Published in final edited form as:

Curr Opin Crit Care. 2008 October ; 14(5): 593–599. doi:10.1097/MCC.0b013e32830f1dd5.

Novel Strategies to Combat Bacterial Virulence

S.V. Lynch¹ and J.P. Wiener-Kronish^{1,2,3,†}

¹ Department of Anesthesia and Perioperative Care, University of California San Francisco, CA 94143, USA

² Cardiovascular Research Institute, University of California San Francisco, CA 94143, USA

³ Department of Medicine, University of California San Francisco, CA 94143, USA

Abstract

Purpose of review—Incidences of antimicrobial resistant infections have increased dramatically over the past several decades and are associated with adverse patient outcomes. Alternative approaches to combat infection are critical, and have led to the development of more specific drugs targeted at particular bacterial virulence systems or essential regulatory pathways. The purpose of this review is to highlight the recent developments in anti-bacterial therapy and the novel approaches toward increasing our therapeutic armory against bacterial infection.

Recent findings—Although classic antibiotic development is not occurring rapidly, alternative therapeutics that target specific bacterial virulence systems are progressing from the discovery stage through the FDA approval process. Here we review novel antibodies that target specific virulence systems as well as a variety of newly discovered small molecules that block bacterial attachment, communication systems (quorum sensing) or important regulatory processes associated with virulence gene expression.

Summary—The success of novel therapeutics could significantly change clinical practice. Furthermore, the complications of collateral damage due to antibiotic administration e.g. suprainfections or decreased host immunity due to loss of synergistic bacterial communities, may be minimized using therapeutics that specifically target pathogenic behavior.

Keywords

Bacterial virulence; antimicrobial therapy; Quorum sensing; type III secretion system; novel therapeutics

Introduction

The rapid increase in bacterial antimicrobial resistance remains a growing problem, decreasing the therapeutic options available for a multitude of pathogenic species that commonly infect the human host. The purpose of this review article is to examine the developing field of alternative therapeutics that combat bacterial virulence systems of specific pathogens associated with poor patient outcomes. This review also examines evidence for polymicrobial communities in a variety of clinical infectious states and discusses the utility of combined therapeutic interventions to improve long-term patient outcome.

Corresponding author: Susan V. Lynch, Department of Anesthesia and Perioperative Care, Box 0542, University of California San Francisco, San Francisco, CA 94143. lynchsv@anesthesia.ucsf.edu. Tel: + 415 476 6784.

[†]Current address: Massachusetts General Hospital, Department of Anesthesia and Critical Care, GRB444, 55 Fruit Street, Boston, MA 02114.

Alternative strategies to combat bacterial infections

Our long term relationship with bacteria initiates during the birthing process and culminates in a human super-organism that contains 10 times more microbial than human cells [1]. Disturbances in this delicate balancing act, shift the host-microbe interaction from one of synergy to infection, resulting in host tissue damage and the need for therapeutic intervention. Since the relatively recent discovery of Penicillin as the active antimicrobial agent produced by *Penicillium* species, a multitude of antibiotics discovered and chemically modified over the past 8 decades have provided the first line of defense against a variety of bacterial infections. Historically, these compounds have dramatically changed our ability to combat infectious agents. However, bacteria are adept at rapidly acclimatizing and developing resistance to a myriad of insults, including antimicrobial agents which they commonly encounter in non-host environments, resulting in more resistant pathogens and increasingly limited treatment options.

Since antibiotic compounds are currently being developed at a significantly slower pace than the evolution of bacterial resistance, alternative strategies are necessary to combat infecting species. Approaches vary from targeting specific virulence systems to manipulating physiological aspects of bacterial lifestyle such as cell-to-cell communication or the need for a specific factor for virulence gene expression. The following, while by no means a comprehensive discussion, provides an overview of some of the current approaches being used to develop alternative antimicrobial therapies to combat bacterial infection in the human host.

Targeting virulence factors

One approach to develop novel antimicrobial compounds is to target specific virulence factors associated with a particular disease state. Secretion systems and toxins provide ideal targets for novel therapies and many studies have focused on developing treatments to neutralize or block these specific physiological factors.

Protection through inactivating antibodies

The type III secretion system (TTSS) found in many pathogenic species is a needle like injection system that facilitates secretion of effector molecules directly into the mammalian host epithelium (Fig. 1A). This specific virulence system in clinical isolates of *Pseudomonas aeruginosa* has been strongly correlated with acute lung injury and the development of ventilator-associated pneumonia in intubated patients [2–5]. Active type III cytotoxin secretion also targets host immune cells e.g. macrophage and neutrophils, allowing organisms evade the immune response and often reducing the numbers of viable host defense cells [6,7].

