available at www.sciencedirect.com

SCIENCE DIRECT®

journal homepage: www.elsevier.com/locate/biochempharm

Commentary

# When will the genomics investment pay off for antibacterial discovery?

*Scott D. Mills* *

*Infection Discovery, AstraZeneca R&D Boston, 35 Gatehouse Drive, Waltham, MA 02451 USA*

ARTICLE INFO

*Keywords:*
Antibacterial
Genomic
Bioinformatic
Target
Resistance
Screening

ABSTRACT

Effective solutions to antibacterial resistance are among the key unmet medical needs driving the antibacterial industry. A major thrust in a number of companies is the development of agents with new modes of action in order to bypass the increasing emergence of antibacterial resistance. However, few antibacterials marketed in the last 30 years have novel modes of action. Most recently, genomics and target-based screening technologies have been emphasized as a means to facilitate this and expedite the antibacterial discovery process. And although no new antibacterials have yet been marketed as result of these technologies, genomics has delivered well-validated novel bacterial targets as well as a host of genetic approaches to support the antibacterial discovery process. Likewise, high throughput screening technologies have delivered the capacity to perform robust screenings of large compound collections to identify target inhibitors for lead generation. One of the principal challenges still facing antibacterial discovery is to become proficient at optimizing target inhibitors into broad-spectrum antibacterials with appropriate in vivo properties. Genomics-based technologies clearly have the potential for additional application throughout the discovery process especially in the areas of structural biology and safety assessment.



## 1. Introduction

There have been several articles in the past few years stating that antibacterial drug discovery is experiencing difficult times, and that many large pharmaceutical companies are closing down or reducing their anti-infective discovery efforts [1–4]. Undoubtedly, there has been a steady decline in new FDA approved antibacterial agents in the last 20 years (71% decrease between 1983 and 2004) [2,5]. Of the 10 antibacterial agents approved since 1998, only linezolid an oxazolidinone and daptomycin a lipopeptide have new modes of action, daptomycin acting at a novel target site [2]. The oxazolidinone class represents the first significant synthetic antibacterial class introduced to the market since the quinolones in the 1960s. Linezolid was approved for human use approximately 20 years after the discovery of the oxazolidinones [6]. Daptomycin, a cyclic lipopeptide fermentation product, was first discovered and developed in the 1980s. It was subsequently licensed in 1997 and approved for use in 2003, more than 15 years after its initial discovery [7]. The underlying message is that the discovery and development of new antibacterial classes has been rare, and has required lengthy research and development time periods before they were ready for market. The vast majority of FDA approved

* Tel.: +1 781 839 4619; fax: +1 781 839 4570.
*E-mail address:* scott.mills@astrazeneca.com.
0006-2952/$ – see front matter 
doi:10.1016/j.bcp.2005.11.025

antibacterial drugs in the last 40 years have been modifications of existing agents that were discovered and developed during the ''Golden Era'' of antibacterial discovery from the late 1930s to the 1960s [8].

### 1.1. *Pre-genomic ''Golden Era''*

It is important to understand the antibacterial industry prior to the ''genomic era'' in order to align expectations from genomics relative to past performance. The ''Golden Era'' of antibacterial discovery represents the time period when most of the major classes of successful antibacterials were discovered [8]. The antibacterials discovered during this period were, for the most part, complex molecules that were produced by fermentation often followed by synthetic modification. For the last 40 years, synthetic modifications have had significant impact on potency, spectrum, efficacy and safety profiles leading to second, third and fourth generation antibacterials with improved properties. However, the industry has experienced difficulty in staying ahead of antibacterial resistance by simply modifying the existing classes.

The most noteworthy synthetic antibacterial class from the ''Golden Era'' came in 1962 when the synthetic quinolones were discovered as nalidixic acid. This class did not have a big impact on the market until being modified into the very successful fluoroquinolone class (norfloxacin) in the 1980s. Again, it has taken considerable time to optimize this class into what it is today with commercial successes such as ciprofloxacin, levofloxacin, moxifloxacin, and gatifloxacin.

