DRUG DISCOVERY TODAY TARGETS

Using microarray gene signatures to elucidate mechanisms of antibiotic action and resistance

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Microarray analyses reveal global changes in gene expression in response to environmental changes and, thus, are well suited to providing a detailed picture of bacterial responses to antibiotic treatment. These responses are represented by patterns of gene expression, termed expression signatures, which provide insight into the mechanism of action of antibiotics as well as the general physiological responses of bacteria to antibiotic-related stresses. The complexity of such signatures is challenging the notion that antibiotics act on single targets and this is consistent with the concept that there are multiple targets coupled with common stress responses. A more detailed knowledge of how known antibiotics act should reveal new strategies for antimicrobial drug discovery.

Antimicrobial drug discovery and screening approaches

Since Alexander Fleming's discovery of the antimicrobial activity of penicillin [1], the field of antimicrobial drug discovery has been largely dominated by whole-cell screening assays, wherein new antimicrobial compounds are chosen for their ability to inhibit the growth of actively multiplying bacteria. Although the mechanism of action of such compounds is not always clear, this approach was successful in the early days of antibiotic development. Whereas this approach still holds potential for the screening of large synthetic chemical libraries with novel chemistries or naturally occurring antimicrobials, including peptides, no major novel leads have resulted in the past 40 years [2,3].

To combat the emergence of bacterial resistance, researchers have taken to modifying the chemical structure of existing antibiotics to yield derivatives that are more potent, have broader spectrum or are more effective *in vivo* (e.g. better oral bioavailability, longer half-life). Although such modifications have resulted in new candidates for clinical development, albeit with seemingly decreased frequency [2], these are only short-term solutions to the fundamental problem of bacterial resistance because resistance to the parent molecule foreshadows resistance development in the derivative, in that the same basic resistance mechanisms can give rise to cross resistance in both the parent and derivative. Moreover, our limited understanding of the target or the mechanism of action of the parent compound often hinders rational improvement of antibiotic structures.

In the past decade, antimicrobial drug discovery research has incorporated a complementary strategy, led by the identification of prospective novel targets important for bacterial growth or survival. An improved understanding in bacterial biology and metabolism and the sequencing of many genes involved in these processes [4] has facilitated the identification of novel targets. Screening systems designed specifically to target individual proteins known to be essential for cell survival are then used to identify inhibitors in a high-throughput target-specific fashion. Such

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target-based screening approaches have some advantages over random screening methods, particularly with respect to their ability to facilitate lead compound optimization, as the target is already understood.

The popularity of such approaches has grown tremendously with the advent of bacterial genome sequencing. The initial sequencing of the *Haemophilus influenzae* genome in 1995 [5], followed in rapid succession by the elucidation of the genomic sequences of more than 200 organisms, including many medically important pathogens, has provided fuel to the notion that novel antibacterial targets are abundant. Armed with a set of criteria that define an ideal antibiotic target, namely conservation across pathogens, target absence in the host, essentiality and accessible location in the microbe, a multitude of tools has been developed to mine this wealth of bacterial genomic information. For example, advanced sequence homology programs, such as *genomic* BLAST (NCBI, www.ncbi.nlm.nih.gov/sutils/genom_table.cgi), have facilitated the identification of genes ubiquitously present across a range of bacterial genomes. Similarly, genomic knockout libraries are defining core sets of genes present in all bacterial genomes that encode proteins essential to bacterial growth and survival; these sets range in number from 50 to 300, depending on the number of genomes compared [6–9]. Such approaches have led to the discovery of inhibitors of targets essential for microbial survival, such as polypeptide deformylase [10] or the fatty-acid biosynthesis pathway [11].

Although bacterial genomics and its associated technologies were believed to be the launching pad for a whole new era in antimicrobial drug discovery [4], there are still no new antimicrobial agents in late clinical development that have originated solely from genomics-based approaches [4,12]. Attempts by several pharmaceutical companies to utilize such strategies in antimicrobial drug discovery have been seemingly without a major return on investment, although a few antimicrobials have recently entered into early clinical development [12]. This lack of success in an area of research with so much potential, leads one to speculate that there might be few new practical antibacterial targets and modes of action left to be discovered [4].

