Small-molecule quorum quenchers to prevent Staphylococcus aureus infection

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Editorial
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Small-molecule quorum quenchers to prevent *Staphylococcus aureus* infection

"The concept underlying antivirulence is to shut down pathogenesis mechanisms in the invading bacteria without impacting growth, thereby enabling the host immune system to clear the infection in the absence of antibiotics."

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Antibiotic-resistant bacteria are one of the most significant current threats to human health. The diminishing antibiotic development pipeline coupled with the propensity for bacterial pathogens to evolve resistance represents an ongoing crisis that needs to be addressed [1]. Resistance development has long been a major concern with *Staphylococcus aureus*, a notorious bacterial pathogen that causes a wide spectrum of acute and chronic disease. The incidence of methicillin-resistant *S. aureus* (MRSA) infections in both hospital and community settings continues to rise [2], and healthcare-associated infections due to MRSA cost more and lead to longer hospital stays than those caused by any other bacterial pathogen [3]. Antibiotic stewardship to preserve the most efficacious treatments for severe bacterial infections needs to be at the forefront of strategies designed to address growing resistance challenges. How can these principles be applied to managing *S. aureus* disease burden? When considering the breadth of infections caused by *S. aureus*, the majority are skin and soft tissue infections (SSTIs), resulting in 11.6 million ambulatory care visits per year [4]. In emergency departments, 76% of *S. aureus* cases are SSTIs and 59% of these are MRSA [5]. Many of these SSTIs would be considered uncomplicated and treated with straightforward courses of antibiotics. Considering these points, the load of antibiotic therapy being directed toward *S. aureus* SSTIs is significant, and it seems probable that this is contributing to resistance development. What if innovative countermeasures for SSTIs that do not drive resistance could be translated into approved therapies? Such a strategy could help preserve the most effective antibiotics for the severe, deep-seated *S. aureus* infections and enable uncomplicated infections to be treated more rapidly and effectively.

Antivirulence strategies represent a promising approach that could address the challenge of antibiotic stewardship [1]. The concept underlying antivirulence is to shut down pathogenesis mechanisms in the invading bacteria without impacting growth, thereby enabling the host immune system to clear the infection in the absence of antibiotics. Quorum-sensing systems in particular have drawn attention as targets for the development of virulence-controlling therapeutics [6]. Although bioactive agents that function in this manner have yet to reach the clinic, animal model studies suggest potential for the approach [7,8]. The strategy of disarming the pathogen could lead to treatments that do not select or enrich for bacterial resistance, while simultaneously preserving the beneficial resident flora. This article covers some of the recent advances in the discovery and development of small-molecule antivirulence agents that inhibit *S. aureus* quorum sensing.

Pathogenesis in *S. aureus* is regulated by the *agr* quorum-sensing system, a cell density-dependent regulator that responds to the extracellular concentration of a peptide signaling molecule (reviewed in [9]). This signaling molecule is a modified peptide (called an autoinducing peptide [AIP]), which is typically 7–9 residues in length and has its last five residues cyclized into a thiolactone ring. The AIP signal is produced from a ribosomal precursor and processed and secreted from the cell by the AgrB membrane endopeptidase and signal peptidase SpsB. Once AIP reaches a critical concentration, it binds to a

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receptor on the AgrC histidine kinase, activating the kinase to phosphorylate the AgrA response regulator and inducing the quorum-sensing system. The primary output of AgrA is a large transcript called RNAIII, driven by the P3 promoter, and high RNAIII levels lead to the production of numerous virulence factors, including toxins, exo-enzymes and immunomodulators. Small molecules that inhibit this system (referred to as 'quorum quenchers') are the focus of this article, and three obvious targets for such compounds are AgrC, AgrA or AgrB. Of these, AgrC has been the subject of most research and development, and its extracellular location and function as the AIP receptor have drawn considerable attention. However, AgrC exhibits signs of being hypermutable [10], potentially meaning a future reservoir of resistance. By contrast, AgrA and AgrB are highly conserved among the Staphylococci and even other Gram-positive pathogens. Targeting these proteins could ultimately prove more fruitful.

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The first identified quorum-quenching agents are the AIPs themselves, which are produced by *S. aureus* and other Staphylococcal strains, and interact directly with AgrC. Through a mechanism coined '*agr* interference', the AIPs produced by one strain can cross-inhibit a strain producing a different *agr* class [9]. This property of the *agr* system has led to the identification of peptide scaffolds that act as AgrC-receptor antagonists and inhibit specific *S. aureus* strains. Such interfering AIP structures have proven effective for treatment of *S. aureus* skin abscess in mouse models [7], suggesting the potential benefit of quorum quenching to prevent SSTI. Development of AIP-based receptor antagonists continues to be an active area of exploration, and numerous inhibiting structures have been identified and optimized (recently reviewed [8]). Chemical screening has also identified synthetic agents thought to act as AgrC-receptor antagonists, such as benzbromarone, a treatment for gout. Benzbromarone is a potent quorum quencher (IC_{50}: 0.1–0.2 μM) and inhibits production of *agr*-regulated virulence factors in all types of *S. aureus* strains [8].