### 1.2. *Antibacterial resistance*

Antibacterial resistance is a key driver in antibacterial discovery as illustrated by the following examples. Penicillin was introduced in the early 1940s and within a few years penicillin-resistant *Staphylococcus aureus* strains were identified. By the late 1960s, greater than 80% of *S. aureus* isolates were penicillin resistant in United States hospitals [5]. In the late 1960s, methicillin was introduced to overcome penicillin-resistant *S. aureus*. In 1974, 2% of hospital-acquired *S. aureus* infections in the United States were methicillin resistant (MRSA), and by 2002 the incidence of hospital-acquired MRSA had increased to 57%. Vancomycin was introduced as a first-line therapy for serious MRSA infections in the 1980s. Vancomycin intermediate (VISA) and now vancomycin resistant (VRSA) MRSA are becoming more frequently documented [5,9,10]. Linezolid and daptomycin were approved for the treatment of infections caused by MRSA and vancomycin-resistant enterococci (VRE). Linezolid resistant VRE and MRSA and daptomycin resistant MRSA were documented within a year of FDA approval [11–13]. There is a growing sentiment that only synthetic antibacterials with new modes of action (against novel targets) will effectively deal with infections caused by multi-drug-resistant bacteria in that preexisting gene-based resistance would be unlikely.

In the past decade, the ''genomic promise'' has been advertised as a means to accelerate the antibacterial discovery process in delivering first-in-class drugs active against resistant bacteria. The ''genomic promise'' has also fueled venture capital-backed biotechnology companies seeking lucrative high tech opportunities in the antibacterial industry. An obvious question is: why would the mature antibacterial discovery industry be led into thinking that the recently established genomics and related technologies could deliver drugs in an unprecedented timeframe? Bacterial genomics has had a considerable impact on the overall paradigm of antibacterial discovery. But does the industry have realistic expectations from genomics?

## 2. The genomic era (1995–present)

The 10th anniversary of the publication of the *Haemophilus influenzae* genomic sequence in 1995 has recently been celebrated [14]. This was the first free-living organism genome to be sequenced and as a result marked the beginning of the ''genomic era''. Later in 1995 the *Mycoplasma genitalium* genome was sequenced, thus facilitating the first comparison of small genomes from two distinctly different pathogens [15], and consequently ushered in the ''minimum-gene-set'' concept and ''comparative genomics'' [16]. As new genomes are completed, the minimum-gene-set is further refined and tested [17]. In 1999, the first intraspecies genomic comparison of two *Helicobacter pylori* strains was published [18]. This facilitated a first glance at strain to strain variation at the whole genome level. Twenty-five genomes, including 11 human bacterial pathogens, were completed in the first 5 years of the genomic era. In the next 5 years 185 genomes, or 90% of the 10 year total, were completed as illustrated in Fig. 1 [19]. It was not until 2001 that the important Gram-positive pathogen genomes of *S. aureus*, *Streptococcus pneumoniae* and *Streptococcus pyogenes* were made publicly available. As of November 2005, 281 microbial genomes have been completed (257 bacteria, 24 archaea) and are publicly available, of which 123 are from strains of human bacterial pathogens. The completed bacterial genomes range in size from 0.58 megabases (*M. genitalium*) to 9.12 megabases (*Streptomyces avermitilis*), and now many bacterial species are represented by genome sequences of multiple clinical isolates. For instance, the skin and soft tissue pathogens *S. aureus* and *S. pyogenes* are represented by 6 and 7 completed genomes, respectively. This has allowed for a more definitive look at clinical isolates of the

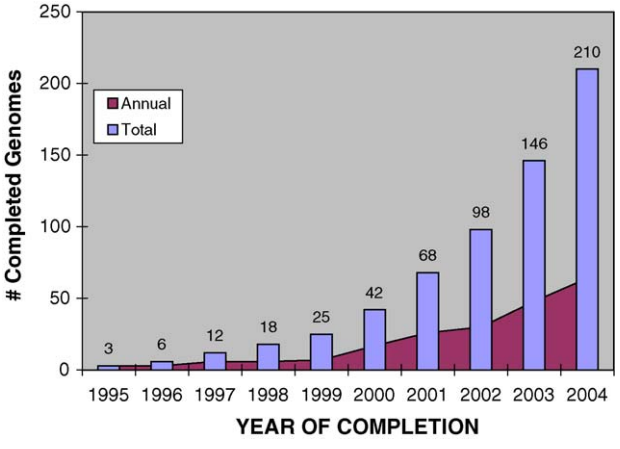


**Fig. 1 – The first 10 years of the genomic era (1995–2004).**

same species [20,21]. In addition to the completed genomes, there are currently 546 genome projects at various stages of completion (521 bacteria, 25 archaea). The genomic era was further showcased in 2001 by the long awaited landmark publications of the human genome sequence by Celera Genomics [22] and The International Human Genome Sequencing Consortium [23].

