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Exploring innate glycopeptide resistance mechanisms in *Staphylococcus aureus*

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Clinical relevance of intermediate resistance to glycopeptides

Staphylococcus aureus is a common cause of hospital-acquired diseases, with some of them due to infection of medical devices such as artificial heart valves, catheters, and orthopedic implants. Multidrug resistance is commonly observed in hospital isolates of methicillin-resistant *S. aureus* (MRSA), which are characterized by a mobile chromosomal element (SCCmec) encoding a penicillin binding protein (PBP) known as PBP2A. Because of its low affinity for β -lactam antibiotics, PBP2a allows cell-wall synthesis at β -lactam concentrations that inhibit other PBPs. For this reason, the glycopeptides antibiotics vancomycin and teicoplanin are frequently used to treat multiresistant MRSA infections. It was anticipated that intensive use of these antibiotics would eventually promote emergence of resistant clones, severely limiting therapeutic options [1]. Interestingly, it took almost 50 years before the first truly vancomycin-resistant clinical isolates were described that carried the *vanA* resistance gene cassette (VRSA), presumably transmitted from *Enterococcus* [1]. In contrast, an intermediate or low-level resistance, ascribed to innate vancomycin resistance in the absence of *vanA*, arises both *in vivo* and *in vitro* in *S. aureus* following prolonged drug exposure [1, 2]. Strains that exhibit vancomycin intermediate resistance (VISA) and glycopeptide intermediate resistance (GISA, referring to both teicoplanin and vancomycin), as well as those displaying intermediate resistance to only teicoplanin have been described.

Morphological changes of VISA/GISA

A fundamental question underlying innate resistance is what changes occur to counter the effects of the glycopeptide antibiotics, which target terminal stages of peptidoglycan assembly outside the bacterial cell membrane. Both vancomycin and teicoplanin bind to the D-alanyl-D-alanine terminus of peptidoglycan murein monomer (lipid II) and prevent condensation of additional monomers to form the nascent peptidoglycan chain [3]. Two morphological changes frequently encountered in clinical and laboratory-derived GISA isolates are: (i) cell wall thickening, which might contribute to glycopeptide resistance by amplifying stem peptide targets or preventing access of the antibiotic to the underlying cell wall targets [4]; and (ii)

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heterogeneous clusters of intensively pigmented colonies on agar plates, which might reflect stress-induced activation of σ^B , an alternative sigma factor [5]. Most likely, both transmembrane signaling and altered gene expression underlie innate responses to glycopeptides.

Current approaches to elucidate resistance mechanisms

Differential transcriptomic profiling of glycopeptide-resistant strains with their presumptive susceptible parents was used to identify resistance-contributing candidate genes [6-8]. As a result, several global regulators and mutations in two-component sensory systems (TCS) have been implicated. Among these, the TCS *vraRS* is known to play a prominent role in mediating resistance to various cell-wall-active antibiotics and as a global regulon of cell-wall biosynthesis pathway(s) in *S. aureus* [6]. A note of caution is warranted when studying cell-wall stress regulons, since the results can differ among the different strains and antibiotic concentrations used [9].

Whole-genome sequencing of serial *S. aureus* isolates that developed glycopeptide resistance either *in vitro* or during patient therapy has helped identify mutations in a few TCS contributing to the VISA/GISA phenotype, such as *vraRS*, *yycF* (WalKR), *agrAC* or *graRS* [10,11]. While this approach is powerful and may be broadly applied in the future as genomic sequencing costs fall, data validation should minimally satisfy the following criteria. First, sequential clinical isolates that develop low-level resistance under glycopeptide therapy should not be exposed to any additional antibiotics. Additionally, sequential, progressively resistant clones from diverse backgrounds should be compared to delineate common genetic changes required for expression of glycopeptide resistance. Finally, the impact of any mutation on gene expression levels should be rigorously assessed by appropriate genetic methods.

Comparison of genetically-stable, glycopeptide-susceptible revertants arising from glycopeptide-resistant parents has proven useful [12]. Comparative genomic hybridization revealed that a spontaneous teicoplanin-susceptible revertant (isolated from an animal model of foreign body infection) carried a specific deletion of two open reading frames, compared to its resistant parent [12]. The role of these genes is presently under investigation.

Perspectives and future implications

We suggest that several issues should be addressed for a better understanding of the molecular basis of innate glycopeptide resistance. First, we lack simple, reliable assays for detecting VISA phenotypes, the expression of which is frequently heterogeneous across bacterial populations. Phenotypic detection of reduced susceptibility to teicoplanin is far easier, but cross-resistance to vancomycin is by no means universal. In contrast, infrequent clinical isolates displaying clear-cut VISA phenotypes are almost uniformly cross-resistant to teicoplanin. The precise reasons for differences in cross-resistance need to be explored.