Complex modes of action

A perusal of medical and microbiology textbooks leads one to believe that all antibiotics work by simple mechanisms, involving single targets. This, however, does not appear to be correct, at least for the bactericidal antibiotics. For example, β-lactams, the class of antibiotics utilized most often in clinical setting, are known to have several molecular targets [13]. β-lactams inhibit the activity of penicillin-binding proteins (PBPs), a group of enzymes important for cell-wall synthesis, and activate murein hydrolases, which are active in bacterial cell-wall degradation. Although the antimicrobial activity of β-lactams, at their minimal inhibitory concentration [14], is often attributed to interaction with a predominant PBP, β-lactams can interact with multiple PBPs. Indeed, the majority of β-lactams bind to several PBPs with similar affinities [13] and it has been suggested that inhibition of more than one PBP could account for the action of these antibiotics [15]. Aminoglycoside antimicrobials have exceptionally complicated modes of action too, which can be best explained by multiple targets [16,17]. Likewise, cationic antimicrobial peptides can act on the permeability barrier of bacterial membranes, cell division or macromolecular synthesis and it has been proposed that peptides affect multiple anionic targets [18,19]. Even antibiotics such as tetracycline and chloramphenicol are known to have secondary effects on bacterial membrane permeability at concentrations above the minimum inhibitory concentration (supra-MIC) [20]. Thus, it appears unlikely that most antibiotics have single, easily definable targets. Rather, such complicated mechanisms of action are indicative of multiple molecular targets contributing to bacterial inhibition. These could be primary targets, leading directly to inhibition of bacterial growth, or secondary targets contributing to the overall inhibitory effect.

Despite their limited impact on antimicrobial discovery to date, genomic methods have shed light on the complexities of antibiotic action, and in so doing have highlighted possible reasons for the void in antimicrobial drug discovery. The value of genomics was illustrated for yeast, for which target validation studies by Marton *et al.* [21] were based on the assumption that some inhibitors act on single targets. Using whole genome yeast microarrays, gene-expression profiles were determined following addition of the inhibitor FK506 to yeast cultures. At low inhibitor concentrations, expression patterns were shown to correlate with the deletion of the gene encoding calcineurin, the FK506-specific target. However, when higher concentrations of FK506 were used, changes in the expression of genes outside the target pathway were also observed, indicating that FK506 had additional secondary targets [21]. Intriguingly, some recent reviews on transcriptional profiling in antibiotic research have advocated the use of sub-inhibitory doses of antibiotics and the analysis of early time points following antimicrobial exposure [22,23] so to rule out complicating components (e.g. inhibition of secondary targets and downstream effects) from an inhibitor's transcriptional profile. Whereas the use of sub-inhibitory concentrations might simplify the experimental output, such concentrations might restrict the discovery of secondary and downstream effects that contribute to the mechanism of action. Thus, it seems prudent to examine simultaneously the transcriptional profiles of inhibitors administered at different physiologically relevant concentrations, so as to learn how 'off-target' effects [21] contribute to the activity of the inhibitor [9]. Furthermore, functional genomic studies have clearly indicated that different compounds acting against the same target do not always have the same

TABLE 1

Groups of gene-expression responses to antibiotic treatment

Gene-expression response group	Group characteristics and examples
1. Direct effects	Characteristic signatures of primary target inhibition, complicated by secondary effects (e.g. antibiotics targeting DNA replication machinery cause DNA damage and elicit SOS DNA-repair response; antibiotics targeting RNA synthesis inhibit transcription and elicit changes in tRNAs and nucleotides, for example).
2. Indirect effects	Triggered when primary target is inhibited, as organism attempts to compensate for changes in its environment (e.g. general stress responses, metabolic changes and resistance mechanisms).
3. Secondary effects	Downstream effects of target inhibition that have no particular role in antibiotic action and thus do not impact on the fate of antibiotic-treated bacteria.
4. Bystander effects	Changes in organism- or antibiotic-specific genes, or in generally unrelated genes.

mechanism of action. For example, comparison of the global gene-expression patterns of two inhibitors of cyclin-dependent kinase 2 in yeast revealed distinct differences in the expression profiles, despite similar *in vitro* activities exhibited by the two inhibitors [9,24].