### 2.1. Genomic sequence and bioinformatics

The obvious short-term return on the genomics investment has been massive amounts of whole genome DNA sequence [19]. However, without the development of powerful computational systems for integration and analysis, the vast quantity of genomic sequence data would be difficult to manage and largely uninterpretable. Computational methods have allowed for automation of the entire process from sequencing and assembly of DNA fragments (contigs) into genomes to prediction of protein coding regions and annotation of function [24]. The development of software for multiple genome analyses has facilitated integration of all of the genomic information with analytical tools that are easy to use and now publicly available in web-based platforms such as MicrobesOnline [25], Comprehensive Microbial Resource [26] and Entrez Genome [19]. In the last 3–5 years, the antibacterial industry has assembled powerful comprehensive platforms based on greater than 100 annotated genomes from human bacterial pathogens and advanced computational methods for their genome-driven target-based discovery programs. These databases form a critical knowledge network that serve as the basis for genomics studies. The databases described above fuel basic and applied discovery programs in academia and the private sector.

## 3. Genomics and target-based antibacterial discovery

### 3.1. Target identification

The development of sophisticated comparative genomic platforms has been key to exploiting bacterial genome sequences for target identification and selection. Bacterial targets are selected based on predetermined criteria with the overall purpose of translating clinical indications into novel molecular targets. The first step in this process is to identify bacterial protein targets that are present in a desired spectrum of bacteria. Comparative genomic analyses are used to cluster targets according to clinical indications. For example, the pathogens primarily responsible for community acquired respiratory tract infections (RTI) include *S. pneumoniae*, *Moraxella catarrhalis* and *H. influenzae* [27]. Therefore, in order to select a target for this indication it must be present in these three pathogens. Moreover, additional value is ascribed to targets that are also present in atypical pathogens that cause respiratory tract infections such as *Mycoplasma pneumoniae*, *Chlamydia pneumoniae* and Legionella spp. Conservation of a target's primary sequence (homology) or predicted secondary structure is taken into account at this stage. Availability of functional and/or structural information may provide meaningful insight into target conservation beyond primary sequence homology. The addition of actual 3D bacterial protein structures, and homology models, greatly enhances the target selection process in helping to identify drugable binding sites. The human genome sequence is typically queried during target selection to determine whether a bacterial target has a human counterpart, and if so its degree of homology, in order to assess the potential risk of target-based toxicity. As such, target identification incorporates risk assessment early in the discovery process by identifying the potential for spectrum and selectivity for a given target.

### 3.2. Target validation

Once targets are selected and ranked based on the target identification criteria (e.g. potential for bacterial spectrum and human toxicity) they should be genetically validated to confirm their essentiality for cell viability. Genomics plays a central role in target validation in that it facilitates genome-scale functional studies to evaluate essentiality in key pathogens, and it facilitates directed genetic studies in clinically relevant organisms to assess individual targets. Genome-scale probing of essentiality has been accomplished in several key pathogens including *Pseudomonas aeruginosa* [28], *Escherichia coli* [29], *H. influenzae* [30], *S. aureus* [31,32], *S. pneumoniae* [33,34], *M. pneumoniae* [35] and nonpathogen *Bacillus subtilis* [36] using a variety of genetic methods all relying on the genomic sequence of these organisms for constructing recombinant strains and mapping putative essential genes of interest. These studies have generated a valuable inventory of essential genes to prioritize for further target validation efforts. In most cases, the inferred essentiality data from the genome-scale studies require direct confirmation of essentiality by more rigorous gene knockout and complementation studies [37–39], and/or controlled gene expression studies [40,41]. Development of genetic models and directed studies of gene essentiality have been greatly impacted by the availability of genomic sequence [42].

