycan even in the presence of beta-lactam antibiotics. This is the basis of beta-lactam resistance of MRSA. The unique PBP2’ is the product of the exogenous gene called mecA carried by a mobile genetic element, SCCmec, which *S aureus* has acquired from an as yet unknown bacterial species by lateral gene transfer.²¹

Glycopeptides inhibition of transpeptidation and nascent peptidoglycan synthesis

By contrast with beta-lactams, glycopeptides bind to D-alanyl-D-alanine residues of the murein monomer (figure 4). There are two classes of binding targets in the *S aureus* cell: firstly, D-alanyl-D-alanine residues in the completed peptidoglycan layers or on the nascent peptidoglycan chain; and secondly, the murein monomers located in the cytoplasmic membrane that serves as the substrates for glycosyltransferase (figure 4). The binding of glycopeptides to the former targets does not inhibit nascent peptidoglycan synthesis, though it may interfere with cross-bridge formation mediated by PBPs. This may be the reason why teicoplanin is synergistic with beta-lactam antibiotics. If glycopeptides bind to murein monomers in the cytoplasmic membrane, peptidoglycan synthesis is completely inhibited, and the cells cease to multiply. However, for the glycopeptide molecules to bind to such targets, they have to pass through about 20 peptidoglycan layers (only two layers are drawn in figures 2–4) without being trapped by the first targets. Since there are many D-alanyl-D-alanine targets in the peptidoglycan layers, many glycopeptide molecules are trapped in the peptidoglycan layers. This compromises the therapeutic effectiveness of glycopeptides. For example, if high numbers of *S aureus* cells are present in the infected tissue of the patient, many glycopeptide molecules will be adsorbed to their cell walls, and tissue concentrations will be
lower than the required therapeutic threshold. Therefore, measures to decrease bacterial cell numbers in the patient’s body by surgical elimination of an abscess or by drainage of pus would frequently be required to make glycopeptide therapy more effective. For the same reason, accurate susceptibility testing of glycopeptides is much more difficult to do than with other antibiotics, because variations of inoculum size (cell number) added to the broth or the agar plates containing glycopeptides can affect the free drug concentration, resulting in variations of MIC values.

Cell-wall thickness is a major contributor to vancomycin resistance

Mechanism of vancomycin resistance has been extensively studied with the first clinical VRSA strain, Mu50.22-24 Biochemical and transmission electron microscopy (TEM) examination of the Mu50 cell, suggested that it produces increased amounts of peptidoglycan. More murein monomers and more layers (probably 30–40 layers as judged by cell-wall thickness observed with TEM) of peptidoglycan are considered to be present in the cell wall (figure 5; only three layers are drawn). As a result, more vancomycin molecules are trapped in the peptidoglycan layers and less reach the cytoplasmic membrane. More vancomycin molecules are trapped in the peptidoglycan layers and less reach the cytoplasmic membrane than usual.

Experiments suggest that the mesh structure of the outer membrane; they displace the older layers outwards so that they are eventually cast off from the cell surface. Autolytic enzymes (peptidoglycan hydrolysing enzymes) are involved in these shedding processes. A VRSA strain isolated from Michigan, USA, has a remarkably reduced autolytic activity that returns to normal with the loss of vancomycin resistance and reduction in the cell wall thickness (L Cui, personal communication). Therefore, increased amounts of peptidoglycan, as seen in Mu50. The other is to reduce peptidoglycan turnover. New peptidoglycan layers are always produced on the surface of the cytoplasmic membrane; they displace the older layers outwards so that they are eventually cast off from the cell surface. Autolytic enzymes (peptidoglycan hydrolysing enzymes) are involved in these shedding processes. A VRSA strain isolated from Michigan, USA, has a remarkably reduced autolytic activity that returns to normal with the loss of vancomycin resistance and reduction in the cell wall thickness (L Cui, personal communication). Therefore, the Michigan strain and Mu50 seem to employ a different strategy to achieve the same goal—ie, thickening of the cell wall.

