Identification of Low-Level Vancomycin Resistance in Staphylococcus aureus in the Era of Informatics

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Vancomycin is currently the standard treatment for presumptive or culture-proven methicillin-resistant Staphylococcus aureus (MRSA) and has been so for the past several decades (1, 2). Several meta-analyses have suggested that the vancomycin MIC positively correlates with treatment failure, regardless of the method used to calculate the MIC. This suggests that the method most commonly used for calculating vancomycin MICs (Etest; bioMérieux Clinical Diagnostics) might be appropriately applied to all MRSA isolates (2-4).

Vancomycin exhibits concentration-independent killing of S. aureus, and the best pharmacodynamic indicator of efficacy is the ratio calculated as the area under the concentration curve (AUC) divided by the MIC (AUC/MIC). Current evidence indicates that clinical success may be reasonably expected if the AUC/MIC ratio exceeds 400 (5, 6). For patients with normal renal function, this target could not be achieved for MRSA isolates exhibiting a vancomycin MIC of \( \geq 2 \) \( \mu g/\text{ml} \) without exposing the patients to toxic levels of vancomycin (5-7). Most MRSA strains exhibit MICs of 1 \( \mu g/\text{ml} \), and several large surveys place 2 \( \mu g/\text{ml} \) precisely on the border of the wild-type MIC distribution for MRSA versus vancomycin (8-10). Thus, precise calculation of the vancomycin MIC for MRSA is an essential element of predicting the clinical success of vancomycin.

Further complicating the use of the vancomycin MIC as a predictor of clinical failure is the fact that standard methods of measuring it are imprecise, limiting the utility of the vancomycin MIC as a clinical decision support tool. High analytic variability of greater than \( \pm 1 \) doubling dilution is typical, in addition to a method-dependent fixed bias of 1 or 2 doubling dilutions (11-16).

VISA and hVISA

Vancomycin-intermediate S. aureus (VISA), exemplified by the type strain Mu50 (17), typically exhibits vancomycin MICs of 4 to 8 \( \mu g/\text{ml} \). Because of a tendency to revert to a wild-type MIC without continuous selective pressure from vancomycin, VISA is difficult to detect by standard MIC-based methods, where the standard practice is to confirm such unusual phenotypes through subculture and retesting in the absence of vancomycin selective pressure.

Heteroresistant VISA (hVISA), exemplified by the type strain Mu3 (18), exhibits vancomycin MICs that overlap the wild-type distribution by standard methods. Identification of the subpopulations of hVISA clones that exhibit elevated MICs is therefore done by cumbersome high-inoculum population analysis (19). Alternate methods such as Etest glycopeptide resistance detection strips, high-inoculum macro Etest, and agar containing vancomycin (20-22) are less labor-intensive but take up to 2 days. This introduces significant delays in the identification or exclusion of VISA or hVISA, during which a patient may be on suboptimal therapy. Rapid nucleic acid-based testing is not available for VISA and hVISA, partly because of genetic heterogeneity (reviewed by Howden and colleagues [23]).

VISA and hVISA are not just theoretical concerns; estimates vary, but the prevalence of hVISA is often placed at around 10% of the total S. aureus isolates in surveys where effective methods are employed to detect the hVISA phenotype (22). The prevalence of VISA is more difficult to estimate because of the phenomenon of \( ex vivo \) reversion to a susceptible phenotype but is likely much lower (10). For this reason, most research focuses on hVISA. There are, however, no surveys of how many clinical laboratories currently screen isolates for the hVISA phenotype. A search of the archives of the ClinMicroNet discussion group (24) revealed scant discussion of this problem, suggesting that hVISA receives little attention in current clinical practice relative to its potential impact.