### 3.3. Hit and lead identification

Once validated, the target gene is cloned and its corresponding protein product expressed in an optimized expression system (e.g. *E. coli*, *Pichia pastoris* or Baculovirus). The target protein is then purified and a robust biochemical assay is developed so that it is suitable for screening a large and diverse collection of low molecular weight compounds in order to identify target inhibitors or ''hits''. The hits are subsequently characterized with respect to potency, mechanism of inhibition, enzyme spectrum and selectivity, and physical properties. When focusing on potential broad-spectrum targets, it is beneficial to express and characterize isozymes of a given target from several genetically diverse bacterial species of interest. This may be a useful predictor of bacterial spectrum potential for a specific compound class. Suitably potent hits with acceptable isozyme spectrum are further screened against a panel of microbes for cellular activity. Two of the primary difficulties in converting an enzyme inhibitor into an antibacterial are penetrating the membrane barriers (permeability) and preventing efflux. In order to determine whether an enzyme

inhibitor can penetrate the bacterial cell, without introducing the complication of efflux, antibacterial activities can be evaluated using recombinant bacterial strains lacking one or more efflux pumps. Again, genomics has been instrumental in identifying potential efflux proteins and subsequently creating efflux pump mutant strains for evaluation of inhibitors [43]. Once whole-cell activity is achieved, it is important to ascertain that the antibacterial effect is due to the inhibition of the intended target and not via an unintended mechanism. This can be accomplished using defined mode of action assays, which become decisive in guiding medicinal chemistry efforts with respect to optimizing potency, spectrum and selectivity.

### 3.4. Whole-cell screening and mode of action

The target-based genomics approach can be combined with traditional whole-cell screening to minimize the risk of not achieving whole-cell activity in hits from high throughput screening. Using this approach, targets are identified and validated in the same manner as described earlier in this paper. However, instead of screening an isolated enzyme, screening would be carried out using a genetically altered bacterial strain such that it would respond in a measurable way when a target of interest is inhibited. The response can be measured as growth inhibition (absorbance) or induction of a linked reporter gene (e.g. luminescence or fluorescence). The former involves constructing recombinant strains that underexpress a target gene of interest so that when inhibited a corresponding impact on growth is observed [44,45]. The latter involves fusion of a reporter gene, such as *gfp* or *lux*, to a promoter that has been identified, for instance, by DNA microarray analysis, to specifically respond to antibiotic stress [46,47]. If successful, target-directed inhibitors with antibacterial activity can be identified directly from HTS. This would allow medicinal chemists to focus on important properties such as antibacterial potency and DMPK properties.

In order to further balance the risks of relying too heavily on target-based screening, genomics can be utilized to identify the target of antibacterial compounds after they are discovered by whole-cell screening. One approach to identifying the target of an antibacterial with an unknown mode of action is to isolate spontaneously resistant mutants and identify the genetic basis of resistance by functional cloning, sequencing and genome mapping [48]. Recently, a more sophisticated approach to genome mapping of resistance mutations to identify targets of novel antibacterial compounds was published [49]. This method utilizes whole genome restriction maps and experimental transformation efficiencies of restriction endonuclease-digested genomic DNA in order to map the mutations and the putative target. DNA microarray analysis has also proven to be useful in focusing in on the target of antibacterial compounds with unknown modes of action [50,51].

Once whole-cell activity is achieved, it is important to ascertain whether the antibacterial effect is due to inhibition of the intended target or by an unintended mode of action. This can be accomplished using defined mode of action assays, such as those described above for target-based whole-cell screening. Additionally, recombinant strains that overexpress a target may be used to study dose response relationships between inhibitors and the intended target in order to confirm the correct mode of action. Genomics has also played a key role in efficiently mapping and characterizing antibacterial resistance including identifying new efflux mechanisms. Well-characterized mutant strains of bacteria resistant to a compound series may be used in later stage projects to monitor the mode of action to ensure that a change in the molecule does not result in an inadvertent or mixed mode of action. Genomics has certainly enhanced the ability of the bacterial geneticist to design and manipulate recombinant strains for whole-cell screening and mode of action assays.

### 3.5. Spectrum

Clearly, bacterial genomics makes its biggest impact at the earlier stages of target-based antibacterial discovery (e.g. target identification and validation). A well-defined process has been established at AstraZeneca for selecting and validating novel protein targets, each with a proposed bacterial spectrum. However, the presence of a functionally equivalent target in multiple bacterial species does not guarantee that each of the corresponding isozymes will be equally inhibited by a single molecule. Novel protein targets should be isolated from several species of interest and shown to be similarly inhibited by lead compounds. It is likely that conservation at the level of 3D protein structure at the site of target inhibition is more important than conservation at the primary sequence level. As structural genomics and homology modeling technologies improve, not surprisingly our knowledge about conservation of ligand binding sites should also advance our ability to predict the drug-ability of broad-spectrum ligand binding sites.

