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Disarming pathogens: benefits and challenges of antimicrobials that target bacterial virulence instead of growth and viability

“...antivirulence drugs are promising to revitalize the antimicrobial drug development pipeline that has been frightfully dry in recent decades.”

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Currently, antibiotics are failing fast while rates of drug-resistant bacteria are increasing worldwide. With almost no new drugs coming to market and with even last-resort antibiotics like colistin becoming ineffective [1], a global scale public health crisis appears unavoidable. In fact, WHO has declared that with no action “a post-antibiotic era – in which common infections and minor injuries can kill – far from being an apocalyptic fantasy, is instead a very real possibility for the 21st century” [2]. With more annual deaths predicted by 2050 from antibiotic-resistant infections than cancer [3], we urgently need effective alternatives to combat this crisis. This has resulted in a resurgence of research activity into various alternative antibacterial approaches, which are at various stages of clinical and preclinical development. Given continued funding and appropriate investment by industry and governments, these alternatives could deliver much-needed antimicrobials for clinical use in the immediate future [4]. One such approach, the antivirulence approach, is promising to provide antimicrobial therapies predicted to be superior to conventional antibiotics [5]. This editorial presents some of the latest evidence to support this notion, highlighting at the same time some of the issues that need to be considered when evaluating antivirulence drugs.

For a more detailed discussion on the topic, readers are referred to current pieces by the author [6] and others [7–9].

Antivirulence drugs target bacterial virulence factors and, unlike classic antibiotics, are aimed at disarming pathogens rather than killing them or inhibiting their growth. Human, animal and plant pathogens rely on a plethora of virulence factors, which they employ at various stages of the infection process to cause disease. Examples of virulence factors include toxins, adhesins, invasins, quorum sensing and protein secretion systems. Inactivation of this (or part of this) arsenal will attenuate pathogens and render them susceptible to natural host defenses; thus, pharmacological inhibition of virulence factors presents an attractive alternative to antibiotics. Several antivirulence strategies are currently under development and have been collectively and individually reviewed extensively over the past few years (most recently by [10]). Importantly, a ‘disarm – don’t kill’ approach promises to deliver antimicrobials that can replace currently failing antibiotics while also overcoming two of their major shortfalls: drug resistance and killing of commensal bacteria.

Bactericidal or bacteriostatic antibiotics impose strong selection pressure on a bacterial population and thus select for muta-

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tions in the population that allow bacteria to survive the drug action. In cases where these mutations can be transferred and shared between different bacteria, resistance mechanisms can spread far and wide, rendering a drug ineffective sometimes soon after its introduction into clinical practice. While resistance to killing or growth inhibition is always a beneficial trait for bacteria, resistance evolution to virulence inhibition is more complex. Whether or not inhibiting a virulence factor will impose strong selection pressure in the pathogen population depends primarily on the net fitness cost that this factor confers to the pathogen, as well as the pathogen's population structure and environmental conditions. To accurately predict this requires an in-depth understanding of the exact contribution of each virulence factor to a pathogen's fitness during infection (temporally) and in different environmental niches (spatially).

“Another advantage of inhibiting virulence as an antimicrobial approach is that it provides a plethora of possible targets for inhibition.”

One of the best-studied examples of resistance evolution to antivirulence drugs involves quorum sensing inhibitors (QSIs), also known as quorum-quenching drugs that disrupt bacterial cell-to-cell communication. Resistance to QSIs has been documented experimentally in *Pseudomonas aeruginosa* strains, including clinical isolates from cystic fibrosis patients not previously exposed to the drugs [11,12], suggesting that such antivirulence compounds are not more evolutionary robust than conventional antibiotics. However, work by the same group demonstrated that when tested in physiologically relevant *in vitro* conditions and not complex rich media, QSIs resulted in growth inhibition of *P. aeruginosa* and not just virulence attenuation [12,13], revealing the true fitness cost of quorum sensing loss to this pathogen. These studies highlight the importance of evaluating resistance evolution in biologically relevant conditions tailored to each different pathogen and of testing diverse clinical strains instead of one/few lab or reference strains. A set of best-practice guidelines was recently proposed for QSI testing [14] and antivirulence drug testing more generally [6]. While QSIs can affect bacterial growth and impact on expression of many genes, other virulence factor inhibitors have proved to be more evolutionary robust. Blocking *P. aeruginosa*'s ability to sequester iron, for example, by quenching the siderophore pyoverdine with gallium was recently shown not to select resistant mutants *in vitro* despite the fact that gallium attenuated both *P. aeruginosa* growth and virulence during *in vivo* infection [15]. Anti-adhesion strategies, such as antibiofilm compounds against the *Escherichia coli* capsule [16] are also proving to be less prone to resistance development and possibly