Recent work has begun to improve upon our understanding of antimicrobials [22]. Genomics, in particular DNA microarray technology, has been prominent in this work.

Expression signatures of bacteria interacting with antimicrobials

Whole-genome expression profiling, facilitated by the development of DNA microarrays [25,26], provides a comprehensive portrait of the bacterial response to any given condition because it allows simultaneous analysis of the expression of all genes in an organism. Microarrays are cDNA- or oligonucleotide-based platforms containing probes to every open reading frame in a given genome. Labeled mRNA samples from an organism grown under a given condition, or cDNAs made therefrom, are hybridized to these arrays. Microarray studies, therefore, provide a snapshot of the genome-wide response of an organism to its environment. The resulting global gene-expression profile, also called the bacterial transcriptome, is, thus, being used to elucidate the bacterial responses to antibiotic stress and to better define the mechanism of action of antimicrobial compounds.

To explain the magnitude and complexity of expression changes elicited by any given antibiotic at a single exposure time point, it is necessary to broadly classify these responses. In principle, affected genes can be separated into four response groupings, based on their relationship to target inhibition (Table 1).

Group 1 responses encompass expression changes in genes that are altered as a direct consequence of target inhibition by the antibiotic. For example, an antibiotic that targets DNA replication is expected to cause DNA damage as a result of less effective replication and a potentially increased error rate. As a result, these antibiotics are likely to elicit an SOS DNA-repair response, as has been demonstrated for fluoroquinolones and coumarins [27–29]. Because antibiotic activity is directed to a protein rather than an mRNA transcript, expression analysis of early time points post-inhibition are not likely to include expression changes in the target itself. However, altered expression of the target gene or of a gene encoding a functionally related protein will eventually occur, as the organism attempts to compensate for the loss of the target protein. Each class of antibiotic induces alterations in the expression of a distinct set of genes reflective of the inhibition of the primary targets for that class. This set of genes, also termed a 'gene signature', can be used to predict whether another antibiotic interacts with the same target. These 'direct-effect' gene signatures might also encompass antibiotic action on secondary targets, which might or might not be class specific, although the majority of antimicrobials within a given class would conceivably have similar secondary targets.

Group 2 responses include expression changes in genes indirectly affected by inhibition of the primary target (Table 1). This group encompasses genes mediating or reflective of general stress responses (e.g. stringent response, heat-shock response) [28]. Also included in this group are genes that represent the attempt of the bacterium to compensate for or bypass antibiotic-induced alterations to its homeostatic environment, such as genes mediating metabolic changes or genes encoding inducible efflux pumps and antibiotic-modifying enzymes that facilitate resistance. Such gene patterns will not be specific to a given antibiotic class because of the overlap expected in the bacterial responses to different environmental stresses.

Group 3 gene-expression responses relate to the downstream (secondary) consequences of target inhibition (Table 1). For example, if the response of a bacterium to antibiotic inhibition of a target protein were to alter the expression of a given regulatory protein, the expression of any co-regulated genes would consequently change. Many of these downstream genes would have no relationship to the action of the antibiotic and would be unlikely to impact on the fate of antibiotic-treated cells.

Group 4 gene-expression responses can be characterized as 'bystander effects' (Table 1) and encompass those responses that are organism- or strain-specific (e.g. virulence genes or other genes specific to a given bacterium), or are caused only by a particular antibiotic in a given class. An

example might be genes responding specifically to second-generation fluoroquinolones but not fourth-generation fluoroquinolones, even though both are considered to have the same target.

If we assume that many or all antibiotics inhibit more than one cellular target, the complexity of gene-expression responses observed is not surprising. Below, we explore how the currently available antimicrobial expression signatures in bacteria illustrate these concepts.

