Available online at www.sciencedirect.com
ScienceDirect
Current Opinion in Microbiology

New screens and targets in antibacterial drug discovery

Shannon B Falconer[1,2] and Eric D Brown[1,2]

As the supply of effective antibiotics dwindles and the emergence of multi-drug-resistant bacteria becomes more commonplace, there is an urgent need to identify novel antibacterial targets and leads with new mechanisms of action. Among the strategies to bolster our current scarcity of effective antibiotics are biochemical and phenotype-based screens, and the rational design of inhibitors. In this review we highlight some recent contributions that these methodologies have yielded, placing particular emphasis on screens capable of identifying novel leads involved in such processes as virulence; underexploited targets that reside in bacterial cell surfaces; the use of bacteriophage as antibiotic adjuvants; and novel targets of essential pathways. We discuss these findings in the context of the field of antibiotic drug discovery and how such discoveries position us to begin to fill the antibiotic gap that has been widening for the last half century.

Addresses
[1] Department of Biochemistry and Biomedical Sciences, McMaster University, 1200 Main Street West, Hamilton, ON, Canada L8N 3Z5
[2] Michael G. DeGroote Institute for Infectious Disease Research, McMaster University, 1200 Main Street West, Hamilton, ON, Canada L8N 3Z5

Corresponding author: Brown, Eric D (ebrown@mcmaster.ca)

Current Opinion in Microbiology 2009, **12**:497–504

This review comes from a themed issue on
Antimicrobials
Edited by Christopher Walsh and Gerry Wright

Available online 3rd August 2009

1369-5274/$ – see front matter

DOI 10.1016/j.mib.2009.07.001

Introduction

Since the deployment of antimicrobial chemotherapy in the 1940s, efforts to combat pathogenic microbes have been steadily mired by the emergence of bacterial resistance. Not only have drug-resistant bacteria now been observed for virtually every antibiotic class [1], particularly pernicious multi-drug-resistant strains have begun to pervade both hospital and community settings. Now more than ever it is acutely apparent that microbial resistance is an unavoidable consequence of antibiotic therapy. This realization means that although the path to antimicrobial drug discovery is well trodden, in order to have constant leverage over bacterial pathogens we must continually blaze new trails with respect to identifying novel mechanisms and targets to treat bacterial infections.

Herein we discuss some of the most recent advances in the field of antibacterial drug discovery (Table 1), placing particular emphasis on novel screening strategies that facilitate the identification of antibiotic lead compounds; as well as examining candidates that have been unknown or underexploited as potential targets for drug discovery.

New screens and new technologies

Phenotype-based screens identify antibacterial leads with novel mechanisms of action

In a 2008 study, Arnoldo *et al.* [2••] performed an ingenious forward genetic approach that permitted the identification of both a *bona fide* small molecule lead and the target of that compound with relative ease, thus circumventing the traditional caveat of phenotype-based drug discovery, which is having to discern the target of the lead compound. Using a yeast cell-based phenotypic assay combined with the power of chemical genomics, Arnoldo *et al.* identified the first known inhibitor of the *Pseudomonas aeruginosa* virulence protein, ExoS. Although this was not the first study to use bakers yeast, *Saccharomyces cerevisiae*, as a model for human bacterial infections, it is the first report to date to employ compound screening against *S. cerevisiae* to identify small molecule inhibitors of human pathogenic bacteria. Briefly, the authors commenced this study with a shortlist of *P. aeruginosa* virulence and essential genes that were then individually transformed into yeast cells and monitored for their ability to impair yeast growth upon overexpression [2••]. Genes detected as causing growth inhibition when overexpressed were then screened against a panel of compounds to assay for small molecules capable of restoring yeast growth, thereby allowing researchers to identify the compound, exosin, and its cellular target, ExoS. The success of this study not only points to the utility in using yeast as a proxy for investigating human pathogens, but also reveals a phenotype-based screen that readily allows for target identification.