cross-resistance to existing mechanisms. While experimental evidence on antivirulence drug resistance is slowly beginning to accumulate, there is still considerable lack of data to conclusively evaluate the evolutionary robustness of different antivirulence strategies. Assessing evolution of resistance to antivirulence drugs and the potential of resistance mechanisms to spread among bacteria are imperative for their further development and successful application as next-generation antimicrobials.

Another advantage of inhibiting virulence as an antimicrobial approach is that it provides a plethora of possible targets for inhibition. Depending on how widespread a target is among different bacteria and how conserved it is in sequence, structure and function, antivirulence drugs can be tailored to be broad- or narrow-spectrum, and target only pathogenic species or even pathogenic strains within a species. For example, antivirulence compounds and neutralizing antibodies against *Clostridium difficile* toxins or Shiga toxins produced by some *E. coli* strains can have a very narrow spectrum of activity, as they do not inhibit any nontoxin-producing bacteria in the host. In addition, depending on the expression pattern of the virulence target, the action of an antivirulence drug during treatment could be restricted to only those host niches where expression of the particular virulence gene would be of benefit to the pathogen. For example, inhibition of staphyloxanthin biosynthesis, the golden pigment of *Staphylococcus aureus*, increases bacterial susceptibility to killing by reactive oxygen species and would attenuate *S. aureus* only in host niches where its presence would result in neutrophil recruitment and reactive oxygen species production, but not in host sites of commensal colonization [17]. Such environmental specificity prevents off-site drug activity and could ensure that selection of resistant mutants is minimized at commensal sites in the host where the target might not be expressed.

Despite the obvious advantages of developing very specific inhibitors against pathogenic species or strains, the successful application of such drugs in the clinic would heavily rely on powerful diagnostics capable of quickly and accurately identifying the infecting species or strain and/or whether it expresses the virulence factor targeted; a task still remaining a major challenge today. Some antivirulence drugs can circumvent this requirement as they target a virulence strategy that is shared by a wide group of pathogens that cause similar types of disease. Type three secretion system inhibitors, for example, have been shown to be active against a wide range of diarrhea-causing pathogens, such as enteropathogenic *E. coli*, enterohemorrhagic *E. coli*, *Salmonella enterica* and *Shigella flexneri* [18]. Moreover, antivirulence drugs can also be developed to have a broader spectrum of activity. Thiazolidinone compounds, for example, can inhibit type II and III protein secretion

systems in a wide range of Gram-negative bacteria [19], while inhibitors of bacterial enzymes that mediate virulence, such as the conserved Gram-positive cysteine transpeptidase sortase A, have also been shown to be active against multiple species [20]. Whether narrow- or broad-spectrum, antivirulence drugs have the potential to minimize off-target effects by not indiscriminately inhibiting a host's resident microbiota. This, however, remains to be demonstrated *in vivo*.

In conclusion, antivirulence drugs are promising to revitalize the antimicrobial drug development pipeline that has been frightfully dry in recent decades. Their potential to be tailored for spectrum-of-activity and the emerging evidence on the evolutionary robustness of certain antivirulence drugs and targets, makes the antivirulence approach an attractive alternative to antibiotics. Engagement of large pharmaceutical companies and

continued investment into research projects addressing antivirulence drug resistance evolution and their ability to preserve natural microbiota while effectively treating or preventing infections, will enhance the identification of lead antivirulence candidates and will guarantee their timely progression into clinical practice.

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