



## The Relationship between Virulence and Antimicrobial Resistance in Bacteria

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A number of recent discoveries have defined the relationship between bacterial virulence (or fitness) and antimicrobial resistance. Although the terms “fitness” and “virulence” are sometimes used interchangeably, they refer to different characteristics in bacteria. Fitness is defined as the ability of a bacterium to reproduce and grow under a variety of environmental conditions, while virulence is the property whereby bacteria exhibit pathogenicity to mammalian and other hosts. In general, organisms which have lost fitness are less virulent, but the reverse is not true. There are a number of ways in which bacterial virulence (or fitness) and antimicrobial resistance can be related. For instance, mutations or acquisition of plasmids encoding antimicrobial resistance may render organisms less fit; but, compensatory mutations may also restore fitness in the presence of antimicrobial resistance. Virulence mechanisms may result in resistance to antimicrobials and resistance itself may contribute to virulence. The presence of resistance and virulence genes on the same plasmid or transposon may lead to coselection in the presence of antimicrobial agents. The acquisition of resistance genes may be accomplished by organisms with preexisting virulence factors. Antimicrobials may also modulate virulence; and finally, genetic regulatory systems may control both the production or expression of virulence factors and antimicrobial resistance. It is the latter phenomenon which will be emphasized in this presentation.

A number of bacteria have well defined quorum sensing mechanisms and in several of these, mutations which alter quorum sensing result in alterations both in virulence and in antimicrobial resistance. For example, mutations in the *fsr* quorum sensing system in *E. faecalis* result in decreased autolysis on exposure to beta-lactams. Perhaps more important is the role of the accessory gene regulator (*agr*) quorum sensing system in *S. aureus*. We have recently shown that mutations affecting the expression of this system can be associated with the acquisition of glycopeptide resistance.

The *agr* system is one of several global regulatory loci in *S. aureus*. Another important system is the *sarA* (staphylococcal accessory regulator) system which regulates the expression of cell surface and virulence genes and can interact with *agr* promoter elements as well. Recent studies of oligonucleotide micro-arrays and Northern blot analysis confirm that both of these systems have an extensive global regulatory function in *S. aureus*. The accessory gene regulator (*agr*) system regulates both quorum sensing and expression of cell surface genes and virulence factors in *S. aureus*. It does so by the expression of quorum sensing octopeptides (also known as autoinducing peptides). These extracellularly-secreted peptides allow cell-cell communication among *S. aureus*; and via interaction with specific targets, they influence the expression of cell surface proteins (e.g., protein A, coagulases, fibronectin-bind-

ing proteins, etc.) and biofilm. This system also regulates the expression of exotoxins. As *S. aureus* reaches the stationary phase of growth, the *agr* system represses the genes encoding for cell surface proteins and activates expression of secreted exotoxin or virulence genes. Based on polymorphisms in the autoinducing peptides, *S. aureus* can be classified into one of four *agr* groups.

The autoinduction of virulence in *S. aureus* is thought to occur as a two-step process. As organisms multiply, an autoinducer known as ribonucleotide activating protein (RAP) accumulates and induces autophosphorylation of its receptor known as TRAP (target for RAP). This in turn upregulates a specific promoter (P2) which leads to the production of RNAII which is the messenger RNA for the production of the four major genes (ABCD) in the *agr* system. This then leads to the production of the autoinducing peptide and its receptor *agrC*. When AIP binds to *agrC*, it leads to phosphorylation of *agrA* which activates P3, which in turn upregulates RNAIII synthesis and down regulates TRAP phosphorylation. The production of RNAIII causes increased production of virulence factors. Recent studies in our laboratory have shown that alterations in the expression of the *agr* system are related to (and likely play a major role in) the development of glycopeptide tolerance and resistance in *S. aureus*.

It has been known for a number of years that the bactericidal activity of the glycopeptides (vancomycin and teicoplanin, in particular) appears less potent against modern staphylococcal isolates than it was 30 or 40 years ago. Moreover, during the past decade, a series of "glycopeptide intermediate" strains of *S. aureus* have been described. These strains have vancomycin MICs of 8-16  $\mu\text{g}/\text{mL}$  and have been associated with therapeutic failures of vancomycin and teicoplanin. These organisms usually exhibit heteroresistance to vancomycin and infections due to these organisms do not respond to vancomycin therapy, even when the serum concentrations of vancomycin are maintained above the minimal inhibitory concentration of vancomycin for the infecting organism. Recent studies in our laboratory have suggested that the majority (but not all) of VISA and hetero-VISA (strains with heteroresistance but MICs of vancomycin of  $\leq 4 \mu\text{g}/\text{mL}$ ) fall into the accessory gene regulator group II. Moreover, we have studied a number of these isolates and found that all of them contain mutations in one or more of the three major genes (A, B, C) in the *agr* system. In addition to their heteroresistance of vancomycin, these strains exhibit decreased production of  $\delta$ -lysin and other virulence factors and demonstrate increased biofilm production and vancomycin tolerance. Although the majority of strains exhibiting this property fall into *agr* Group II, we and others have also found similar heteroresistance among strains in *agr* Group I. Thus the enrichment of these isolates for *agr* Group II may simply be a phenomenon of the clonal spread of organisms which are subject to the development of glycopeptide resistance. Nonetheless, when we studied MRSA isolates to determine if the Group II polymorphism at the *agr* locus demonstrated a relationship with the clinical efficacy of vancomycin, we found that among 36 clinically evaluable patients with the *agr* Group II polymorphism, 31 had an infection that failed to respond to vancomycin, whereas only five had an infection that responded successfully to vancomycin. This finding suggests that VISA and hetero-VISA clinical isolates in the United States and Japan are enriched for *agr* Group II polymorphism and suggests possible intrinsic survival advantage of some *S. aureus* clones with this genetic marker under vancomycin selective pressure. These findings also explain why the VISA and hetero-VISA strains may exhibit diminished virulence (we have observed a number of patients with prolonged bacteremia - up to 30 days - due to these organisms in the absence of death or metastatic infection). Thus when there are mutations in the *agr* system (particularly in *agr* Group II), the *agr* functions abnormally and the usual alteration in expression of genes mediated by this system is impossible. These organisms produce large amounts of biofilm, produce decreased toxins such as  $\delta$ -lysin (possibly explaining their diminished virulence), and

express glycopeptide tolerance, which under conditions of prolonged exposure to glycopeptides leads to additional mutations that ultimately raise the minimal inhibitory concentration of the glycopeptide, resulting in true glycopeptide resistance (currently known as VISA or GISA). This also is consistent with the clinical settings in which VISA and GISA have been noted. These include persistent forms of infection and/or bacteremia due to MRSA (sternal wound, IV lines, peritoneal dialysis infections, prosthetic valve endocarditis, etc.). There is usually the presence of a foreign body (which makes the enhanced biofilm production of these organisms even more effective in pathogenesis). Many of the patients have renal failure and thus receive intermittent (and sometimes inadequate) doses of glycopeptides and all of them have received long-term therapy with vancomycin or teicoplanin before the emergence of the GISA or VISA strains.

## References

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