The type III injectosome is comprised of multiple proteins which anchor the needle complex in the bacterial membrane and facilitate toxin secretion. For example, the PcrV protein facilitates *P. aeruginosa* cytotoxin secretion and is located at the tip of the needle apparatus [8]. This protein is believed to play a role in binding mammalian membrane receptors (Fig. 1B) and is essential for effective cytotoxin secretion by this species. A novel antibody (anti-PcrV) which binds this protein exhibits excellent efficacy in protecting both acutely infected and burned mice against type III-mediated epithelial damage [9–13], presumably by blocking binding of the needle complex to the mammalian cell membrane preventing effector translocation (Fig. 1C). This antibody has been humanized to increase its half-life and reduce immunogenicity and is currently in human phase I/II clinical trials. Use of this antibody could conceivably provide a three-fold mechanism of protection by **1.** preventing acute lung injury thus **2.** reducing the inflammatory response which can also contribute to host epithelial injury and **3.** preserving macrophage and neutrophil activity. A viable host

defense system with greater numbers of functional immune cells would permit enhanced clearance of co-habiting species, particularly pathogenic ones that through their combined activity contribute to the disease state.

A similar approach has been used to develop antibodies against type III secretion by *Yersinia pestis* the causative agent of bubonic plague. LcrV, a protective antigen and the *Y. pestis* ortholog of PcrV, is also located at the tip of the needle-like type III injection apparatus. Like PcrV, LcrV facilitates secretion of the cytotoxic *Yersinia* protein effectors (Yops) [14,15]. An antibody against this protein has demonstrated protection against lethal doses of a virulent *Y. pestis* strain in a mouse model of bubonic plague preventing host cell apoptosis and promoting phagocytosis [16]

Other antibody-based therapeutics in development include Valortim, a human monoclonal antibody which exhibits prophylactic and post-infection efficacy against *B. anthracis* toxins in rabbit and non-human primate models [17]. This antibody has successfully completed Phase I safety and pharmacokinetic evaluation and has been fast-tracked by the FDA to facilitate rapid regulatory review. The anti-virulence antibody, “BAT” (heptavalent botulism antitoxin) has been developed to provide protection against 7 botulinum toxins (A, B, C, D, E, F and G). This improved antibody whose efficacy has been assessed in animal studies, currently has “investigational drug” (IND) status from the FDA for studies in human subjects (<http://www.cnw.ca/fr/releases/archive/September2007/27/c6071.html>).

Blocking microbial attachment

Part of the typical bacterial lifecycle involves attachment to a surface and biofilm formation. Biofilms are broadly defined as communities of bacterial cells encased in a protective extracellular matrix. Due to the inherent increased resistance of biofilms to conventional antimicrobial therapy, clearance of these bacterial reservoirs is problematic, leading to subsequent infections. In the human host, this mode of bacterial lifestyle contributes substantially to chronic infections, such as airway colonization of cystic fibrosis patients [18–21] and recurrent urinary tract infections[22,23]. The initial stages of biofilm formation involve attachment of the bacterial cell to the host cell surface, which is mediated by adhesion proteins. Blocking this initial attachment step provides a strategy for preventing colonization of the epithelial surface. Unfortunately, a multitude of adhesin proteins are produced by various bacterial species, thus, development of a common anti-adhesion therapy that would universally block bacterial attachment to host cells is unlikely. However, some pathogenic species such as *P. aeruginosa* and *Candida albicans* do appear to possess adhesions that have homologous receptor-binding domains[24]. This aspect has been exploited to develop therapeutics effective against both species in parallel [25]. Other anti-adhesion therapies that target species-specific adhesions e.g. those of uropathogenic *Escherichia coli*, the primary cause of recurrent urinary tract infection, are also currently in development.

Pili or fimbriae, hollow extracellular bacterial appendages composed of oligomeric pilin proteins, also play a prominent role in initial bacterial attachment, by binding receptors on the mammalian host cell surface [26–29]. Both Gram positive and Gram negative bacterial species produce pili making them an attractive target for novel therapeutics. Adhesins such as lectin are commonly found on the pili and facilitate binding to oligosaccharides on the mammalian cell surface [30]. Approaches to prevent initial attachment events involving pili include blocking binding by the pili-associated adhesions. Naturally occurring and synthetic carbohydrate derivatives have exhibited effective pili-blocking activity in a variety of assays [31–33], and represent a viable option for novel anti-adhesive therapeutics [34]. An alternative approach is to inhibit pilus formation by blocking the activity of chaperone-usher systems necessary for their biogenesis. Pilicides are in development for a number of

bacterial species including *Escherichia coli* [35–38]. Given the key role pili play in initial adhesion and the fact that the chaperone-usher system plays a pivotal role in pili biogenesis for a number of pathogenic species, it is hoped that pilicides may be effective against multiple species, providing a broad-range antibacterial therapeutic option.

Identification of molecules that provide protection against specific virulence factors

A recent study to examine the molecular basis of how commensal species become pathogenic, demonstrated inhibition of *Shigella* species virulence gene expression by quinolinate. This compound produced by the *nadA* and *nadB* genes of *Shigella* species acts as an anti-virulence factor that inhibits invasion by blocking type III secretion of two effector proteins IpAB and IpAC that are key to mammalian cell invasion. In addition, quinolinate also prevented cell-to-cell spread of *S. flexneri* 5a and its ability to induce polymorphonuclear neutrophil transepithelial migration [39].