Other factors are also known to contribute to vancomycin resistance in Mu50, though to a lesser degree than the cell-wall thickness. Enhanced supply of murein monomers in Mu50 cells is associated with a decrease in the intracellular glutamate level. Glutamine is consumed by the increased activity of one of the key enzymes (glucosamine
6-phosphate synthetase) of the murein monomer synthesis pathway. This results in the increased synthesis of structurally altered murein monomers (the non-amidated form) that are inefficient substrates for cross-bridge formation by PBPs. The final outcome of this sequential event is a raised proportion of D-alanyl-D-alanine residues in the peptidoglycan layers. In fact, about 2-4 times the amount of D-alanyl-D-alanine residues are found in a unit weight of purified peptidoglycan of Mu50 compared with VSSA strains. This means that a single cell of Mu50, with its 1-5 times thickened cell wall, can trap as many as 3-6 times more vancomycin molecules than a VSSA cell. Reduced cross-linkage of peptidoglycan has also been shown in a VRSA strain obtained in vitro. In this study a drastic decrease in peptidoglycan cross-linkage due to mutational inactivations of the PBP genes (PBP2* and PBP4) is associated with vancomycin resistance in a VRSA strain generated in vitro, called VM. However, both the mutant strain VM and MRSA strain COL, from which the former mutant was derived, have an unusually thickened cell wall as far as we can judge from the published electron microscopy. In our experiments, reduction of peptidoglycan cross-linking alone does not cause glycopeptide resistance. Its contribution is effective only when the strain has a thickened cell wall. Cell wall thickening is considered the prerequisite for vancomycin resistance.

We also found that the non-amidated murein monomer has an increased binding affinity for vancomycin compared with the normal murein monomer. Therefore, the production of the abnormal murein monomers also contributes to the vancomycin resistance of Mu50 by enhancing the affinity-trapping, and clogging the peptidoglycan mesh.

The genetic basis for vancomycin resistance has not been elucidated yet. My research group has identified some novel genes whose expression is either increased or decreased in Mu3 and/or Mu50, compared with vancomycin-susceptible strains. More information will be available by comparing the whole genome sequences of Mu50 and N315, (the latter is a vancomycin-susceptible Japanese MRSA strain), since the strains are closely related and only different in a few phenotypes, including vancomycin resistance. One thing now apparent is that the SCCmec element carrying the mecA gene is not required for vancomycin resistance. The precise deletion of the element from Mu50, Mu3, and other Japanese hetero-VRSA strains did not alter the level and patterns of vancomycin resistance. Recent isolation of a vancomycin-resistant, metillin-resistant strain further indicates that vancomycin resistance is not necessarily confined to MRSA.

**Teicoplanin resistance**

Teicoplanin and vancomycin belong to the glycopeptide class of antibiotics. Both exert antimicrobial activity by binding to the D-alanyl-D-alanine residue of murein monomer. Therefore, a common resistance mechanism for the two antibiotics is to be expected. In fact, all the VRSA strains analysed possess teicoplanin resistance (defined by MIC ≥8 mg/L). Cell-wall thickness also contributes to teicoplanin resistance as expressed by the VRSA strains (MIC 8–32 mg/L) and resistance decreases when cell-wall thickness decreases. However, about half of the vancomycin-susceptible revertants of VRSA strains still maintain intermediate levels of teicoplanin resistance (MIC 8 or 16 mg/L). This finding suggests that there may be other mechanisms than cell-wall thickness for teicoplanin resistance.

In support of this suggestion is the historical overview of glycopeptide resistance in *S aureus*. Historically, *S aureus* acquired teicoplanin resistance before it acquired vancomycin resistance. There are quite a few MRSA strains that are resistant to teicoplanin but are still “susceptible” to vancomycin as judged by MIC values. However, acquisition of teicoplanin resistance is frequently accompanied by a small increase in vancomycin resistance; in fact, hetero-VRSA strains belong to this category of strains (see below).