Clinical outcome data related to the hVISA phenotype are scarce, and quantification of its impact is limited both by a lack of ability to detect the phenotype and by the small sample sizes used in individual studies. In a larger and more recent meta-analysis than those previously cited, an MIC of \( \geq 1.5 \) \( \mu g/\text{ml} \) was not associated with an increased mortality rate when subgroup analysis...
controlled for additional factors, including the method used to calculate vancomycin MICs, MIC cutoffs, whether strains were MRSA or methicillin-susceptible S. aureus (MSSA), and the presence of the hVISA phenotype (25). Paradoxically, in the seven studies that assessed the impact of the hVISA phenotype, a small decreased risk difference was found for hVISA over non-hVISA (−7.0% [95% confidence interval, −14.6% to 0.6%]). Although these findings are not formally significant (P = 0.07), they reveal a trend that may suggest lower virulence of hVISA strains and/or a heretofore unappreciated fact about the susceptibility of the hosts it infects. Indeed, in one study, patients infected with MSSA with elevated vancomycin MICs who were treated with fluoroquinolone had higher rates of mortality than those infected with strains with lower MICs. This was similar to the mortality rate of patients infected with MRSA with elevated vancomycin MICs who were treated with vancomycin, suggesting that the severity of illness and/or the virulence of the infecting strain may be a more important clinical outcome predictor than the vancomycin MIC (26).

Collectively, these findings illustrate the need for improved methods to separate vancomycin-susceptible S. aureus from VISA and hVISA strains so that a more effective assessment of their impact on clinical care may be made. High-quality clinical evidence tying vancomycin MICs to outcomes is lacking, and this shortcoming is driven partly by a lack of microbiological methods to define vancomycin MICs and the VISA and hVISA phenotypes. With the burgeoning number of clinically effective alternative antimicrobial therapies for MSSA and MRSA infections (reviewed in references 27 and 28), the decision to treat with vancomycin or an alternative is an increasingly prevalent dilemma that deserves better support from the clinical microbiology laboratory.

**PROXY SUSCEPTIBILITY TESTING BY MALDI-TOF**

The matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) instruments now commonly employed in clinical microbiology laboratories have limited mass resolution and cannot achieve definitive molecular identifications by, for example, fragmenting proteins to determine their amino acid sequences. The functionality of off-the-shelf MALDI-TOF systems is limited to comparison of whole-cell mass spectra to reference spectra to obtain the identification of unknown organisms. Still, the mass fingerprint produced in a MALDI-TOF experiment is rich enough to allow productive comparison of the mass spectra of identical strains collected before and after experimental treatment and those of unique strains if the conditions are well controlled.

Most susceptibility-by-MALDI-TOF work to date has either (i) identified beta-lactamase activity through direct observation of antibiotic hydrolysis or (ii) associated a resistance phenotype with the observation of changes in bacterial spectra after exposure to drugs (reviewed in reference 29). This allows common acquired resistance mechanisms, especially those seen in Gram-negative organisms, to be identified within hours. Resistance in Gram-positive bacteria, such as vancomycin resistance in enterococci, methicillin resistance in staphylococci, and the VISA and hVISA phenotypes, are often the result of more complex mechanisms that do not involve easily observed enzymatic activity. Identification of strains harboring these phenotypes using clinical-grade MALDI-TOF instruments requires the collection of spectra from large sets of well-characterized strains with and without the phenotype of interest, as well as identification of differential “features” (which most often remain unidentified but may represent proteins, lipids, or other small molecules within the observable mass range of the instrument) within those spectra that are associated with the phenotype of interest. Individual features must generate a prominent signal, have within- and between-run repeatability, be robust to routine preanalytical variability, and be sensitive and specific markers of the phenotype of interest. In this respect, a mass spectrometric “feature” or collection of features is similar to any commonly employed laboratory test (30).

However, as opposed to a single-analyte lab test, the best MALDI-TOF methods used to identify phenotypes from whole-cell mass spectra often analyze hundreds of spectral features by using custom computer codes. The classification performance of combinations of these features is established through statistical “machine learning” algorithms that map large numbers of unidentifiable but useful features into multidimensional space. “Normal ranges” and “cutoffs” do not make intuitive sense, though once trained, a machine learning classifier can be validated and deployed like a routine laboratory test with the familiar performance characteristics of sensitivity and specificity.