Similarly, broad-spectrum enzymatic inhibition does not imply broad-spectrum antibacterial activity. Taxonomically unrelated bacteria exhibit different cell wall and membrane characteristics that result in differential permeability and efflux potential of small molecules. A new set of structure–activity relationships (SAR) will likely influence compound permeability and efflux relative to biochemical potency.

### 3.6. Examples of target-based programs

The target-based discovery paradigm encompasses many key technologies. Genomics has led the way with comprehensive informatics platforms, genetically validated novel targets and a host of genetic approaches to antibacterial discovery [42]. High throughput screening has generated key advances in assay development and detection systems, miniaturization and robotics, and compound management systems [52]. And synthetic chemistry has generated advances in methods such as combinatorial chemistry and high throughput parallel synthesis for generating large, diverse compound libraries for screening [53]. It is clearly the interplay between these technologies that is crucial to the success of lead generation via the target-based paradigm. Lead optimization and candidate drug delivery remain as key challenges that are for the most part independent of genomics. Even so, there are several

examples in the antibacterial discovery arena of novel targets being exploited to identify and optimize synthetic scaffolds with potent antibacterial activity, as well as favorable in vivo properties. Some selected examples include FabI/FabK and FabH (fatty acid biosynthesis) [54,55], PDF (peptide deformylase) [56,57], LpxC (lipid A biosynthesis) [44] and tRNA synthetases [58].

## 4. Prospects

In the last 10–15 years there has been a significant shift in the antibacterial discovery paradigm. In many instances, there is less focus on natural product screening and/or modifying existing antibacterial classes, and more focus on discovering first-in-class antibacterials to deal with resistance more effectively. Genomics has been publicized as a means to accelerate the entire discovery process. But, is it realistic to expect to discover and develop novel first-in-class agents in an unprecedented timeframe based on genomics? Genomics is one of several technologies being applied to drug discovery. It has not, and will not, deliver drugs on its own. Many of the challenges facing the antibacterial industry would remain challenges with or without genomics.

Genomics has paid off for antibacterial discovery in many ways. Genomics has fed directly into antibacterial discovery in the form of innovative technologies such as high throughput automated DNA sequencing, improved bioinformatics and comparative genomics software, and transcriptional profiling with DNA microarrays. Significantly, genomics has facilitated the development of robust genome-scale genetic approaches. Genomics has been a catalyst for ideas and concepts that have delivered key insights into bacterial gene regulation [59], physiology and metabolism [60,61], and evolution of pathogenicity and environmental adaptation [62,63]. Genomics and genetics have delivered validated essential targets that fit a number of different clinical indications and risk profiles. In turn, these targets have fed into target-based HTS drug-hunting campaigns. Genomics is used to determine the mode of action of compounds with antibacterial activity and to characterize mechanisms of resistance. It is playing a fundamental role in structural biology, and will continue to feed into structural genomics efforts [64]. As the human genome is better characterized, and complex biological interactions better understood, genomics will undoubtedly play an important role in drug discovery and development in the form of pharmacogenomics [65,66] and toxicogenomics [67].

The genomics-driven target-based antibacterial discovery paradigm will undoubtedly improve on its efficiencies as it has greatly matured in the last 3–5 years. It stands poised to deliver novel compounds to the development pipeline as long as there remains a long-term vision and commitment to applying innovative technologies to antibacterial discovery.

## Acknowledgements

I would like to thank Richard Alm, Lawrence MacPherson and Paul Manning for their useful comments on this article.