In an effort to conduct an antibiotic screen that not only detects small molecules that work traditionally (i.e. by targeting genes that are essential), but also has the capacity to detect compounds that target virulence, enhance host defense or function as prodrugs, Moy *et al.* [3] presented an elegant assay that details a screen of more than 7000 compounds using the live-animal infection model, *Caenorhabditis elegans*. Upon infecting *C. elegans* with the human opportunistic bacteria *Enterococcus faecalis*, the authors identified 25 compounds that stimulated nematode survival. Interestingly, Moy *et al.* observed that a handful of these same molecules either

Table 1

A summary of both new and promising antibiotic targets, and screens that allow for the identification of inhibitors against novel pathways

Novel antibiotic targets

Target	Associated pathway	Novel inhibitors	References
LpxC	Lipid A of lipopolysaccharide	CHIR-090	[5•,6–8]
Glucosyltransferase	Peptidoglycan	Moenomycin	[9••,10–13]
Intracellular steps of peptidoglycan synthesis	Peptidoglycan		[14–17]
Late stage tag/tar genes	Wall teichoic acid		[25,27,28,32,34]
LtaS	Lipoteichoic acid		[39••,40•]
Acetyltransferase domain of GlmU	Precursor for lipopolysaccharide, peptidoglycan, and teichoic acid		[41]
SOS response, oxidative stress response pathway, biofilm formation	Nonessential gene pathways	Engineered bacteriophage	[45••]
Peptide deformylase	Peptide synthesis	Actinonin, BB83698, LBM415	[46–49]
FabF, FabH	Type II fatty acid synthesis	Platensimycin, platencin	[51,52,53•]

Novel phenotype-based screens

Model organism and pathogen	Targets identified	Associated pathway	References
S. cerevisiae, *P. aeruginosa*	ExoS	Virulence	[2••]
C. elegans, *E. faecalis*	Unknown	Virulence, host innate immunity	[3]

did not show any effect on the inhibition of *C. elegans* growth *in vitro*, or that inhibition occurred at a much higher MIC than observed *in vivo*. These findings support the idea that *in vitro* screening methods are limited in their ability to detect compounds with such novel mechanisms of action as targeting virulence or enhancing host innate immunity, and that *in vivo* screening techniques hold ample promise as a means to expand the current paucity of antimicrobials that function via innovative routes.

Novel targets and leads with new mechanisms of action

Bacterial cell surfaces

The indispensable role of the cell wall in bacterial viability has rendered it a choice target for the development of antimicrobials. Indeed, since the dawn of the antimicrobial era, drugs such as the β-lactams and the glycopeptides have been in use as potent biosynthetic inhibitors of peptidoglycan — a major cell wall component that is responsible for both maintaining structural integrity and protecting bacteria against osmotic pressure (for review, see Vollmer *et al.* [4]). However, in addition to peptidoglycan, bacteria possess other cell surface features that represent potential targets for novel antibacterial leads, such as lipid A and lipopolysaccharide (LPS), which provide the membrane anchor of LPS and decorate the outer membrane of Gram-negative bacteria, respectively, and teichoic acid found on the cell wall of Gram-positive organisms. The following describes some of the research that is being conducted to identify novel antibacterial leads that target the biosynthesis of lipid A and LPS, peptidoglycan and teichoic acid.

As the efficacy of traditional antibiotics targeting DNA replication, cell wall and protein biosynthesis steadily dwindles, inhibitors against underexploited essential bacterial processes are attracting much attention. The lipid A moiety of LPS, an essential molecule and the major lipid found in the outer leaflet of the outer membrane, is one such prime example and is considered to be among the most promising novel antibacterial targets of Gram-negative bacteria [5•,6]. LpxC is responsible for the committed step in lipid A biosynthesis and catalyzes the deacetylation of UDP-3-*O*-(*R*-3-hydroxyacyl)-*N*-acetylglucosamine. Although a number of LpxC inhibitors have been found to be lethal against certain Gram-negative organisms including *Escherichia coli*, these compounds were unable to inhibit the growth of *P. aeruginosa* and hence not developed as clinical antibiotics [7]. Indeed, it was not until McClerren *et al.* [8] reported CHIR-090 that an LpxC inhibitor displaying activity against *P. aeruginosa* was identified. Since its initial discovery, CHIR-090 has been shown *in vitro* to inhibit LpxC orthologs from the additional pathogenic Gram-negative bacteria, *Neisseria meningitidis* and *Helicobacter pylori* at concentrations as low as the nanomolar range [6], and a solution structure of the LpxC–CHIR-090 has recently been reported [5•]. Given its potent and broad-spectrum activity against Gram-negative pathogens, CHIR-090 represents an exciting new lead as a novel LpxC inhibitor.

The final stages of peptidoglycan assembly occur via the activity of two enzymes: namely, glucosyltransferases (GT) and transpeptidases (TP), the latter of which are also referred to as penicillin-binding proteins (PBPs) and are the main targets of the β-lactam group of antibiotics. Although TP proteins have been well characterized, it has