As a technique for identifying bacterial phenotypes, rapid classification based on MALDI-TOF spectra has the appeal of speed (minutes for analysis) and ease, given the use of commonly available clinical instruments and the potential to reuse the same spectrum for bacterial identification. As Mather et al. have demonstrated (31), for VISA and hVISA, even an imperfect classifier has a very high negative predictive value (99%), potentially allowing definitive therapy to be prescribed with confidence much earlier than with culture-based methods. This technology has the potential for broad application to problems other than VISA and hVISA, but there are a number of barriers to widespread adoption.

**MICROBIOLOGY INFORMATICS AND FUTURE CHALLENGES**

With this new technology, most clinical microbiologists will find their skill sets challenged by unfamiliar terms, tools, and means of understanding. The exemplary study by Mather and colleagues in this issue (31) offers a view of how old and new techniques intersect in the development of a MALDI-TOF method to detect VISA and hVISA. In their study, the authors employed classical methods and type strains, new culture-based innovations (vancomycin-containing agar plates), and informatics-based innovations to construct a new way to rapidly identify the VISA and hVISA phenotypes both rapidly and accurately.

There are several notable innovations in this study that deserve comment. Mather and colleagues used clinical-grade equipment and open-source computer programs that are free for others to use. The programming code itself is text that can be straightforwardly cut, pasted, and run and is provided in its entirety as supplemental material. The authors have also provided comments to assist others in deploying it in their own research, thereby allowing interested parties to generate a free-of-charge (minus time and materials) replica of these authors’ work in minutes to days. The code published here is also modular; spectral processing, machine learning, and classification can all be broken out, modified, and improved upon. Provision of source code is therefore a notable advance that has rarely been seen in the clinical microbiology literature to this point. Publication of source code should be encouraged by scientific journals for its potential to improve the reproducibility of studies that employ custom computer code (32).

This advance, however, presents new dilemmas that must be addressed by a scientific publishing enterprise that has not had to
manage authors wishing to give away free machines for peer review and scientific advancement. Given variations in implementation, only the original source code is a perfect representation of itself that allows exact replication of an experiment. Given this, if a manuscript contains source code that is critical to the conclusions drawn, but it has not at least been provided for peer review, has that manuscript been adequately peer reviewed? Taken a step further, should authors be required to deposit source code and, if so, where? Open-access code repositories such as Dryad (Durham, NC, USA) and figshare (London, United Kingdom) offer persistent repositories for source code, allowing long-term accessibility of the code referred to in published works. What about proprietary code or code that contains intellectual property? These questions should be addressed in formal journal policies such as those of the Nature Publishing Group (33).

As informatics-based technologies are increasingly brought into the clinical microbiology laboratory, a related challenge will be in adequately training clinical microbiologists to appreciate these tools to identify VISA, hVISA, and other resistance phenotypes. This work reinforces the need for the implementation of tools to identify VISA, hVISA, and other resistance phenotypes more rapidly and effectively, as well as the need for a broad effort to train and certify informatics-literate clinical microbiologists to address this gap. As the rise of next-generation sequencing and whole-slide imaging, evolving pathology informatics curricula should focus more sharply on data handling and the manipulation of data through programming (35). This imparts a level of informatics literacy, if not proficiency, that will be necessary in the practice of clinical microbiology in the future. The American Society for Microbiology, College of American Pathologists, and affiliated groups and journals should develop standards for informatics training and expand educational offerings at the resident, fellow, and continuing medical education levels.

The results reported in this issue by Mather et al. (31) are a clear indication that progress is being made at the intersection of rapid AST, clinical MALDI-TOF, and pathology informatics in addressing shortcomings of the classical laboratory treatment of VISA and hVISA. This work reinforces the need for the implementation of tools to identify VISA, hVISA, and other resistance phenotypes more rapidly and effectively, as well as the need for a broad effort to train and certify informatics-literate clinical microbiologists capable of building upon the type of scientific tools published here.

REFERENCES