Signatures characteristic of direct target inhibition

Global transcription profiles of bacteria following antimicrobial exposure reveal that the bacterial response to a particular antibiotic often reflects the 'direct' response of the cell to inhibition of a particular physiological function as targeted by the antimicrobial (Table 1, Group 1). As an example, DNA-gyrase inhibitors of the quinolone class elicit the bacterial SOS DNA-repair response as a consequence of the DNA damage caused by the interaction of these agents with DNA gyrase [27–29]. Similarly, exposure of bacteria to different translation inhibitors, such as mupirocin or puromycin, results in transcriptional changes in genes corresponding to the step targeted in protein synthesis [30,31]. Likewise, the expression profile of *Mycobacterium tuberculosis* treated with antimicrobial agents that inhibit various enzymes in mycolic acid biosynthesis is characteristic of such target inhibition and includes genes encoding type II fatty-acid synthase enzymes [32,33]. Thus, bacterial gene-expression signatures can be used to validate the antimicrobial target and its proposed mechanism of action based on expression changes in genes directly affected by target inhibition.

The expression changes in genes directly affected by target inhibition are often complicated by inhibition of secondary targets. These secondary effects convey a complex mode of action, reflecting mixed target activity, with one classical mechanism dominating but others contributing to the inhibitory action of the antibiotic at the MIC. Mixed targets and complex mechanisms of action are already well known for some antimicrobials, including penicillin [15,34]. Similarly, analysis of the expression signatures elicited in response to acivicin [35] and 4,5-dihydroxy-2-cyclopenten-1-one [36], for example, indicates a mixed mechanism of action. Even the expression signatures for well-characterized antimicrobials like fluoroquinolones include numerous changes in genes outside of the signature response, including a considerable number of genes of unknown function [27,29].

Whereas these additional responses have the potential to provide insight into the function of these unknown genes and, thus, represent useful starting points for the identification of new targets for antimicrobial drug discovery [23], they also suggest off-target effects of antibiotics. Moreover, antimicrobials with unrelated mechanisms of action have been shown to produce overlapping expression signatures. *Escherichia coli* cultures treated with bactericidal concentrations of the β-lactam ampicillin and the fluoroquinolone ofloxacin, for example, elicited overlapping changes in the expression of 161 genes, mostly of unknown function [37]. Therefore, the induction of these genes cannot be associated with a single mechanism of action or target. Instead, the induction of shared genes by unrelated antimicrobials is likely to be indicative of common cellular responses to antimicrobial stresses and overlapping secondary targets.

Comparison of expression signatures induced by novel antimicrobial agents with those from compounds with known modes of action can be used to identify or validate the mechanism of action of novel inhibitors [22,27,33,38]. Databases or compendia of expression profiles in bacteria are beginning to be generated [27,39,40] and have been used to assign mechanisms of action to compounds such as a novel phenyl-thiazolylurea derivative [39], among others [27].

Signatures beyond direct target inhibition

Bacterial responses, and thus bacterial expression profiles following antibiotic treatment, typically contain substantially more genes than those directly targeted by the antibiotic. Among these, there are numerous genes 'indirectly' affected by the antibiotic (Table 1, Group 2) but nonetheless relevant to the response of the organism to the antibiotic-induced stress (i.e. genes involved in general stress responses). For example, the heat-shock response which helps the cell survive the consequence of high temperatures, as well other unrelated stress conditions, is often induced in response to various antibiotics. The aminoglycoside kanamycin elicits a very strong heat-shock response in *E. coli* [29]. Similarly, the cell-wall specific agents bacitracin, D-cycloserine and oxacillin, all cause stress responses in *Staphylococcus aureus* [41], as does puromycin in *Streptococcus pneumoniae* [30].

Secondary and bystander effects also impact on expression signatures (Table 1, Groups 3 and 4). Because there is great variety in the lifestyle, genomes and cellular structures of bacteria, it is no surprise that gene-expression signatures for antibiotics vary from bacterium to bacterium. This is especially true for the major subdivisions of bacterial organisms (i.e. Gram-negative versus Gram-positive bacteria). Utaida *et al.* [41], for example, observed a cell-wall stress regulon in Gram-positives that was induced by treatment with different antibiotics acting at distinct stages of cell-wall synthesis. By contrast, cell-wall specific agents, like ampicillin, evaluated in the Gram-negative bacterium *E. coli*, appeared to induce a different response pattern [29].

Towards expression signature libraries

With these signature groupings in mind, investigators have started to develop compendia of gene-expression signatures for a range of antimicrobials [27,39,40]. Although in some instances modes of action for novel compounds

can be predicted on the basis of this compendium, difficulties in prediction arise if the database does not contain expression profiles for a broad enough complement of antibiotics.

