The Multiple Paths to Heteroresistance and Intermediate Resistance to Vancomycin in Staphylococcus aureus

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(See the major article by Vidaillac et al on pages 67–74.)

Keywords. vancomycin; intermediate resistance; heteroresistance; Staphylococcus aureus; mechanisms

A comprehensive understanding of the mechanisms by which Staphylococcus aureus achieves intermediate levels of resistance to vancomycin could foster the development of improved diagnostic tests and therapeutic agents, with potentially improved clinical outcomes. Vidaillac et al, in this issue of the Journal [1], used well-characterized successive S. aureus clinical isolates that had acquired serial mutations together with reduced susceptibility during vancomycin treatment in order to compare the in vivo evolution of resistance in these isolates to that seen in vitro [2]. They reasoned that the patient’s isolates, all recovered from blood, may not have been fully representative of organisms present at tissue site(s) of infection. Using an in vitro pharmacokinetic/pharmacodynamic (PK/PD) model with simulated vegetation, they were able to observe phenotypic changes, including reduced viability, loss of pigmentation, and changes in colony size and hemolytic activity, prior to the identification of the detection of resistant mutants. With the appearance of the latter, daptomycin minimal inhibitory concentrations (MICs) rose, along with those of vancomycin, and this was accompanied by thickened cell walls and reduced autolysis, decreased surface anionic charge, and susceptibility to cationic peptides. Whole-genome sequencing demonstrated that the vancomycin-intermediate S. aureus (VISA) phenotype was acquired via at least 3 different genetic pathways and that, in one of the 2 cases, the pathway differed from that seen in vivo.

High-level resistance of S. aureus to vancomycin, resulting from horizontal transfer of transposon Tn1546 carrying the vanA operon from Enterococcus species, is well understood but rare [3]. In contrast, intermediate resistance is more common, more complex, and less well understood. VISA and vancomycin-heteroresistant S. aureus (hVISA; heteroresistance to vancomycin is considered a transitional state to intermediate resistance) exhibit a number of typical phenotypic characteristics, including cell wall and septal thickening, abnormal cell division, reduced cell wall turnover and autolysis, reduced hemolytic activity, and reduced virulence in animal models [4]. The vancomycin heteroresistance and intermediate resistance phenotypes are believed to result from altered cell envelope charge and diminished penetration of the drug through the thickened cell wall. The excess d-ala-d-ala targets in the cell wall act as a molecular sink, impairing vancomycin from accessing its pentapeptide target [5, 6].

Mutations associated with the intermediate resistance phenotype have been identified in VraSR (vancomycin resistance–associated sensor/regulator), GraSR (glycopeptide resistance–associated sensor/regulator), WalKR (also known as “YycGF” and “VicKR”), as well as rpoB and others. VraSR, GraSR, and WalKR are each 2-component regulatory systems (TCS). TCS are ubiquitous among bacteria, allowing them to sense and respond to their environment [7]. They possess a cell membrane histidine kinase acting as a sensor/transducer that responds to its external signal by autophosphorylation in the presence of adenosine triphosphate. The phosphoryl group is then transferred to a response regulator, generally a DNA-binding protein that regulates transcription. S. aureus encodes 16 such signal transduction systems in its chromosome, and SCCmec encodes an additional one [7, 8].

VraSR had previously been found to be upregulated in hVISA strain Mu3 and VISA strain Mu50 [8, 9]. It acts under the regulation of yvqF as an on-off switch of

Received and accepted 1 March 2013; electronically published 28 March 2013.
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The Journal of Infectious Diseases 2013:208:7–9
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DOI: 10.1093/infdis/jit136
a large cell wall–stress stimulon that responds to cell wall–active antibiotics, among other triggers [10]. Of note is that overexpression of vraSR is also associated with reduced susceptibility of S. aureus to daptomycin [11], consistent with the observation of a frequent correlation between vancomycin and daptomycin MICs [5].