Screening libraries of natural products, small synthetic molecules and peptides is proving to be a fertile area for novel antimicrobials. To date, small molecule library screening has led to the identification of a compound, virstatin, which effectively inhibits expression of the *toxT* gene, a transcription factor that activates expression of cholera toxin and pilus production by *Vibrio cholerae* [40]. In separate studies, compounds (peptides and small molecules) that block anthrax toxin by inhibiting the enzymatic activity associated with activity have been identified [41–43] while others (Amiodarone and Bepridil) block endosomal acidification – a crucial step in toxin entry to the mammalian cell [44]. In another study, Nordfelth *et al* screened a library of small molecules to identify a compound that would inhibit Type III secretion by *Yersinia pseudotuberculosis*. This approach led to the identification of an acylated hydrazone that specifically blocks TTSS effector protein secretion by this species [45].

Quorum Sensing Inhibitors (QSI's)

Bacteria are masters of primordial sensory perception; this ability is facilitated through a communication system called quorum sensing (QS) that permits individual cells of a single species to sense either “self” (cell density of same species) or “non-self” (distinct microbial neighbors). The complex hierarchical genetic network that underlies this signaling system provides exquisite regulatory control of specific subsets of genes, including virulence factors, permitting defined responses to changing environmental conditions [46]. QS by both Gram negative and Gram positive bacterial species share the same principles, production of and sensing molecular signaling compounds. However, the mechanism by which these molecules are produced and sensed and the signal molecules themselves vary across species. QS has been associated with biofilm formation [47–49] and with regulation of the virulence factors associated with this sessile lifestyle [46].

In 1993, de Nys *et al* [50] reported that a marine alga, *Delisea pulchra*, produced a variety of halogenated furanones that inhibit bacterial quorum sensing and provided antifouling protection. This was the first report of quorum sensing inhibitors (QSI's). Efforts to chemically modify these compounds to increase their activity against clinically relevant species such as *P. aeruginosa* resulted in identification of two compounds C-56 and C-30 which have demonstrated efficacy in clearance of this species in a mouse model of chronic infection [51,52]. Other QSI's have since been identified in herbal and fungal foods [53,54], these compounds also demonstrate efficacy *in vitro* and *in vivo* in a murine model of infection. Recently, a QSI molecule, hamamelitannin, that occurs as a natural product in the bark of witch hazel was discovered by structure-based “virtual screening” [55]. This compound demonstrated QSI activity and was shown to inhibit biofilm formation on in-dwelling devices in a rat model. What was particularly exciting about this compound was

that it prevented infection by Methicillin Resistant *Staphylococcus aureus* (MRSA) and Methicillin resistant *Staphylococcus epidermidis* (MRSE) – two of the most problematic and intractable clinically relevant pathogens [55]**.

To date, no study has examined the effect of QSI compounds on reciprocally regulated virulence factors that may be induced due to blocking QS-controlled biofilm-associated pathogenicity e.g. type III secretion in *P. aeruginosa* [56] or their effects on host polymicrobial community diversity or function. While these compounds hold promise, caution must be taken against extrapolating single species results with multi-species clinical reality. Inter-species cross-talk has been demonstrated between *P. aeruginosa* and *Burkholderia cepacia* [57] and between *Serratia liquefaciens* and *P. aeruginosa* [58]. Molecules that block or potentially mimic these inter-species communication molecules may provide a means to manipulate the physiology of multiple species in parallel and another avenue for therapeutic development.

The “Trojan horse” strategy

Another promising therapeutic approach aimed at the clinically relevant pathogen *P. aeruginosa*, targets a specific facet of this organisms physiology essential for its pathogenicity. The human immune response limits iron (Fe^{3+}) availability in an attempt to attenuate virulence of infecting pathogens. However, Fe^{3+} plays a key role in many clinically-relevant pathogenic species and a variety of systems to scavenge Fe^{3+} from the environment have developed in these organisms. Recent work by a group in Seattle used a “Trojan Horse” strategy to deceive the bacterial cell and reduce iron uptake [59]. The transition metal gallium (Ga^{3+}) is chemically similar to iron, but unlike Fe^{3+} it does not under go redox reactions and therefore is biologically inactive and unable to perform the cellular functions of Fe^{3+} within the bacterial cell. Provision of gallium to established biofilms of *P. aeruginosa* *in vitro* resulted in a dose dependent clearance of these sessile communities [59]. This approach has also demonstrated effectiveness *in vivo* in two murine models of infection [59]. Presence of gallium reduces Fe^{3+} uptake therefore repressing iron-dependent transcriptional regulators such as *pvdS* that control a number of virulence factors in *P. aeruginosa*. Two benefits of using this approach in human hosts are that gallium is already approved for use in large concentrations by the FDA and since Fe^{3+} is required by a multitude of clinically relevant pathogens it is likely that this therapeutic will be efficacious against co-infecting pathogens in polymicrobial communities.