Second, detailed physiological studies of isolates that are resistant to teicoplanin and sensitive to vancomycin should be carried out in comparison to strains resistant to both glycopeptides. For instance, a connection appears to exist between glycopeptide resistance and an altered surface charge in *S. aureus*. Mutants in either *graRS* (encoding a TCS [7]) or the adjacent genes *vraFG* (coding for an ABC transporter) have a more negative surface charge and are hypersensitive to vancomycin and polymyxin B [13,14].

Third, co-emergence of daptomycin resistance and the VISA phenotype has been observed in some isolates [15]. Uncovering the molecular basis of this linkage will certainly open new avenues of research.

In summary, despite intense study, a global model for innate glycopeptide resistance in *S. aureus* remains elusive. It is likely that multiple genetic changes occur during acquisition and maintenance of the resistance phenotype. Understanding the nature and overlap of these events may provide important insights to future therapy of multiresistant *S. aureus*.

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References

1. Tenover FC, Moellering RC Jr. The rationale for revising the Clinical and Laboratory Standards Institute vancomycin minimal inhibitory concentration interpretive criteria for *Staphylococcus aureus*. Clin. Infect. Dis 2007;44:1208–1215. [PubMed: 17407040]
2. Hiramatsu K. Vancomycin-resistant *Staphylococcus aureus*: a new model of antibiotic resistance. Lancet Infect. Dis 2001;1:147–155. [PubMed: 11871491]
3. Allen NE, Nicas TI. Mechanism of action of oritavancin and related glycopeptide antibiotics. FEMS Microbiol. Rev 2003;26:511–532. [PubMed: 12586393]
4. Cui L, et al. Cell wall thickening is a common feature of vancomycin resistance in *Staphylococcus aureus*. J. Clin. Microbiol 2003;41:5–14. [PubMed: 12517819]
5. Bischoff M, Berger-Bächi B. Teicoplanin stress-selected mutations increasing sigma(B) activity in *Staphylococcus aureus*. Antimicrob. Agents Chemother 2001;45:1714–1720. [PubMed: 11353616]
6. Kuroda M, et al. Two-component system VraSR positively modulates the regulation of cell-wall biosynthesis pathway in *Staphylococcus aureus*. Mol. Microbiol 2003;49:807–821. [PubMed: 12864861]
7. Cui L, et al. DNA microarray-based identification of genes associated with glycopeptide resistance in *Staphylococcus aureus*. Antimicrob. Agents Chemother 2005;49:3404–3413. [PubMed: 16048954]
8. McAleese F, et al. Overexpression of genes of the cell wall stimulon in clinical isolates of *Staphylococcus aureus* exhibiting vancomycin-intermediate-*S. aureus*-type resistance to vancomycin. J. Bacteriol 2006;188:1120–1133. [PubMed: 16428416]
9. McCallum N, et al. Strain dependence of the cell wall-damage induced stimulon in *Staphylococcus aureus*. Biochim. Biophys. Acta 2006;1760:1475–1481. [PubMed: 16891058]
10. Mwangi MM, et al. Tracking the *in vivo* evolution of multidrug resistance in *Staphylococcus aureus* by whole-genome sequencing. Proc. Natl. Acad. Sci. U. S. A 2007;104:9451–9456. [PubMed: 17517606]
11. Howden BP, et al. Genomics reveals a point mutation in the two-component sensor gene *graS* that leads to vancomycin-intermediate resistance in clinical *Staphylococcus aureus*. Antimicrob. Agents Chemother 2008;52:3755–3562. [PubMed: 18644967]
12. Renzoni A, et al. Identification by genomic and genetic analysis of two new genes playing a key role in intermediate glycopeptide resistance in *Staphylococcus aureus*. Antimicrob. Agents Chemother 2009;53:903–911. [PubMed: 19104009]
13. Meehl M, et al. Interaction of the GraRS two-component system with the VraFG ABC transporter to support vancomycin-intermediate resistance in *Staphylococcus aureus*. Antimicrob. Agents Chemother 2007;51:2679–2689. [PubMed: 17502406]
14. Herbert S, et al. Molecular basis of resistance to muramidase and cationic antimicrobial peptide activity of lysozyme in staphylococci. PLoS. Pathog 2007;3:e102. [PubMed: 17676995]
15. Cui L, et al. Correlation between reduced daptomycin susceptibility and vancomycin resistance in vancomycin-intermediate *Staphylococcus aureus*. Antimicrob. Agents Chemother 2006;50:1079–1082. [PubMed: 16495273]