The *Bacillus subtilis* database compiled by Hutter *et al.* [27], for example, is extensive and comprises expression signatures induced by 37 'well-characterized' agents, representing six distinct classes of antibiotics, allowing prediction of the mechanism of action for a series of test compounds. Predictive patterns were identified bioinformatically using support vector machine methodology, and the data are summarized in Table 2. Twenty-seven of the antimicrobials could be fitted into only four of the original six major mechanisms of action. The remaining ten antimicrobials elicited expression patterns that were either paradoxical to their known or assumed mechanisms of action, or that were unclassifiable (underlined in Table 2). A so-called topoisomerase pattern, defined by a DNA-damage–SOS response, was observed for the quinolones and coumarins. Likewise, a protein-synthesis inhibition signature was evident for the known protein synthesis inhibitors. A cell-wall specific pattern was observed for the cell-wall synthesis inhibitors, but surprisingly it was also observed for dapsone and sulfacetamide, agents that target folate biosynthesis. Conversely, the classic cell-wall synthesis inhibitor amoxicillin appeared to induce an expression pattern similar to that expected of a folate biosynthesis inhibitor. The authors attributed this misclassification to the small training sets used to define the predictors for folate acid biosynthesis agents. A membrane-specific pattern was also observed for membrane-active compounds. However, cerulenin, a fatty-acid biosynthesis inhibitor, clarithromycin, a protein biosynthesis inhibitor, and the DNA gyrase inhibitor coumermycin A1, also exhibited expression signatures characteristic of these membrane-active agents. Further classification also showed that the test compounds hydrogen peroxide, doxorubicin and azaserine, but not ethidium bromide, clustered with the quinolones, based on comparison of their expression signatures. Whereas these compounds are all known to cause DNA damage, their classification with type II topoisomerase inhibitors indicates that the predictors used by Hutter *et al.* [27] were not specific for topoisomerase inhibitors, such as quinolones, but encompassed in a broader sense stress caused by DNA damage (although the predictor clearly did not assign all types of DNA stress to this class, as evidenced by rejection of the DNA intercalating agent, ethidium bromide). Taken together, the findings from this detailed study serve to illustrate the merits and limitations of mechanism-of-action predictions based on genomic expression signatures.

Until a larger reference compendium of expression signatures is generated and made freely available to the research community, caution must be taken when assigning mechanisms of action to compounds based solely on gene-expression signatures because the comparison is limited to mechanisms employed by the antibiotics represented in the available databases. In our view, it makes little sense that each group of researchers interested in such studies develops independent databases, and we encourage investigators in this area to adopt the MIAME (minimum information about a microarray experiment) protocols, which are a set of uniform protocols for reporting and banking microarray information [42], and to place non-proprietary information in publicly accessible databases (e.g. ArrayExpress at EBI or GEO at NCBI) [43,44]. The ability to characterize the mechanism of action of novel antimicrobials will clearly be limited until the reference compendium is expanded. Efforts to increase the reference compendium of expression signatures with transcriptome profiles from bacterial mutants showing low-level expression of potential target proteins have been initiated [39,45]. These mutants mimic inhibition of the target protein by small molecule inhibitors and provide additional expression signatures not observed with the current selection of antimicrobials because they target only a limited number of proteins. Freiberg *et al.* [39] used this mutant-based compendium of expression signatures to propose a novel mechanism of action for moiramide B, based on the inhibition of the acetyl coenzyme A carboxylase of the fatty-acid biosynthesis system. Similarly, RNA-interference techniques could be used to construct a set of conditional mutants and expand the reference compendium [45,46].