No bug is an island – the issue of polymicrobial communities

One key issue with development of species-specific therapies is that bacterial species do not exist in isolation in the human host. The evidence that co-habiting bacteria or polymicrobial communities occupy specific host niches has increased rapidly over the past 5 years. Prior to this, our perception of infection was limited to a handful of primary pathogens occurring in isolation in these niches. The advent of applying culture-independent approaches to examine clinical samples has revealed a wealth of previously unappreciated microbial diversity. For example, a recent survey of pediatric cystic fibrosis airways revealed that 65 different bacterial species could be identified and coincidence of known respiratory pathogens was consistently seen across patient samples [60]. Recent culture-independent analyses of gastrointestinal microbial communities demonstrated the presence of 395 bacterial phylotypes, 62% of which represented novel lineages and that shifts in bacterial community composition underlie disease states [61–64]. Another study has demonstrated pathogen co-incidence in airway polymicrobial communities of patients developing ventilator-associated pneumonia [65]. These and many other studies have demonstrated the complexity of our relationship with microbial inhabitants and underscores the likelihood that pathogenesis is mediated by a mixed population rather than a single species in isolation.

While the usual pathogenic suspects remain key species in these bacterial communities, our perception of infection is changing from one of a single species phenomenon to that of a dynamic polymicrobial state composed of multiple co-incident pathogens. Bacteria possess a multitude of two-component signal transduction systems that allow them to sense environmental signals and respond appropriately. In infectious states, these signals may come from the host immune response or the microbial neighbors co-habiting the same ecological niche. These insights now allow us to consider therapeutic approaches that block specific virulence factors or dull the sensor-response systems of the resistant species within the polymicrobial community preventing host cell damage.

Culture-independent tools are permitting greater insight into how interventions such as antimicrobial administration cause substantial collateral damage to microbial diversity, often with little effect on the target pathogen [65]. For example, antimicrobial administration to patients developing ventilator-associated pneumonia leads to loss of potentially protective species and proliferation of opportunistic pathogens [65], this is also true of antibiotic dependent *C. difficile* proliferation [66]. Given that bacterial species are adept at sensing and responding to their environment, including microbial diversity, we are starting to appreciate, using culture-independent tools, how specific treatments impact the polymicrobial community and these shifts in community composition affect primary pathogen behavior. Comprehensively examining the effect of novel therapeutics on the microbial community diversity and function will yield unprecedented information on the effects of these treatments and provide valuable insight into unforeseen side-effects such as pathogen proliferation or unanticipated induction of specific virulence systems.

Conclusion

Development of therapeutics that target specific aspects of bacterial physiology, represent a promising approach to combating pathogenesis. Ultimately, understanding bacterial physiology at both the single species level and within the context of a dynamic mixed-species population in the human host will provide further therapeutic targets and potentially the means to manipulate and analyze whole communities behavior with the aim of reducing virulence gene expression, combating infection and improving host health.

Acknowledgments

SVL is funded by an American Lung Association award and a NIH award (AI075410). JWK is funded by NIH grants SCCOR HL 74005 and HL 69809, HL074005 (SCCOR Project 4).

Bibliography

*, special interest;

**, outstanding interest.

1. Sears CL. A dynamic partnership: celebrating our gut flora. *Anaerobe* 2005 Oct;11(5):247–51. [PubMed: 16701579]
2. Roy-Burman A, Savel RH, Racine S, Swanson BL, Revadigar NS, Fujimoto J, et al. Type III protein secretion is associated with death in lower respiratory and systemic *Pseudomonas aeruginosa* infections. *The Journal of infectious diseases* 2001 Jun 15;183(12):1767–74. [PubMed: 11372029]
3. Finck-Barbancon V, Goranson J, Zhu L, Sawa T, Wiener-Kronish JP, Fleiszig SM, et al. ExoU expression by *Pseudomonas aeruginosa* correlates with acute cytotoxicity and epithelial injury. *Molecular microbiology* 1997 Aug;25(3):547–57. [PubMed: 9302017]