Shlaes and colleagues demonstrated that PBP2 is overproduced in a teicoplanin-resistant *S aureus* mutant strain (MIC 16 mg/L) compared with its parent clinical strain. Over-production of PBP2 is also observed in Mu50 and the hetero-VRSA strain Mu3—both are resistant to teicoplanin. We demonstrated that experimental over-expression of PBP2* in a VSSA strain causes the vancomycin MIC to increase by 1 mg/L (from 1 to 2 mg/L), whereas that of teicoplanin increased significantly from 2 to 8 mg/L. In agreement with its marginal contribution to vancomycin resistance, over-expressed PBP2* alone does not lead to cell-wall thickening. On the other hand, it increases the rate of cross-linking of cell-wall peptidoglycan (K Hiramatsu, unpublished observation). This finding highlights again the difference between the two glycopeptides. It may be that teicoplanin is more prone to inhibiting transglycosylation than vancomycin, and vancomycin more inclined to inhibit transglycolputation.

**Hetero-VRSA**

**Clinical significance of hetero-VRSA**

Mutant strains having vancomycin MIC of 8 mg/L are not obtainable in vitro by one-step selection of vancomycin in VSSA strain. However, some Japanese clinical MRSA strains having susceptible vancomycin MIC values (<8 mg/L) generate VRSA at a resistance frequency of 10^6 or greater. These strains, represented by strain Mu3, are considered as precursor strains for VRSA. When strain Mu3 is grown overnight in drug-free medium to 10^6 cells/mL, several hundred cells are found growing in samples plated on agar plates containing 4 mg/L of vancomycin, implying that the MIC values of these cells are equal to or greater than 8 mg/L. Mu3 also contains a subpopulation of cells that are resistant to various other concentrations of vancomycin as illustrated in figure 6. Therefore, Mu3 has a “heterogeneous” population of cells with different levels of vancomycin susceptibility including vancomycin-resistant cells (MIC ≥8 mg/L). Thus, the Mu3 strain is designated hetero-VRSA. Population analysis is the standard method for identifying hetero-VRSA. It analyses as many as 10^7–9 CFU (colony-forming units) by contrast with about 10^4 CFU in standardised methods used today. Standard MIC...
methods cannot quantitatively detect the resistant cell sub-population present in hetero-VRSA strains, which constitutes only 1/10^6 of the entire population due to the low inoculum density used.

Conventional susceptibility tests (MIC, disk diffusion tests, &c) cannot discriminate between VSSA and hetero-VRSA. However, the two may behave in a significantly different manner in response to vancomycin therapy. Figure 7 illustrates a test tube experiment comparing Mu3 with a VSSA strain 87/20. Mu3 has a slightly higher vancomycin MIC value (2 mg/l) than the MRSA strain 87/20 (MIC=1 mg/L). Inspite of this minor difference in MIC value, 10 mg/L of vancomycin is required to completely suppress the growth of about 2 × 10^6 cells/mL of Mu3, whereas 2 mg/L was sufficient to suppress an equivalent number of cells of strain 87/20. This difference is due to a sub-population of cells in Mu3 that are resistant to vancomycin (figure 7). It is also important to observe that Mu3 cell number decreases in the initial 72 h of exposure to 5 mg/L of vancomycin, but increases substantially thereafter. This correlates with the unique clinical case from whom Mu3 was isolated, where the patient’s pneumonia initially responded favorably to vancomycin therapy, but became exacerbated after the 9th day of vancomycin therapy.35

Resistance heterogeneity is not confined to vancomycin but is also well described for other antibiotics such as beta-lactams, and aminoglycosides.17 In the era of antibiotic resistance, the concept of hetero-resistance is important not only for the prediction of clinical effectiveness of therapy for individual patients, but also for the detection of trends of emerging resistance. Because hetero-resistance escapes detection by conventional susceptibility tests, it is not an acceptable reason to neglect its importance from a clinical and epidemiological viewpoint.

