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Productive steps toward an antimicrobial targeting virulence

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Targeting virulence factors has gained increasing attention as a potential approach to new antibiotics. Small molecule inhibitors of virulence have been shown to change the course of disease in whole organism infection models. Recently, key advances in the field include the identification of novel targets within cell signaling pathways, a new class of anti-virulence compounds that target bacterial defenses against host immunity, and a growing body of *in vivo* data to support the general approach of anti-virulence therapies. Additionally, there has been a distinct trend toward developing broader spectrum anti-virulence compounds, in particular agents with activity against diverse Gram-negative organisms. Herein we provide an update on the status of the field with a focus on recent advancements.

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Introduction

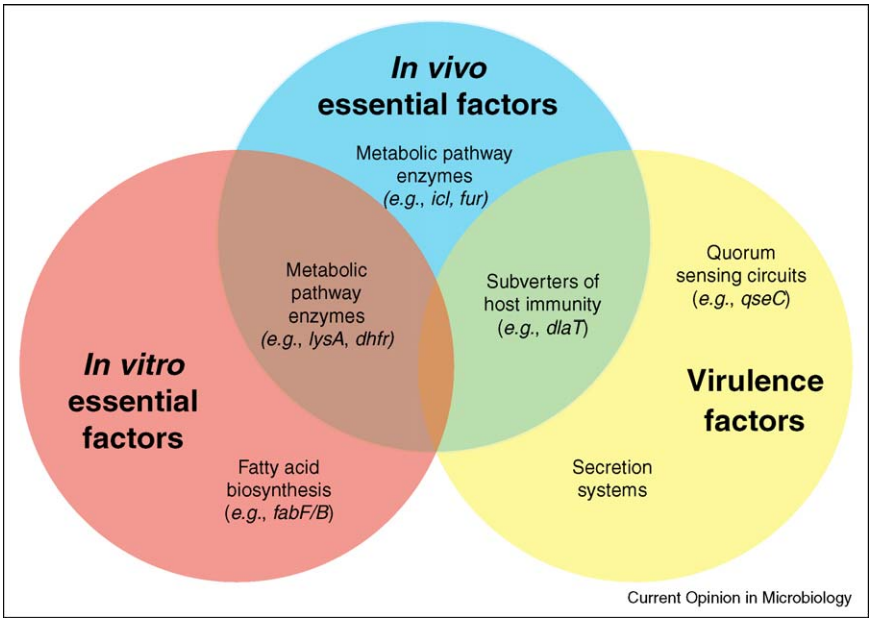
Since the first use of penicillin in the 1940s, clinical drug resistance has quickly followed the introduction of any new antibiotic. Highly resistant bacteria, including methicillin-resistant *Staphylococcus aureus* [1], extended-spectrum beta-lactamase producing Gram-negative organisms [2], and extensively drug resistant tuberculosis [3] now pose an increasing threat to public health with limited treatment options. New antimicrobial agents are clearly needed; however, recent approaches to drug discovery have been unsuccessful [4]. New paradigms for therapeutics are warranted, including strategies that target bacterial virulence in the battle against resistant organisms.

Targeting *in vitro* essential genes, *in vivo* essential genes, or virulence factors

The goal of any antibiotic is the clearance or prevention of infection within the context of the host. However, most traditional antibiotics were identified based on their *in vitro* antimicrobial activity under laboratory culture conditions. As a result, most antibiotics target processes essential for *in vitro* growth, with the implicit assumption that the same processes are also essential for *in vivo* infection. New work questions the validity of this assumption, as exemplified in the studies of fatty acid biosynthesis (FAB) inhibitors. Recent interest in targeting FAB as a strategy for antibiotic discovery is based on both evidence for its essentiality under traditional laboratory growth conditions and the knowledge that isoniazid, a potent antituberculosis drug, targets mycolic acid biosynthesis [5]. Thus, great excitement surrounded the identification of the natural product platensimycin and its derivatives as FabF/B inhibitors [6,7]. A recent study however, suggests that FAB may not be equally essential *in vivo* where organisms are able to scavenge fatty acids from their host microenvironment. Inhibitors of the biosynthetic enzymes FabI and FabB did not impair the growth of *Streptococcus agalactiae* in the presence of unsaturated fatty acids, which are present in human serum. Additionally, strains lacking FabI or FabB were not attenuated in a mouse model of neonatal meningitis [8]. These results cast doubt on the relevance of fatty acid biosynthesis as an antimicrobial target and bring into sharp relief the potential disparity between requirements for *in vitro* and *in vivo* bacterial survival.