4. Kurahashi K, Kajikawa O, Sawa T, Ohara M, Gropper MA, Frank DW, et al. Pathogenesis of septic shock in *Pseudomonas aeruginosa* pneumonia. *The Journal of clinical investigation* 1999 Sep; 104(6):743–50. [PubMed: 10491409]
5. Pankhaniya RR, Tamura M, Allmond LR, Moriyama K, Ajayi T, Wiener-Kronish JP, et al. *Pseudomonas aeruginosa* causes acute lung injury via the catalytic activity of the patatin-like phospholipase domain of ExoU. *Critical care medicine* 2004 Nov;32(11):2293–9. [PubMed: 15640644]
6. Dacheux D, Toussaint B, Richard M, Brochier G, Croize J, Attree I. *Pseudomonas aeruginosa* cystic fibrosis isolates induce rapid, type III secretion-dependent, but ExoU-independent, oncosis of macrophages and polymorphonuclear neutrophils. *Infection and immunity* 2000 May;68(5):2916–24. [PubMed: 10768989]
7. Geddes K, Cruz F, Heffron F. Analysis of cells targeted by *Salmonella* type III secretion in vivo. *PLoS pathogens* 2007 Dec;3(12):e196. [PubMed: 18159943]
8. Broz P, Mueller CA, Muller SA, Philippsen A, Sorg I, Engel A, et al. Function and molecular architecture of the *Yersinia* injectisome tip complex. *Molecular microbiology* 2007 Sep;65(5): 1311–20. [PubMed: 17697254]
9. Faure K, Fujimoto J, Shimabukuro DW, Ajayi T, Shime N, Moriyama K, et al. Effects of monoclonal anti-PcrV antibody on *Pseudomonas aeruginosa*-induced acute lung injury in a rat model. *Journal of immune based therapies and vaccines* 2003 Aug 13;1(1):2. [PubMed: 12943554]
10. Frank DW, Vallis A, Wiener-Kronish JP, Roy-Burman A, Spack EG, Mullaney BP, et al. Generation and characterization of a protective monoclonal antibody to *Pseudomonas aeruginosa* PcrV. *The Journal of infectious diseases* 2002 Jul 1;186(1):64–73. [PubMed: 12089663]
11. Imamura Y, Yanagihara K, Fukuda Y, Kaneko Y, Seki M, Izumikawa K, et al. Effect of anti-PcrV antibody in a murine chronic airway *Pseudomonas aeruginosa* infection model. *Eur Respir J* 2007 May;29(5):965–8. [PubMed: 17301098]
12. Neely AN, Holder IA, Wiener-Kronish JP, Sawa T. Passive anti-PcrV treatment protects burned mice against *Pseudomonas aeruginosa* challenge. *Burns* 2005 Mar;31(2):153–8. [PubMed: 15683685]
13. Sawa T, Yahr TL, Ohara M, Kurahashi K, Gropper MA, Wiener-Kronish JP, et al. Active and passive immunization with the *Pseudomonas* V antigen protects against type III intoxication and lung injury. *Nature medicine* 1999 Apr;5(4):392–8.
14. Cornelis GR, Wolf-Watz H. The *Yersinia* Yop virulon: a bacterial system for subverting eukaryotic cells. *Molecular microbiology* 1997 Mar;23(5):861–7. [PubMed: 9076724]
15. Brubaker RR. Interleukin-10 and inhibition of innate immunity to *Yersiniae*: roles of Yops and LcrV (V antigen). *Infection and immunity* 2003 Jul;71(7):3673–81. [PubMed: 12819047]
16. Zauberman A, Cohen S, Levy Y, Halperin G, Lazar S, Velan B, et al. Neutralization of *Yersinia pestis*-mediated macrophage cytotoxicity by anti-LcrV antibodies and its correlation with protective immunity in a mouse model of bubonic plague. *Vaccine* 2008 Mar 20;26(13):1616–25. [PubMed: 18304706]
17. (SEC). USaEC. Pharmathene, Inc. Form 10-Q, Quarter Ended September 30, Commission File Number 001-32587. 2007. SEC web site [online], http://wwwsecgov/Archives/edgar/data/1326190/000110465907083136/a07-29212_110qhtm#Item2_103948
18. Lam J, Chan R, Lam K, Costerton JW. Production of mucoid microcolonies by *Pseudomonas aeruginosa* within infected lungs in cystic fibrosis. *Infection and immunity* 1980 May;28(2):546–56. [PubMed: 6772562]
19. Guina T, Purvine SO, Yi EC, Eng J, Goodlett DR, Aebersold R, et al. Quantitative proteomic analysis indicates increased synthesis of a quinolone by *Pseudomonas aeruginosa* isolates from cystic fibrosis airways. *Proceedings of the National Academy of Sciences of the United States of America* 2003 Mar 4;100(5):2771–6. [PubMed: 12601166]
20. Hassett DJ, Cuppoletti J, Trapnell B, Lyman SV, Rowe JJ, Yoon SS, et al. Anaerobic metabolism and quorum sensing by *Pseudomonas aeruginosa* biofilms in chronically infected cystic fibrosis airways: rethinking antibiotic treatment strategies and drug targets. *Advanced drug delivery reviews* 2002 Dec 5;54(11):1425–43. [PubMed: 12458153]