**Biological significance of hetero-VRSA**

VRSA is obtained through two-step selection of vancomycin resistance in Japanese MRSA strains (figure 8). The hetero-VRSA strains obtained by in-vitro selection with 1 mg/L of vancomycin maintained the hetero-resistance phenotype for a week’s serial passage in drug-free media. However, the strains tend to lose the resistant subpopulations by the second week. This observation contrasts with the stability of...
the hetero-resistance of Mu3, which is stably expressed even after 80 days’ serial passage in a drug-free medium. Therefore, it suggests that there are two types of hetero-VRSA: stable and unstable (figure 8). The stable variant of hetero-VRSA may be established either by cycles of exposure to vancomycin or by one-step acquisition of a single but stable genetic alteration. In-vitro experiments predict that many unstable hetero-VRSA strains are produced in the clinical setting. These strains express hetero-resistance directly after isolation from patients who undergo glycopeptide therapy, but the phenotype tends to be lost during strain storage in drug-free media, contributing to the underestimation of the prevalence of hetero-VRSA. However, it would still be important to detect stable hetero-VRSA strains, because they would prevail more easily in the hospital environment eventually causing more vancomycin therapeutic failures by generating VRSA at high frequency during the therapy.

Vancomycin-resistance in the VRSA phenotype also tends to revert. Experiments with 16 VRSA strains from different parts of the world showed that the vancomycin MIC returned to “susceptible” levels (2 mg/L) after 10 to 84 days’ serial passages. Therefore, the stability of VRSA phenotype can vary significantly. A notable observation was that the “susceptible” revertants obtained from 15 of the 16 VRSA strains still expressed heterogeneous vancomycin resistance similar to that of Mu3—one strain returned to a susceptibility level with a VSSA-type population curve characteristic (see the population curve of FDA209P in figure 6). Exposure of these revertants to 4 mg/L of vancomycin, however, selected VRSA strains at very high frequencies of about $10^{-5}$ to $10^{-7}$ (L Cui, unpublished observation). This finding indicates that, though the VRSA strain may not disseminate itself as a stable resistance phenotype that tends to return to the hetero-VRSA status, it can readily revert to VRSA when exposed to vancomycin (figure 8).

From December 1998 to January 1999, an outbreak of VRSA was recorded in a Brazilian hospital (G A Oliveira, General Hospital of Vila Penteado and School of Pharmaceutical Sciences, University of Sao Paulo, Sao Paulo, Brazil, personal communication). The incidence indicated that VRSA can transmit from patient to patient if vancomycin is used liberally for burn-unit patients housed in the same room. Aside from this special case, it would be the hetero-VRSA that would disseminate in the hospital and contribute to the incidence of vancomycin-refractory infection. In fact, many hetero-VRSA strains were found even in the Brazilian hospital case besides the multiple VRSA isolates. The emergence of clinically significant VRSA could be the tip of the iceberg, and a sign of the more widespread and insidious prevalence of hetero-VRSA in the hospital.

From a biological point of view, the hetero-VRSA status seems to be a successful ecological achievement of S aureus for the procurement of survival against vancomycin.
pressure. Although vancomycin suppresses the growth of 99.9% of the population of hetero-VRSA, the rest of the population survives and grows in the presence of 4 mg/L of vancomycin, which is almost the upper limit of vancomycin concentration achieved in most infected tissues. Some subpopulation of cells can grow even in 9 mg/L of vancomycin (figure 6). Those VRSA cells spend much energy to produce thickened cell walls, to survive the vancomycin pressure. Once the vancomycin pressure is alleviated, the VRSA cells return to the hetero-VRSA status to conserve energy. \textit{S. aureus} seems to have chosen an effective survival mechanism to prevail as a successful parasite of people. \textit{S. aureus} may not need to acquire the \textit{van} genes from vancomycin-resistant enterococci (VRE), since associated with selective pressure are limited tissue concentrations and the limited cytotoxicity activity of vancomycin.