Bacterial functions that are required to cause disease *in vivo* can fall into two categories: those required for *in vivo* survival — which may or may not also be essential *in vitro* — and those required to cause tissue damage and disease, which are classically considered to be virulence factors (Figure 1). In the first category, *in vivo* essential genes frequently fall along metabolic pathways that make or scavenge for required nutrients that are scarce within the host microenvironment. Those nutrients or their precursors may be readily available in culture media, obviating those pathways *in vitro*. For example, *Mycobacterium tuberculosis* deficient in both isocitrate lyase isozymes grows similarly to wild-type strains in standard culture media, but grows poorly in macrophages and is rapidly cleared in infected mice [9]. Other genes that are required *in vivo* include those that scavenge iron within the host, where levels may be low. As an example, *Vibrio cholerae* strains

Figure 1



Overlap between *in vivo* essential factors, *in vitro* essential factors, and virulence factors. Categorization of example bacterial targets.

unable to produce the siderophore vibriobactin cannot colonize the intestine or cause diarrhea in a mouse infection model, yet grow normally *in vitro* [10]. Isocitrate lyase and the biosynthetic enzymes that produce vibriobactin would thus be considered essential *in vivo* but not *in vitro*, and would be potentially good targets for antibiotic development.

The second category of bacterial functions required to cause disease *in vivo* includes proteins that are classically referred to as virulence factors because they contribute directly to disease pathogenesis. While *in vivo* essential genes do not actively interact with host cells or functions, virulence factors actively damage host cells or interfere with host cell functions. For example, *Salmonella* effector proteins SopE and SopB, secreted into host cells through type III secretion (T3S) machinery, reorganize the eukaryotic actin cytoskeleton, modulating bacterial uptake [11]. More subtly, some virulence factors may interfere with host immune functions. In *M. tuberculosis*, for example, dihydrolipoamide transferase (DlaT) neutralizes reactive nitrogen intermediates, key components of host immunity, by reducing peroxynitrites [12]. Because of the active mechanism by which DlaT subverts host function, we would consider it to be a virulence mechanism.

Distinguishing between *in vivo* essential functions and virulence mechanisms can sometimes be challenging. Since they both can effect *in vivo* bacterial survival, targeting either one is a viable therapeutic strategy. However, the remainder of this review will focus on targeting specific virulence factors as novel therapeutic strategies.

Pros and Cons of targeting virulence

Targeting virulence factors has several theoretical advantages over standard antibiotic treatment. First, a resistant clone's survival advantage in the presence of traditional antibiotic drives selection for that clone. Theoretically, non-bactericidal drugs may not similarly select for resistance. If the targeted virulence factor is not essential for survival *in vivo*, mutations resulting in resistance should have no impact on relative bacterial fitness [13]. Second, because many virulence factors are organism-specific and virulence-targeting drugs are unlikely to be bactericidal, host commensal flora would be minimally impacted. Preserving commensals would reduce the risk of both secondary infections with organisms such as *Clostridium difficile* and colonization with drug-resistant organisms. Finally, the narrow spectrum of some anti-virulence therapies, while criticized as a potential drawback, can also be advantageous. Using new, limited spectrum antibiotics where clinically appropriate could restrict the use of broader spectrum antibiotics to instances of necessity, slowing the evolution of resistance to broad-spectrum agents.

While there are multiple potential advantages to virulence inhibitors as therapeutics, questions about their utility remain. Whether they will work best as prophylactic agents, solo therapeutic agents, or therapeutic agents in conjunction with conventional antibiotics has