The output of AgrC is the phosphorylation of the AgrA response regulator and, in turn, the upregulation of RNAIII to induce *S. aureus* virulence. Identification of agents that target ArgA directly or prevent the induction of RNAIII has yielded promising leads. The DNA-binding domain structure of the AgrA response regulator has been solved, and this structure has been used as a template for nuclear magnetic resonance-based inhibitor screening [11]. The best AgrA inhibitors were confirmed in electrophoretic mobility shift assays using purified protein. Computer docking has also yielded promising AgrA small-molecule inhibitors, which were verified in follow-up studies [12]. Using RNAIII production as an output, the synthetic small-molecule quorum quencher named savirin, for *S. aureus* virulence inhibitor, was identified by combinatorial library screening [13]. Savirin is a potent inhibitor of RNAIII transcription (IC_{50}: ~1 μM) and simultaneously prevents the production of virulence factors α-toxin and phenol soluble modulins. Most importantly, savirin attenuated MRSA in a mouse model of SSTI and showed no potential for resistance development through successive rounds of *in vivo* testing [13].

Another strategy of quorum quenching that has yielded preliminary success is targeting the production of the AIP signal. The Type I signal peptidase SpsB is essential for AIP biosynthesis, and linear peptide inhibitors of this peptidase were found to quench the *agr* system across strains [14]. The fungal metabolite ambuic acid was identified in screens for compounds that inhibited the production of the peptide quorum-sensing signal in *Enterococcus faecalis* [15]. In follow-up analysis, ambuic acid successfully prevented endopeptidase activity in the AgrB homolog FsrB, and it acted as a quorum quencher by inhibiting AIP production in *S. aureus* and other Gram positives [15].

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Natural products have proven to be a rich source of anti-infective agents, with approximately 77% of drugs for this purpose derived from natural sources [16]. The concept of screening based on quorum quenching activity is relatively new to the natural products community; nonetheless, some promising findings

have recently been published. One example is the previously mentioned AgrB inhibitor ambuic acid, which is a polyketide-derived epoxyquinone produced by fungi of *Monochaetia* spp. and *Pestalotiopsis* spp [15]. Other examples include cyclized dipeptide molecules secreted by *Lactobacillus reuteri*, which are thought to inhibit AgrC-A two-component action [17], and the compound norlichexanthone, which is produced by the fungus *Penicillium algidum* [18]. Botanical extracts and their components have also been shown to possess quorum quenching activity against *S. aureus*. In one study, multiple plant extracts were found to inhibit the production of δ-toxin, a small peptide encoded in the RNAIII transcript [19], and in another, extracts from the medicinal plant goldenseal (*Hydrastis canadensis*) were found to inhibit RNAIII and α-toxin production, and reduce toxicity to human skin epithelial cells [20]. In both of the aforementioned studies, however, the specific compounds responsible for activity were not identified.

Although quorum quenching in *S. aureus* has made substantial progress over the past decade, there remain many undeveloped areas that need to be addressed. AgrC receptor antagonists are the most advanced candidates, but the undesirable pharmacokinetic properties of peptide inhibitors and the penchant for AgrC to evolve mutations raise questions about their long-term therapeutic utility [10]. AgrB is the most unique component of the *agr* system across Gram-positive pathogens [9], yet ambuic acid is the only reported AgrB inhibitor, and it has only limited efficacy [15]. In all *S. aureus* strains, AgrA is arguably the most conserved component of the system and is divergent from other classes of bacterial response regulators [11], but only a handful of moderately potent inhibitors have been identified for this target [11,12]. Thus, there remains significant untapped potential for both AgrB and AgrA as screening targets. Unfortunately, the mechanism of action of some of the most effective *S. aureus* quorum quenchers remains unclear, and to resolve this uncertainty and improve future screening, assays that provide more mechanistic information are needed. The limited body of currently published quorum quenching compounds includes both synthetic and natural product compounds, and there is reason to assume that both pools present possible sources of novel antivirulence therapies. Ultimately, recent successful *in vivo* studies suggest the promise of antivirulence strategies for addressing the immensely problematic issue of antibiotic-resistant *S. aureus* infections. The challenge of ongoing studies will be the translation of these basic research concepts into approved clinical therapies that can preserve our most important antibiotics for future use.

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