A significant increase of MRSA was noticed in the early 1980s in Japan when the third-generation cephalosporins were used widely throughout Japan. Based on a prediction from a plausible mathematical model on the prevalence of resistant bacteria in hospitals, unrestricted use of the broad-spectrum cephalosporins with weak antimicrobial activity against MRSA might have promoted this quick rise in MRSA.\(^3\) Since, vancomycin and arbekacin (an aminoglycoside antibiotic approved in 1990 for MRSA infection) were not available in Japan until 1991, it became general practice in Japan to treat MRSA infection with a class of beta-lactam antibiotic, such as imipenem, flomoxef, and cefmetazole, which have good MIC values against hetero-MRSA.\(^3\) From MIC data, these beta-lactams appeared effective since MRSA strains in the early 1980s were heterogeneously resistant to meticillin (hetero-MRSA).\(^2\) Towards the end of the 1980s, this practice led to a clone having a high meticillin resistance (homo-MRSA)\(^2\), which became the dominant clone in Japan in the 1990s.\(^2\)

In-vitro selection of hetero-MRSA strains of clonotype II-A with imipenem, cefmetazole, or flomoxef, yields homoconverted mutants at a high frequency of \(10^{-4}\) to \(10^{-5}\).\(^4\) This observation agrees with the historical rise of clonotype-II-A MRSA in Japan.\(^2\) Unexpectedly about 5–10% of the homo-MRSA mutants are hetero-VRSA (figure 9) which indicates that hetero-VRSA might have emerged in Japan in the late 1980s from the MRSA strains exposed to the beta-lactams used at the time. Indeed, several hetero-VRSA strains were found in the late 1980s in Japan before the introduction of vancomycin. There are several genetic mechanisms underlying hetero-to-homo conversion of meticillin resistance.\(^4\) Evidently, there exists some common genetic mechanisms that confer both the hetero-to-homo conversion of meticillin resistance and VSSA to hetero-VRSA conversion. With regard to the relationship between meticillin and vancomycin resistance, Sieradzki and Tomasz reported a phenomenon in which raised vancomycin resistance is associated with reduced meticillin resistance.\(^5\) This phenomenon is observed with an in-vitro VRSA strain produced in the laboratory, which is based on the inactivation of PBPs and a drastic decrease in peptidoglycan cross-linkage. In this condition, it is understandable that the cells become very vulnerable to the action of beta-lactam antibiotics. But, in the case of 16 clinical VRSA strains, the level of meticillin resistance remains high (oxacillin MICs \(\geq 64\) mg/L) except for one strain PC-3 isolated in New York, whose oxacillin MIC is 8 mg/L (L. Cui, personal communication).

The glycopeptide-resistance expression of hetero-VRSA is also influenced by the exposure to beta-lactams. Practically all beta-lactam antibiotics when used at an optimal concentration, increases vancomycin resistance of the hetero-VRSA strain, Mu3.\(^6\) This antagonism which can be demonstrated in vitro, poses a potential problem in the use of combination regimens of beta-lactam and vancomycin against VRSA. It is surprising that there is no antagonism shown between beta-lactams and teicoplanin.\(^4\) The reason for this difference is unknown, but may be correlated with the difference in the resistance mechanism for the two glycopeptides. It would be worthwhile to further explore this difference, in an effort to optimise a combined use of teicoplanin and beta-lactams in the treatment of MRSA infections.

**Strategies to counter VRSA infection**

The nature of the resistance mechanism of VRSA—production and accumulation of excess amounts of cell-wall peptidoglycan—indicates that VRSA would not be prevalent in an environment where the glycopeptide selective pressure is not strong. If a hospital reduces the consumption of glycopeptides, VRSA should not prevail in the hospital. However, this action does not solve the problem completely, because hetero-VRSA may be capable of dissemination without glycopeptide pressure. Therefore, it is necessary to expand antibiotic prescription policy to include beta-lactam antibiotics as well. If we reduce consumption of broad-spectrum cephalosporins (which are ineffective against MRSA), and, this measure combined with effective infection control, the number of MRSA in the hospital would decrease according to a mathematical model developed by Lipsitch et al.\(^7\) Reducing the total number of MRSA is the most effective measure for preventing emergence of VRSA and hetero-VRSA. Recently, successful reduction of MRSA was achieved in a Japanese hospital by cutting the total use of broad-spectrum cephalosporins by half without compromising infection outcome.\(^7\)