21. Yoon SS, Hennigan RF, Hilliard GM, Ochsner UA, Parvatiyar K, Kamani MC, et al. *Pseudomonas aeruginosa* anaerobic respiration in biofilms: relationships to cystic fibrosis pathogenesis. *Developmental cell* 2002 Oct;3(4):593–603. [PubMed: 12408810]
22. Opal SM. Communal living by bacteria and the pathogenesis of urinary tract infections. *PLoS medicine* 2007 Dec;4(12):e349. [PubMed: 18092887]
23. Wright KJ, Seed PC, Hultgren SJ. Development of intracellular bacterial communities of uropathogenic *Escherichia coli* depends on type 1 pili. *Cellular microbiology* 2007 Sep;9(9):2230–41. [PubMed: 17490405]
24. Yu L, Lee KK, Paranchych W, Hodges RS, Irvin RT. Use of synthetic peptides to confirm that the *Pseudomonas aeruginosa* PAK pilus adhesin and the *Candida albicans* fimbrial adhesin possess a homologous receptor-binding domain. *Molecular microbiology* 1996 Mar;19(5):1107–16. [PubMed: 8830267]
25. Lee KK, Yu L, Macdonald DL, Paranchych W, Hodges RS, Irvin RT. Anti-adhesin antibodies that recognize a receptor-binding motif (adhesintope) inhibit pilus/fimbrial-mediated adherence of *Pseudomonas aeruginosa* and *Candida albicans* to asialo-GM1 receptors and human buccal epithelial cell surface receptors. *Canadian journal of microbiology* 1996 May;42(5):479–86. [PubMed: 8640607]
26. Martinez JJ, Mulvey MA, Schilling JD, Pinkner JS, Hultgren SJ. Type 1 pilus-mediated bacterial invasion of bladder epithelial cells. *The EMBO journal* 2000 Jun 15;19(12):2803–12. [PubMed: 10856226]
27. Garofalo CK, Hooton TM, Martin SM, Stamm WE, Palermo JJ, Gordon JI, et al. *Escherichia coli* from urine of female patients with urinary tract infections is competent for intracellular bacterial community formation. *Infection and immunity* 2007 Jan;75(1):52–60. [PubMed: 17074856]
28. Sauer FG, Mulvey MA, Schilling JD, Martinez JJ, Hultgren SJ. Bacterial pili: molecular mechanisms of pathogenesis. *Current opinion in microbiology* 2000 Feb;3(1):65–72. [PubMed: 10679419]
29. Manetti AG, Zingaretti C, Falugi F, Capo S, Bombaci M, Bagnoli F, et al. *Streptococcus pyogenes* pili promote pharyngeal cell adhesion and biofilm formation. *Molecular microbiology* 2007 May;64(4):968–83. [PubMed: 17501921]
30. Pan YT, Xu B, Rice K, Smith S, Jackson R, Elbein AD. Specificity of the high-mannose recognition site between *Enterobacter cloacae* pili adhesin and HT-29 cell membranes. *Infection and immunity* 1997 Oct;65(10):4199–206. [PubMed: 9317027]
31. Firon N, Ashkenazi S, Mirelman D, Ofek I, Sharon N. Aromatic alpha-glycosides of mannose are powerful inhibitors of the adherence of type 1 fimbriated *Escherichia coli* to yeast and intestinal epithelial cells. *Infection and immunity* 1987 Feb;55(2):472–6. [PubMed: 3542836]
32. Hyland RM, Griener TP, Mulvey GL, Kitov PI, Srivastava OP, Marcato P, et al. Basis for N-acetyllactosamine-mediated inhibition of enteropathogenic *Escherichia coli* localized adherence. *Journal of medical microbiology* 2006 Jun;55(Pt 6):669–75. [PubMed: 16687583]
33. Hakkarainen J, Toivanen M, Leinonen A, Frangsmyr L, Stromberg N, Lapinjoki S, et al. Human and bovine milk oligosaccharides inhibit *Neisseria meningitidis* pili attachment in vitro. *The Journal of nutrition* 2005 Oct;135(10):2445–8. [PubMed: 16177210]
34. Sharon N. Carbohydrates as future anti-adhesion drugs for infectious diseases. *Biochimica et biophysica acta* 2006 Apr;1760(4):527–37. [PubMed: 16564136]
35. Aberg V, Almqvist F. Pilicides-small molecules targeting bacterial virulence. *Organic & biomolecular chemistry* 2007 Jun 21;5(12):1827–34. [PubMed: 17551629]
36. Larsson A, Johansson SM, Pinkner JS, Hultgren SJ, Almqvist F, Kihlberg J, et al. Multivariate design, synthesis, and biological evaluation of peptide inhibitors of FimC/FimH protein-protein interactions in uropathogenic *Escherichia coli*. *Journal of medicinal chemistry* 2005 Feb 24;48(4):935–45. [PubMed: 15715464]
- 37*. Pinkner JS, Remaut H, Buelens F, Miller E, Aberg V, Pemberton N, et al. Rationally designed small compounds inhibit pilus biogenesis in uropathogenic bacteria. *Proceedings of the National Academy of Sciences of the United States of America* 2006 Nov 21;103(47):17897–902. Rational design of bicyclic 2-pyridones to block chaperone-usheer pathways responsible for pili