Mutations in GraSR were first identified in association with reduced susceptibility to vancomycin, but they are also important in the resistance of S. aureus to cationic antimicrobial peptides [12, 13]. GraSR controls genes involved in the stress response and also controls the expression of several virulence genes. In addition, it has significant regulatory overlap with the WalKR TCS [13]. WalKR (also known as “YtxGf”) controls cell wall degradation and turnover (which leads to activation of the innate immune response, with counterbalancing evasion through control of the SacSR regulon). WalKR, which controls a number of virulence genes, is the only TCS demonstrated to be essential for the viability of S. aureus [14, 15]. Mutations in Walk or WalR are associated with the intermediate resistance phenotype, as well as with reduced susceptibility to daptomycin and attenuated virulence [15].

rpoB, another relevant gene, albeit one that does not encode a TCS, encodes the β subunit of RNA polymerase. A mutation in rpoB (different from those mutations causing rifamycin resistance) is associated with the intermediate resistance phenotype, as well as with reduced susceptibility to daptomycin [16]. This has been associated with increased expression of the dlt operon, which elevates the surface membrane charge and may account for cross-resistance of vancomycin and daptomycin, which are functionally cationic molecules (the anionic daptomycin acts like a cation in the presence of Ca++ ) [6].

Mutations in ≥1 of these 3 TCS or in rpoB can be identified in almost all VISA and hVISA. Sequencing of candidate loci of 22 VISA and 9 hVISA clinical isolates identified a great diversity of nonsynonymous single-nucleotide polymorphisms (SNPs), with an association with clonal background [17]. Mutations in GraR, GraS, and WalR were detected only in VISA isolates. The MIC was correlated with the number of SNPs in an individual isolate.

A large number of additional SNPs have been observed in hVISA and VISA strains, but the evidence for a causal role is generally lacking. Exceptions include mutations in clpP, a regulatory protease that is reported to be responsible for the raised vancomycin MIC in a laboratory-derived VISA strain and that appears to act together with WalK mutations to elevate the MIC to a greater extent than that seen with each individually [18]. ClpP has been found to be necessary for stress tolerance and biofilm formation in Actinobacillus pleuropneumoniae [19]. Mutation in the gene encoding P2C phosphatase has also been associated with reduced susceptibility to both vancomycin and daptomycin in a USA300 clinical isolate [20]. Mutations in the gene encoding serine-threonine phosphatase, stp-1, which is involved in regulation of cell wall metabolism and virulence, have also been associated with reduced vancomycin susceptibility [21].

While the emergence of hVISA and VISA is generally assumed to be the result of exposure to vancomycin, hVISA was present in Japan prior to the introduction of vancomycin or teicoplanin therapy in that country [22]. This may have been the result of exposure to other cell envelope–active antibiotics, such as β-lactams [23], that are known to affect a number of TCS, but it can also be speculated that it resulted from natural selection of mutants by other molecules that activate stress mechanisms. The environment of the nares, a preferred niche, contains cationic antimicrobial peptides (CAMPs) [24]. CAMPs induce the VraSR regulon in S. aureus [25], consistent with previous evidence that the natural CAMP, LL-37, strongly activates LiaRS, a homolog of VraSR in Bacillus subtilis [26]. In addition to the presence of CAMPs in nasal secretions, colonizing S. aureus coexist in the nares with other bacteria that may produce bacteriocins, which can also cause cell envelope stress in bacteria [27]. These stressful environmental conditions may have set the conditions for the natural selection leading to the presence of hVISA in the absence of prior exposure to vancomycin.

These observations and their interrelationships are incomplete, yet remarkably complex, reflecting the remarkable adaptive ability of S. aureus.

The ability to observe the evolutionary steps in the development of reduced vancomycin susceptibility in vitro, as demonstrated with the PK/PD method used by Vidaillac et al [1], is an important technological step toward increased understanding of this resistance phenotype [1]. The evidence developed by these and other investigators clearly demonstrates that the emergence of hVISA and VISA can occur via a plethora of pathways. As a consequence of these polygenic routes to reduced vancomycin activity, there is no signature mutation whose absence can reliably inform the clinician that an organism is fully susceptible to vancomycin. The clinician is further hampered by the fact that vancomycin MIC determinations in S. aureus are method dependent, making even the phenotypic identification of such isolates potentially problematic [28].

Note
Potential conflict of interest. S. D. is on scientific advisory boards of Pfizer, RibX, Merck, and Cerexa.
The author has submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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