## REFERENCES

[1] Projan SJ. Why is big pharma getting out of antibacterial drug discovery? Curr Opin Microbiol 2003;6:427–30.
[2] Spellberg B, Powers JH, Brass EP, Miller LG, Edwards JE. Trends in antimicrobial drug development: implications for the future. Clin Infect Dis 2004;38:1279–86.
[3] Overbye KM, Barrett JF. Antibiotics: where did we go wrong? Drug Discov Today 2005;10:45–52.
[4] Norrby SR, Nord CE, Finch R. Lack of development of new antimicrobial drugs: a potential serious threat to public health. Lancet Infect Dis 2005;5:115–9.
[5] Infectious Diseases Society of America. Bad Bugs, No Drugs. As antibiotic discovery stagnates—A public health crisis brews; 2004. 35 p.
[6] Moellering RC. Linezolid: the first oxazolidinone antimicrobial. Ann Intern Med 2003;138:135–42.
[7] LaPlante KL, Rybak MJ. Daptomycin—a novel antibiotic against Gram-positive pathogens. Expert Opin Pharmacother 2004;5:2321–31.
[8] Walsh C. Where will new antibiotics come from? Nat Rev Microbiol 2003;1:65–70.
[9] Sakoulas G, Moise-Broder PA, Schentag J, Forrest A, Moellering RC, Eliopoulos GM. Relationship of MIC and bactericidal activity to efficacy of vancomycin for treatment of methicillin-resistant *Staphylococcus aureus* bacteremia. J Clin Microbiol 2004;42:2398–402.
[10] Centers for Disease Control and Prevention. *Staphylococcus aureus* resistant to vancomycin-United States, 2002. Morb Mortal Wkly Rep 2002;51:565–67.
[11] Gonzales RD, Schreckenberger PC, Graham MB, Kelkar S, DenBesten K, Quinn JP. Infections due to vancomycin-resistant *Enterococcus faecium* resistant to linezolid. Lancet Infect Dis 2001;357:1179.
[12] Tsiodras S, Gold HS, Sakoulas G, Eliopoulos GM, Wennersten C, Venkataraman L, et al. Linezolid resistance in a clinical isolate of *Staphylococcus aureus*. Lancet Infect Dis 2001;358:207–8.
[13] Mangili A, Bica I, Snydman DR, Hamer DH. Daptomycin-resistant, methicillin-resistant *Staphylococcus aureus bacteremia*. Clin Infect Dis 2005;40:1058–60.
[14] Fleischmann RD, Adams MD, White O, Clayton RA, Kirkness EF, Kerlavage AR, et al. Whole-genome random sequencing and assembly of *Haemophilus influenzae* Rd. Science 1995;269:496–512.
[15] Fraser CM, Gocayne JD, White O, Adams MD, Clayton RA, Fleischmann RD, et al. The minimal gene complement of *Mycoplasma genitalium*. Science 1995;270:397–403.
[16] Koonin EV. How many genes can make a cell: the minimal-gene-set concept. Annu Rev Genomics Hum Genet 2000;1:99–116.
[17] Gil R, Silva FJ, Pereto J, Moya A. Determination of the core of a minimal bacterial gene set. Microbiol Mol Biol Rev 2004;68:518–37.
[18] Alm RA, Ling LS, Moir DT, King BL, Brown ED, Doig PC, et al. Genomic-sequence comparison of two unrelated isolates of the human gastric pathogen *Helicobacter pylori*. Nature 1999;397:176–80.
[19] The National Center for Biotechnology Information's Entrez Genome Project web page, http://www.ncbi.nlm.nih.gov/genomes/lproks.cgi.
[20] Cassat JE, Dunman PM, McAleese F, Murphy E, Projan SJ, Smeltzer MS. Comparative genomics of *Staphylococcus aureus* musculoskeletal isolates. J Bacteriol 2005;187:576–92.
[21] Lindsay JA, Holden MTG. *Staphylococcus aureus:* superbug, supergenome? Trends Microbiol 2004;12:378–85.