- biogenesis. Lack of pili blocks initial attachment and biofilm formation by uropathogenic *Escherichia coli*, common cause of recurrent urinary tract infections. [PubMed: 17098869]
38. Svensson A, Larsson A, Emtenas H, Hedenstrom M, Fex T, Hultgren SJ, et al. Design and evaluation of pilicides: potential novel antibacterial agents directed against uropathogenic *Escherichia coli*. *Chembiochem* 2001 Dec 3;2(12):915–8. [PubMed: 11948880]
 39. Prunier AL, Schuch R, Fernandez RE, Mumy KL, Kohler H, McCormick BA, et al. nadA and nadB of *Shigella flexneri* 5a are antivirulence loci responsible for the synthesis of quinolate, a small molecule inhibitor of *Shigella* pathogenicity. *Microbiology (Reading, England)* 2007 Jul; 153(Pt 7):2363–72.
 - 40*. Hung DT, Shakhnovich EA, Pierson E, Mekalanos JJ. Small-molecule inhibitor of *Vibrio cholerae* virulence and intestinal colonization. *Science (New York, NY)* 2005 Oct 28;310(5748): 670–4. A high-throughput, phenotypic screen identified a small molecule 4-[N-(1,8-naphthalimide)]-n-butyric acid, virstatin. Virstatin inhibits the transcriptional regulator ToxT of *Vibrio cholerae* preventing expression of both the cholera toxin and the toxin co-regulated pilus. Administration of virstatin protected infant mice from intestinal colonization by *V. cholerae*.
 41. Panchal RG, Hermone AR, Nguyen TL, Wong TY, Schwarzenbacher R, Schmidt J, et al. Identification of small molecule inhibitors of anthrax lethal factor. *Nature structural & molecular biology* 2004 Jan;11(1):67–72.
 42. Tonello F, Seveso M, Marin O, Mock M, Montecucco C. Screening inhibitors of anthrax lethal factor. *Nature* 2002 Jul 25;418(6896):386. [PubMed: 12140548]
 43. Turk BE, Wong TY, Schwarzenbacher R, Jarrell ET, Leppla SH, Collier RJ, et al. The structural basis for substrate and inhibitor selectivity of the anthrax lethal factor. *Nature structural & molecular biology* 2004 Jan;11(1):60–6.
 - 44*. Sanchez AM, Thomas D, Gillespie EJ, Damoiseaux R, Rogers J, Saxe JP, et al. Amiodarone and bepridil inhibit anthrax toxin entry into host cells. *Antimicrobial agents and chemotherapy* 2007 Jul;51(7):2403–11. Cell based assay to identify new molecules that inhibit anthrax toxin resulted in identification of two potent antitoxic compounds, amiodarone and bepridil. Both drugs act by blocking endosomal acidification, thereby preventing anthrax toxin entry. Lethal toxin-challenged mice exhibited greater survival when treated with Amiodarone. [PubMed: 17485504]
 45. Nordfelth R, Kauppi AM, Norberg HA, Wolf-Watz H, Elofsson M. Small-molecule inhibitors specifically targeting type III secretion. *Infection and immunity* 2005 May;73(5):3104–14. [PubMed: 15845518]
 46. Carty NL, Layland N, Colmer-Hamood JA, Calfee MW, Pesci EC, Hamood AN. PtxR modulates the expression of QS-controlled virulence factors in the *Pseudomonas aeruginosa* strain PAO1. *Molecular microbiology* 2006 Aug;61(3):782–94. [PubMed: 16803594]
 47. Huber B, Riedel K, Hentzer M, Heydorn A, Gotschlich A, Givskov M, et al. The cep quorum-sensing system of *Burkholderia cepacia* H111 controls biofilm formation and swarming motility. *Microbiology (Reading, England)* 2001 Sep;147(Pt 9):2517–28.
 48. Lynch MJ, Swift S, Kirke DF, Keevil CW, Dodd CE, Williams P. The regulation of biofilm development by quorum sensing in *Aeromonas hydrophila*. *Environmental microbiology* 2002 Jan;4(1):18–28. [PubMed: 11966822]
 49. Sakuragi Y, Kolter R. Quorum-sensing regulation of the biofilm matrix genes (pel) of *Pseudomonas aeruginosa*. *Journal of bacteriology* 2007 Jul;189(14):5383–6. [PubMed: 17496081]
 50. de Nys RW, AD, König GM, Sticher O. New halogenated furanones from the marine alga *Delisea pulchra*. *Tetrahedron* 1993;49:11213–20.
 51. Hentzer M, Wu H, Andersen JB, Riedel K, Rasmussen TB, Bagge N, et al. Attenuation of *Pseudomonas aeruginosa* virulence by quorum sensing inhibitors. *The EMBO journal* 2003 Aug 1;22(15):3803–15. [PubMed: 12881415]
 52. Wu H, Song Z, Hentzer M, Andersen JB, Molin S, Givskov M, et al. Synthetic furanones inhibit quorum-sensing and enhance bacterial clearance in *Pseudomonas aeruginosa* lung infection in mice. *The Journal of antimicrobial chemotherapy* 2004 Jun;53(6):1054–61. [PubMed: 15117922]
 53. Bjarnsholt T, Jensen PO, Rasmussen TB, Christophersen L, Calum H, Hentzer M, et al. Garlic blocks quorum sensing and promotes rapid clearing of pulmonary *Pseudomonas aeruginosa* infections. *Microbiology (Reading, England)* 2005 Dec;151(Pt 12):3873–80.

