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PERSPECTIVE

Important Complexities of the Antivirulence Target Paradigm: A Novel Ostensibly Resistance-Avoiding Approach for Treating Infections

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Use of antivirulence therapy has assumed that inhibition of bacterial fitness at the site of infection without directly affecting viability will minimize the development of resistance. However, selection for resistant strains is much more likely to occur at sites of colonization or in the environment following excretion of the therapeutic agent. Data are needed regarding whether the drug's target promotes fitness among bacteria in (drug-exposed) niches other than sites of infection. Furthermore, in vivo studies of resistance selection should assess off-target selection for resistance (eg, within the microbiome). Only when such data are available can the risk for development of resistance be gauged appropriately.

Keywords. antivirulence therapy; antimicrobial resistance; antimicrobial targets; virulence factors; microbiome; resistance selection.

In this era of increasing antimicrobial resistance and declining development and approval of new classes of antimicrobials, the threat of a postantibiotic era looms large. Existing antibiotics kill bacteria by inhibiting bacterial functions that are essential to cellular survival. This killing of susceptible bacteria results in strong selective pressure for escape variants, thereby driving the emergence of resistance. Consequently, efforts have been made to develop new therapeutic approaches that minimize selection pressure for resistance while maintaining therapeutic efficacy.

Since drugs that kill bacteria also create the selective pressure that drives resistance, an alternative approach to treating infections that may result in less resistance is to target nonessential virulence factors, thereby impairing the organism's ability to cause disease, without killing it. The assumption is that if inhibition of a virulence factor (eg, an adhesin, toxin, specialized secretion system, virulence regulator, or quorum-sensing molecule) does not directly affect pathogen viability, selection pressure for resistance will be minimized, and the development of resistance will be curtailed [1–7]. It is intuitive that modulating bacterial virulence should result in less selective pressure for resistance than attempting to kill the bacteria; nevertheless, this concept has not yet been well validated.

Thus far, the intellectual model driving the development of antivirulence therapy has focused almost exclusively on minimizing the selective pressure for resistance at the site of infection against the causative pathogen. However, it is unlikely that the emergence of resistance occurs predominantly at the site of infection among bacteria causing the active infection. Indeed, rarely does antimicrobial resistance arise among bacteria at the site of infection during treatment. Exceptions include treatment of cavitary tuberculosis with a single active agent and treatment of certain Enterobacteriaceae with agents that derepress or induce AmpC β-lactamases (eg, third-generation cephalosporins and *Enterobacter* organisms).

In contrast, in most instances of antimicrobial use the selection and clonal amplification of resistant strains is much more likely to occur off target (ie, at areas other than the infection site), either at mucosal or cutaneous sites of microbial colonization in both human and animal hosts or in the inanimate environment following excretion of the therapeutic agent or its metabolic breakdown products. This would occur with commensals, as well as with any acquired colonizing strains. These are ideal settings for selection of resistant variants, since microbial loads are enormous (particularly in the intestines), concentrations of the antimicrobial (or their metabolites) are low, and there is maximal biodiversity, enabling exchange of genetic information.

The site at which the target microbial factor is expressed and contributes to growth or persistence is critical for the development of resistance. If a factor that is required for virulence within the human host also contributes to persistence on an epithelial surface, a therapeutic agent directed against it could impose undesired selective pressure for resistance at the epithelial surface, away from the site of infection. Such selection would

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happen only if the therapeutic agent achieved active levels at the epithelial surface in addition to the site of infection.

Most human pathogens cause infection only incidentally, functioning instead primarily as commensals. Accordingly, the evolution of their virulence factors likely has not been mainly in response to the internal human nutritional or host defense environment or to promote pathogenesis. Therefore, it would not be surprising if bacterial traits that enable or enhance human infection also play an important role for bacterial growth/survival on an epithelial surface or in the environment. By extension, it also would not be surprising if interventions directed against such traits, with the goal of preventing or modulating disease, have unanticipated effects in the (colonized epithelium) commensal niche.

