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Anticipating Resistance to Antivirulence Compounds Employing Directed Evolution for Drug Development

Michael L Fisher*
Biology Department, Southern Connecticut State University, New Haven, USA

***Corresponding author**: Michael L Fisher, Biology Department, Southern Connecticut State University, New Haven, USA, E-mail: Fisherm10@southernct.edu

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Description

The need for new antibiotics and new approaches to treating bacterial infection is critical due to the rise in antimicrobial resistance (AMR) [1].Traditional antibiotics target essential physiological processes and do not discriminate between pathogenic bacteria and the commensal microbiome. This approach disrupts normal microbial flora and places tremendous selective pressure on susceptible bacteria. Thus, resistance can arise either within the target population or the microbiome. Mutations in either population can subsequently spread through horizontal gene transfer [2]. A promising approach to combat AMR is the use of Anti-virulence therapies (AVTs).These compounds specifically target virulence factors and do not affect the normal microbial flora. The rationale behind AVTs is that they prevent selective pressure on non-pathogenic microbial flora. Thus, resistance will likely arise more slowly or not at all [3,4].This notion is predicated on the fact that many virulence targets are specifically upregulated in the host or are only essential in the host environment. In the absence of a virulence target, AVTs would exert no evolutionary pressure to select for resistance. Although AVTs diminish virulence, resistant mutants have already been isolated in the lab [5]. Here we discuss the need to predict evolutionarily favorable mutations and a strategy to primitively generate rationally designed compounds effective against these mutations

Targets of AVTs include: Structural components (i.e. adhesins, secretion systems), regulatory proteins of virulence-specific regulons and signaling molecules [6]. An important example of resistance to AVTs is represented by quorum sensing (QS) inhibitors. QS is an important virulence mechanism in the opportunistic pathogen, *Pseudomonas aeruginosa* [7].This model system has provided valuable evidence of evolution in response to AVTs [8].For instance, QS regulated virulence is diminished in the presence of furanone derivatives, which inhibit the transcriptional regulator, *LasR* [9]. Furthermore, these compounds significantly reduced virulence-associated phenotypes *in vitro*as well as bacterial colonization *in vivo* [9]. However, strains resistant to QS inhibition have been isolated with mutations in both *lasR* and its co-regulator *rhlR* [10,11].These data suggest that we should anticipate rapid evolution of AVT targets leading to resistant strains after clinical application.

Another well-studied target of antivirulence compounds are type three secretion systems (TTSSs) [12,13].The TTSS is a broadly utilized virulence mechanism of enteric pathogens including species of Yersinia, Salmonella, Shigella and Escherichia. This needle-like secretion system allows for the delivery of toxins into host cell to modulate cell function for the benefit of the pathogen. The TTSS makes an attractive target as it is the primary virulence mechanism of many Grams negative pathogens [14].Additionally, its conserved nature allows some AVTs to act across multiple species.For instance, 5-cyano-6-(4-methylbenzylthio) picolinic acid and 1,3-bis[3-(4,5-dihydro-1H-imidazol-2-yl)phenyl]urea inhibit secretion of toxins from both Y.*pestis*as well as Enteropathogenic E. *coli* (EPEC) [15].

Yersinia spp has been particularly well studied in regard to the TTSS and AVTs. Small molecule inhibitors that have been identified in Yersinia include: the ATPase that powers toxin translocation(YscN) [16],the transcriptional regulator of the TTSS and toxins (LcrF) [17,18] and the pore forming tip of the TTSS (YopD) [19].Although AMR has not been reported to date, we can surmise from work in *P. aeruginosa* that it will.To prepare for this eventuality we can make use of the crystal structures of AVT targets, which have been completed for *LasR* [20] and InvC, (YscN homologue from Salmonella).This structural data will allow for in *silico* modeling of the inhibitors with their target. Specifically, we can select for resistant mutants in the lab and model drug-target interactions in *silico* allowing for iterative design of new compounds.

A likely reason we have not seen more reports of strains resistant to is that AVTs is that they were developed outside the context of and active immune response. However, *in vivo*, AVTs would disarmed pathogens of their virulence factors. Pathogens will then be subjected to a powerful immune response that will select for resistance. Our group is pursuing a strategy for the directed evolution of Yersinia by passaging them in the presence of macrophages and/or TTSS inhibitors. Upon elucidating the specific mutations elicited by different AVTs, the starting compounds can be predictively remodeled. Indeed, such a platform for this modeling has described for *LasR*in *P. aeruginosa* [21].

Currently, AVTs represent 8% of the pre-clinical pipeline of 407 antibiotic development projects [22].The difficulty of establishing clinical trials decreases the likelihood that many of these compounds will be clinically available. Furthermore, it is inevitable that resistance to these compounds will arise as demonstrated by *lasR* mutations in Pseudomonas. Thus, we must not wait for potential AVTs to become quickly obsolete, but proactively determine the most likely mutations to occur by combining *ex vivo* experimentation with *in silico* drug modeling.

Antivirulence therapies offer an exciting approach for treating infectious disease, which promises to reduce the spread of resistance *via* horizontal gene transfer. Furthermore, we can anticipate that in the context of an actual infection, resistance will arise just as it has to traditional antibiotics. However, using sequence from laboratory derived mutations, we can anticipate the most evolutionarily favored mutations. This will allow *in silico* modeling to preemptively modify small inhibitory molecules to interact with newly defined virulence targets. This work will all us to determine if can we supplement AVTs with second or third generation derivatives that target not only wild type virulence targets, but also their subsequent mutant form(s). While

the development of "evolution proof" therapeutics is implausible, coupling prudent antibiotic use with AVT and AVT derivatives will extend the usefulness of our emerging cohort of these important antimicrobial compounds.

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