It may also be possible to reduce the selection of vancomycin resistance in MRSA isolates in the hospital by substituting cephalosporins and carbapenems with penicillins that have a relatively strong anti-MRSA activity among beta-lactam antibiotics.\(^8\) In our hospital, use of ampicillin/subactam was encouraged as a substitute for broad-spectrum cephalosporins and carbapenems for surgical prophylaxis after the emergence of VRSA in 1996.\(^3\) As a result, penicillinase-producing MRSA increased from 47% in 1996–1997 to 96% in 1999–2000 (note that both Mu3 and Mu50 are characteristically non-producers of penicillinase). At the same time, MRSAs strains having vancomycin MICs of 4 mg/L or above decreased from 0.43% to 0.08% of total MRSA isolates during this period (T Oguri, and J Igari, Clinical Laboratory, Juntendo University, Tokyo, Japan, personal communication). Evidently, this is only a preliminary observation. A well-designed clinical
study would be required to confirm this anecdotal decrease in vancomycin-resistance among MRSA isolates. In any such clinical study in this direction, however, it would be important to monitor the prevalence of hetero-VRSA by alternative methods that can detect subtle changes in glycopeptide susceptibility of a high number of MRSA isolates.

Treatment of VRSA infection

New agents are being developed against MRSA. Some of them are expected to have considerable activity against hetero-VRSA and VRSA strains as well. Synercid has potent activity against hetero-VRSA and VRSA strains. A new quinolone antibiotic, DU-6859a, has MICs of 0.5 and 1 mg/L against Mu3 and Mu50 which are resistant to other quinolones such as levofloxacin, ciprofloxacin, sparfloxacin, and tosufloxacin. Linezolid also has good activity against MRSA, and is expected to be useful for cases where vancomycin therapy fails. However, linezolid does not have a cytokilling effect against MRSA.

While we do not have any single cytotoxic anti-MRSA antibiotic that exceeds the potency of vancomycin, it is important for us to explore several antibiotic combination therapies. Ampicillin/sulbactam in this regard would be a good partner for vancomycin; even the agent alone has good anti-microbial activity against VRSA in an experimental infection model. Linezolid has either a synergistic or additive effect on hetero-VRSA and VRSA strains when combined with ampicillin/sulbactam. This combination therapy, as well as arbekacin and ampicillin/sulbactam, takes advantage of ampicillin’s high affinity to MRSA PBPs. When we use vancomycin, it is evident from its mechanism of action and mechanism of resistance that we have to try and reduce the bacterial burden from the patient’s body with such procedures as surgical drainage, debridement, and removal of contaminated lines, foreign bodies, or prosthetic materials. When vancomycin therapy is still unsuccessful with these procedures, we use triple therapy of vancomycin, rifampicin (oral) and co-trimoxazole (oral).

Future perspectives

This year, the whole genome sequences of VRSA strain Mu50 and MRSA strain N315 were published. The latter strain, susceptible to vancomycin, is closely related to Mu50; 96% of the nucleotide sequences are identical between the two. By comparing the sequences of N315 and Mu50, we should be able to identify the genetic basis for glycopeptide resistance. These genome data will serve as an invaluable source of information for the future development of novel antibiotics as well as an S aureus vaccine. However, the information inscribed in the Mu50 and N315 genomes also showed that S aureus is an extremely flexible pathogen. It is capable of acquiring any useful genes for survival across species barriers, and updating its armamentarium by multiplying toxin genes by successive gene duplication to attack any people of diverse immune history and genetic backgrounds. We may never be free from the threat of S aureus since part of the benign natural flora when we are healthy becomes a formidable intruder when our body defences become compromised. The best strategy is to maintain a healthy ecological balance with effective intervention strategies to reduce the organisms need to acquire resistance to the best antibiotics. Decreasing the total consumption of antibiotics both in the hospitals and community, and, using them with good rationale based on accurate diagnosis and susceptibility testing information may help preserve mankind’s precious drug.

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Review