Data addressing this concept are limited since antivirulence therapy has yet to be used in the treatment of infections in humans. However, the experience with use of anticapsular vaccines against *Streptococcus pneumoniae* is a useful surrogate. These vaccines have been quite efficacious for decreasing the incidence of invasive disease but also have had profound effects on nasopharyngeal colonization. The resultant overall decrease in colonization with strains targeted by the vaccine has been beneficial. However, this commensal niche selection pressure has also led to escape variants that possess capsular epitopes not targeted by the vaccine. Interestingly and predictably, this has occurred both by outgrowth of nontargeted clones and by epitope switching among targeted strains [8–11], which can be considered a form of acquired resistance to the vaccine. This process has led, in turn, to a need for updated vaccine formulations designed to additionally target these new variants. Another example that supports this concept has been observed with the use of the traditional antimicrobial imipenem (Table 1). Treatment has been shown to select for resistant derivatives at a site of colonization, subsequently resulting in infection [12].

In contrast, a target that is expressed and/or contributes to fitness only during infection within the human host and does not promote epithelial or environmental colonization would be ideal for minimizing selection pressure and, thus, optimizing antimicrobial durability. Examples could include bacterial factors that are required to obtain or produce essential nutrients critical for microbial growth and survival within the nutrient-sequestered human host (eg, iron). Such factors are required for microbial virulence in the host but are typically noncritical in nutrient-rich settings such as the external environment (eg, sewage) or epithelial surfaces within the gastrointestinal tract.

Given these complex dynamics, we propose 4 conceptual categories of antimicrobials, based on their effect in different compartments (Table 1). The first category includes agents that have a lethal effect on bacteria at sites of infection but lack activity at epithelial and environmental sites of colonization. Such agents are largely hypothetical at this time, but we predict that they will exert minimal selection for resistance. The second category includes agents that, like virtually all currently available antimicrobials, have a lethal effect on bacteria at sites of infection but also at sites of colonization. Such agents select for resistance among colonizing strains. The third category includes agents that decrease the rate of or inhibit pathogen growth and/or decrease the degree of or inhibit virulence at sites of infection without killing the bacteria and that have no activity at sites of colonization. Such agents should exert minimal selective pressure driving resistance, although we also note that the efficacy of such agents has yet to be established outside of animal models. The fourth category includes antivirulence agents that have activity at both sites of infection and sites of colonization. Although such agents may select for resistance, the selection pressure likely would be less than with agents that are lethal for bacteria at the site of colonization (category 2). Of course, this hypothesis remains to be tested.

With respect to categories 1 and 3, it is logical to assume that targets that are not expressed on epithelial surfaces or in the environment are optimal for avoiding emergence of resistance to cognate interventions. By targeting such bacterial traits, the risk of altering the microbiome should be negligible and the subsequent short-term consequences (eg, an increased risk of *Clostridium difficile* infection) and

Table 1. Conceptual Categories of Antimicrobial Drugs by Site of Effect, With Associated Site-Specific Risk of Resistance Selection

Proposed Drug Category	Antimicrobial Effect of Drug, by Site		Risk of Resistance Selection, by Site		Established or Hypothesized Example
	Infection	Commensal/ Environmental	Infection	Commensal/ Environmental	
1	Lethal effect	No effect	Minimal	Minimal	Inhibitors of iron acquisition or other growth-limiting nutrients at infection site
2	Lethal effect	Lethal effect	Minimal	Medium to high	Inhibitors of cell wall or protein synthesis (eg, traditional antimicrobials, such as β-lactams, carbapenems, aminoglycosides)
3	Inhibit growth/virulence	No effect	Minimal	Minimal	Inhibitors of iron acquisition or other growth-limiting nutrients at infection site
4	Inhibit growth/virulence	Inhibit growth/virulence	Minimal	Low to medium	Inhibitors of quorum sensing

potential long-term consequences should be minimized.

Significant knowledge gaps exist regarding the site(s) at which selection pressure for antimicrobial resistance is greatest, the environments in which a microbial factor enhances fitness, and whether a treatment that impairs bacterial fitness also affects the evolution of resistance in a niche-dependent manner. We propose that, as an essential part of antimicrobial development, increased attention should be paid to whether the drug's target contributes to fitness in (drug-exposed) niches other than sites of infection. For example, does a target-deficient derivative have a decreased ability to survive within the gastrointestinal tract, nasopharynx, or other sites of colonization? Furthermore, in vivo studies of resistance selection should seek evidence of off-target selection for resistance within the host's (or a broader) microbiome, rather than focusing solely on the target pathogen at the site of infection. These data could be generated by site-appropriate colonization of animals, followed by subsequent treatment and selection for resistant derivatives of the colonizing strain, if present. Only when such data are available can the risk for development of resistance be gauged appropriately.

Notes

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