[22] Venter JC, Adams MD, Myers EW, Li PW, Mural RJ, Sutton GG, et al. The Sequence of the human genome. Science 2001;291:1304–51.
[23] Lander ES, Linton LM, Birren B, Nusbaum C, Zody MC, Baldwin J, et al. Initial sequencing and analysis of the human genome. Nature 2001;409:860–921.
[24] Bansal AK. Bioinformatics in microbial biotechnology—a mini review. Microb Cell Factories 2005;4:19.
[25] Alm EJ, Huang KH, Price MN, Koche RP, Keller K, Dubchak IL, et al. The MicrobesOnline Web site for comparative genomics. Genome Res 2005;15:1015–22 [http://www.microbesonline.org].
[26] The Institute for Genomic Research's Comprehensive Microbial Resource web page, www.tigr.org.
[27] Ball P. Epidemiology and treatment of chronic bronchitis and its exacerbations. Chest 1995;108:43–52.
[28] Wong SM, Mekalanos JJ. Genetic footprinting with mariner-based transposition in *Pseudomonas aeruginosa*. Proc Natl Acad Sci USA 2000;97:10191–6.
[29] Hare RS, Walker SS, Dorman TE, Greene JR, Guzman LM, Kenney TJ, et al. Genetic footprinting in bacteria. J Bacteriol 2001;183:1694–706.
[30] Akerley BJ, Rubin EJ, Novick VL, Amaya K, Judson N, Mekalanos JJ. A genome-scale analysis for identification of genes required for growth or survival of *Haemophilus influenzae*. Proc Natl Acad Sci USA 2002;99:966–71.
[31] Ji Y, Zhang B, Van Horn SF, Warren P, Woodnut G, Burnham MKR, et al. Identification of critical Staphylococcal genes using conditional phenotypes generated by antisense RNA. Science 2001;293:2266–9.
[32] Forsyth RA, Haselbeck RJ, Ohlsen KL, Yamamoto RT, Xu H, Trawick JD, et al. A genome-wide strategy for the identification of essential genes in *Staphylococcus aureus*. Mol Microbiol 2002;43:1387–400.
[33] Thanassi JA, Hartman-Neumann SL, Dougherty TJ, Dougherty BA, Pucci MJ. Identification of 113 conserved essential genes using a high-throughput gene disruption system in *Streptococcus pneumoniae*. Nucleic Acids Res 2002;30:3152–62.
[34] Song JH, Ko KS, Lee JY, Baek JY, Oh WS, Yoon HS, et al. Identification of essential genes in *Streptococcus pneumoniae* by allelic replacement mutagenesis. Mol Cells 2005;19:365–74.
[35] Hutchison CA, Peterson SN, Gill SR, Cline RT, White O, Fraser CM, et al. Global transposon mutagenesis and a minimal mycoplasma genome. Science 1999;286:2165–9.
[36] Kobayashi K, Ehrlich SD, Albertini A, Amati G, Andersen KK, Arnaud M, et al. Essential *Bacillus subtillis* genes. Proc Natl Acad Sci USA 2003;100:4678–83.
[37] Link A, Phillips D, Church G. Methods for generating precise deletions and insertions in the genome of wild-type *Escherichia coli*: application to open reading frame characterization. J Bacteriol 1997;179:6228–37.
[38] Xia M, Lunsford RD, McDevitt D, Lordanescu S. Rapid method for the identification of essential genes in *Staphylococcus aureus*. Plasmid 1999;42:144–9.
[39] Saeed-Koithe A, Yang W, Mills SD. Development of an essential gene knockout and complementation system for *Haemophilus influenzae* using riboflavin synthase (*ribC*) as a model. Appl Environ Microbiol 2004;70:4136–43.
[40] Dervyn E, Suski C, Daniel R, Bruand C, Chapuis J, Errington J, et al. Two essential DNA polymerases at the bacterial replication fork. Science 2001;294:1716–9.
[41] Chan PF, O'Dwyer KM, Palmer LM, Ambrad JD, Ingraham KA, So C, et al. Characterization of a novel fucose regulated promoter (PfcsK) suitable for gene essentiality and antibacterial mode-of-action studies in *Streptococcus pneumoniae*. J Bacteriol 2003;185:2051–8.
[42] Miesel L, Greene J, Black TA. Genetic strategies for antibacterial drug discovery. Nat Rev Genet 2003;4:442–56.
[43] Paulsen IT, Chen J, Nelson KE, Saier Jr MH.. Comparative genomics of microbial drug efflux systems. J Mol Microbiol Biotechnol 2001;3:145–50.
[44] Clements JM, Coignard F, Johnson I, Chandler S, Palan S, Waller A, et al. Antibacterial actives and characterization of novel inhibitors of LpxC. Antimicrob Agents Chemother 2002;46:1793–9.
[45] DeVito JA, Mills JA, Liu VG, Agarwal A, Sizemore CF, Yao Z, et al. An array of target-specific screening strains for antibacterial discovery. Nat Biotechnol 2002;20:478–83.
[46] Fischer HP, Brunner NA, Wieland B, Paquette J, Macko L, Ziegelbauer K, et al. Identification of antibiotic stress-inducible promoters: a systematic approach to novel pathway-specific reporter assays for antibacterial drug discovery. Genome Res 2004;14:90–8.
[47] Tsui WH, Yim G, Wang HH, McClure JE, Surette MG, Davies J. Dual effects of MLS antibiotics: transcriptional modulation and interactions on the ribosome. Chem Biol 2004;11:1307–16.
[48] Mils SD, Yang W, MacCormack K. Molecular characterization of benzimidazole resistance in *Helicobacter pylori*. Antimicrob Agents Chemother 2004;48:2524–30.
[49] Lerner CG, Kakavas SJ, Wagner C, Chang RT, Merta PJ, et al. Novel approach to mapping of resistance mutations in whole genomes by using restriction enzyme modulation of transformation efficiency. Antimicrob Agents Chemother 2005;49:2767–77.
[50] Freiberg C, Fischer HP, Brunner NA. Discovering the mechanism of action of novel antibacterial agents through transcriptional profiling of conditional mutants. Antimicrob Agents Chemother 2005;49:749–59.
[51] Hutter B, Schaab C, Albrecht S, Borgmann M, Brunner NA, Freiberg C, et al. Prediction of mechanisms of action of antibacterial compounds by gene expression profiling. Antimicrob Agents Chemother 2004;48:2838–44.
[52] Liu B, Li S, Hu J. Technological advances in high-throughout screening. Am J Pharmacogenomics 2004;4:263–76.
[53] Gribbon P, Sewing A. High-throughput drug discovery: what can we expect from HTS? Drug Discov Today 2005;10:18–22.
[54] Payne DJ. The potential of bacterial fatty acid biosynthetic enzymes as a source of novel antibacterial agents. Drug News Perspect 2004;17:187–94.
[55] Me Z, Perretta C, Lu J, Su Y, Margosiak S, Gajiwala KS, et al. Structure-based design, synthesis, and study of potent inhibitors of beta-ketoacyl-acyl carrier protein synthase III as potential antimicrobial agents. J Med Chem 2005;48:1596–609.
[56] Hackbarth CJ, Chen DZ, Lewis JG, Clark K, Mangold JB, Cramer JA, et al. N-alkyl urea hydroxamic acids as a new class of peptide deformylase inhibitors with antibacterial activity. Antimicrob Agents Chemother 2002;46:2752–64.
[57] Chen D, Hackbarth C, Ni ZJ, Wu C, Wang W, Jain R, et al. Peptide deformylase inhibitors as antibacterial agents: identification of VRC3375, a proline-3-alkylsuccinyl hydroxamate derivative, by using an integrated combinatorial and medicinal chemistry approach. Antimicrob Agents Chemother 2004;48:250–61.
[58] Jarvest RL, Berge JM, Berry V, Boyd HF, Brown MJ, Elder JS, et al. Nanamolar inhibitors of *Staphylococcus aureus* methionyl tRNA synthetase with potent antibacterial activity against Gram-positive pathogens. J Med Chem 2002;45:1959–62.
[59] Herrgard MJ, Covert MW, Palsson BØ. Reconstruction of microbial transcriptional regulatory networks. Curr Opin Biotechnol 2004;15:70–7.