54. Rasmussen TB, Skindersoe ME, Bjarnsholt T, Phipps RK, Christensen KB, Jensen PO, et al. Identity and effects of quorum-sensing inhibitors produced by *Penicillium* species. *Microbiology* (Reading, England) 2005 May;151(Pt 5):1325–40.
- 55**. Madanahally KD, Adikesavan NV, Cirioni O, Giacometti A, Silvestri C, Scalise G, et al. Discovery of a quorum sensing inhibitor of drug resistant staphylococcal infections by structure-based virtual screening. *Molecular pharmacology*. 2008 Feb 26; *In silico* screening of peptide analogs from a library of 300,000 molecules using the Integrated Scientific Information System (ISIS) software from Elsevier MDL led to the identification of a compound, Hamamelitannin. This small non-peptide molecule effectively blocks a quorum sensing regulator and prevents infection by methicillin-resistant *Staphylococcus* species.
56. Augustin DK, Song Y, Baek MS, Sawa Y, Singh G, Taylor B, et al. Presence or absence of lipopolysaccharide O antigens affects type III secretion by *Pseudomonas aeruginosa*. *Journal of bacteriology* 2007 Mar;189(6):2203–9. [PubMed: 17209027]
57. Riedel K, Hentzer M, Geisenberger O, Huber B, Steidle A, Wu H, et al. N-acylhomoserine-lactone-mediated communication between *Pseudomonas aeruginosa* and *Burkholderia cepacia* in mixed biofilms. *Microbiology* (Reading, England) 2001 Dec;147(Pt 12):3249–62.
58. Holden MT, Ram Chhabra S, de Nys R, Stead P, Bainton NJ, Hill PJ, et al. Quorum-sensing cross talk: isolation and chemical characterization of cyclic dipeptides from *Pseudomonas aeruginosa* and other gram-negative bacteria. *Molecular microbiology* 1999 Sep;33(6):1254–66. [PubMed: 10510239]
- 59**. Kaneko Y, Thoendel M, Olakanmi O, Britigan BE, Singh PK. The transition metal gallium disrupts *Pseudomonas aeruginosa* iron metabolism and has antimicrobial and antibiofilm activity. *The Journal of clinical investigation* 2007 Apr;117(4):877–88. Use of gallium to elicit molecular subterfuge. *P. aeruginosa* relies on iron to regulate expression of a number of important virulence genes. Gallium, which is similar in ionic radius and charge is taken up by the bacterial cell but, unlike iron, fails to induce bacterial gene expression, resulting in inhibition of *P. aeruginosa* growth and biofilm formation in vitro. [PubMed: 17364024]
60. Harris JK, De Groote MA, Sagel SD, Zemanick ET, Kapsner R, Penvari C, et al. Molecular identification of bacteria in bronchoalveolar lavage fluid from children with cystic fibrosis. *Proceedings of the National Academy of Sciences of the United States of America* 2007 Dec 18;104(51):20529–33. [PubMed: 18077362]
61. Eckburg PB, Bik EM, Bernstein CN, Purdom E, Dethlefsen L, Sargent M, et al. Diversity of the human intestinal microbial flora. *Science* (New York, NY) 2005 Jun 10;308(5728):1635–8.
62. Ley RE, Backhed F, Turnbaugh P, Lozupone CA, Knight RD, Gordon JI. Obesity alters gut microbial ecology. *Proceedings of the National Academy of Sciences of the United States of America* 2005 Aug 2;102(31):11070–5. [PubMed: 16033867]
63. Ley RE, Turnbaugh PJ, Klein S, Gordon JI. Microbial ecology: human gut microbes associated with obesity. *Nature* 2006 Dec 21;444(7122):1022–3. [PubMed: 17183309]
64. Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI. An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature* 2006 Dec 21;444(7122):1027–31. [PubMed: 17183312]
65. Flanagan JL, Brodie EL, Weng L, Lynch SV, Garcia O, Brown R, et al. Loss of bacterial diversity during antibiotic treatment of intubated patients colonized with *Pseudomonas aeruginosa*. *Journal of clinical microbiology* 2007 Jun;45(6):1954–62. [PubMed: 17409203]
66. Halsey J. Current and future treatment modalities for *Clostridium difficile*-associated disease. *Am J Health Syst Pharm* 2008 Apr 15;65(8):705–15. [PubMed: 18387898]

Fig. 1.

The Type III secretion system of *P. aeruginosa* involves a needle-like complex that traverses the bacterial bi-layer, crowned by PcrV proteins at the distal tip. **B.** Binding of the needle is believed to occur through interaction with PcrV and facilitates injection of cytotoxins directly into the mammalian cell cytoplasm. **C.** Anti-PcrV antibody binds PcrV and blocks cytotoxin secretion.

Fig. 2.

A. Iron (Fe^{3+}) is scavenged by pathogens and plays a key regulatory role in the expression of virulence genes. Galium (Ga^{3+}), which is similar to Fe^{3+} , is also imported into the bacterial cell but cannot induce virulence gene expression. **B.** Pili, extracellular bacterial appendages, facilitate attachment through adhesions located on the surface of the pili. Small molecules that block these adhesions, also block attachment. Pilicides, compounds that prevent pili formation also block bacterial attachment.