[60] Covert MW, Knight EM, Reed JL, Herrgård MJ, Palsson BØ. Integrating high-throughput and computational data elucidates bacterial networks. Nature 2004;429:92–6.
[61] Schilling CH, Covert MW, Famili I, Church GM, Edwards JS, Palsson BØ. Genome-scale metabolic model of *Helicobacter pylori* 26695. J Bacteriol 2002;184:4582–93.
[62] Fitzgerald JR, Musser JM. Evolutionary genomics of pathogenic bacteria. Trends Microbiol 2001;9:547–53.
[63] Moran NA. Microbial minimalism: genome reduction in bacterial pathogens. Cell 2002;108:583–6.
[64] Schmid MB. Seeing is believing: the impact of structural genomics on antimicrobial drug discovery. Nat Rev Microbiol 2004;2:739–46.
[65] Ziebuhr W, Xiao K, Coulibaly B, Schwarz R, Dandekar T. Pharmacogenomic strategies against resistance development in microbial infections. Pharmacogenomics 2004;5:361–79.
[66] Phillips KA, Van Bebber SL. Measuring the value of pharmacogenomics. Nat Rev Drug Discov 2005;4:500–9.
[67] Gant TW, Zhang SD. In pursuit of effective toxicogenomics. Mutat Res 2005;575:4–16.