Potential use of expression signatures in drug discovery

Detailed expression signatures contain an immense amount of information and arguably might open up avenues for antimicrobial drug discovery. For example, the comparison of gene-expression signatures will continue to be useful in predicting mechanisms of action for novel compounds. Such data mining will also be useful in identifying potential novel antimicrobial targets among the plethora of uncharacterized genes present in expression signatures. For a given class of antibiotics, recurrence of an uncharacterized gene within the 'direct-effect' group of expression signatures might be indicative of the gene's importance in the antimicrobial activity of the antibiotic. Gene-expression signatures could help in predicting potential resistance mechanisms as well. It is still unknown whether two drugs that elicit the same expression signature might also exhibit the same resistance profile. Attempts by the bacterium to resist the antimicrobial assault would almost certainly be reflected in the expression signature, with stress responses and SOS responses alongside specific resistance responses presumably representing such survival adaptations. It would, therefore, be instructive to study the antibiotic-induced signatures of defined, isogenic-resistant and -susceptible strains to understand the physiological changes that are associated with resistance, and to assist in differentiating 'direct' consequences of

TABLE 2

Predicting mechanism of action from gene-expression signatures[a]

Mechanism of action	Antimicrobial[b]	Predicted mechanism of action[c]
Cell-wall synthesis inhibitors	Amoxicillin	Folate synthesis inhibitor
	Cefalexin	✓
	Cefotaxime	✓
	Cefoxitin	✓
	Cycloserine	✓
	Oxacillin	✓
	Penicillin G	✓
	Phosphomycin	✓
	Ristocetin	✓
	Vancomycin	✓
DNA topoisomerase inhibitors	Ciprofloxacin	✓
	Coumermycin A1	Membrane-active compound
	Moxifloxacin	✓
	Nalidixic acid	✓
	Norfloxacin	✓
	Novobiocin	✓
	Azaserine[d]	✓
	Doxorubicin[d]	✓
	Ethidium bromide[d]	Unclassified
	H_2O_2[d]	✓
Fatty-acid synthesis inhibitors	Cerulenin	Membrane-active compound
	Triclosan	Unclassified
Folate synthesis inhibitors	Dapsone	Cell-wall synthesis inhibitor
	Sulfacetamide	Cell-wall synthesis inhibitor
	Sulfamethizole	Unclassified
	Trimethoprim	Unclassified
Membrane-active compounds	Gramicidin A	✓
	Monensin	✓
	Nigericin	Unclassified
	Nitrofurantoin	✓
	Polymyxin B	✓
	Triton X-114	✓
Protein synthesis inhibitors	Chloramphenicol	✓
	Clarithromycin	Membrane-active compound
	Clindamycin	✓
	Erythromycin	✓
	Fusidic acid	✓
	Neomycin	✓
	Puromycin	✓
	Spectinomycin	✓
	Tetracycline	✓

[a]Data from Ref. [27].

[b]Antimicrobials that were either paradoxical to their known or assumed mechanisms of action, or that were unclassifiable are underlined.

[c]Key: ✓, correctly predicted.

[d]Test compound. Mechanism of action for test compounds was predicted from the expression signatures of known antibiotics.

target inhibition from 'indirect responses'. Cataloguing such expression signatures would allow for the identification of an expression profile associated with the generation of resistance, a profile which could then be avoided in the design of new antimicrobials. Conversely, expression signature comparisons could be used to screen for antimicrobials that have multiple targets, possibly leading to compounds with lower probability of resistance

development. (Note, this would not have to be done using microarray methods, but could be conducted by examining only a few genes representative of particular signatures in reporter strains [47,48].)

Not all cellular processes, however, are controlled at the level of gene expression; some processes, such as protein synthesis and modification, occur post-transcriptionally. Furthermore, isolation of stable bacterial mRNA remains technically challenging because of low RNA stability and the absence of polyadenylation tails. Thus, protein profiling provides a complementary approach to the use of DNA microarrays [9] and efforts are underway to generate reference databases of proteomes [40]. However, the throughput capacity of either transcriptome or proteome studies still limits the usefulness of these technologies in routine drug discovery. Nonetheless, tremendous progress in understanding bacterial responses to antimicrobial challenge has already been achieved, and expression signature profiling will continue to facilitate antibiotic drug discovery approaches in the future [22].

Conclusions

Genomics and its associated technologies are not only providing the tools to drive antimicrobial drug discovery as it applies to whole-cell based and target-screening approaches, but are providing new insights into antimicrobial mechanisms of action. Although such analyses have exposed our limited understanding of the mechanisms of action of even well-known antimicrobials, they also open up new avenues for antimicrobial drug discovery. Expression signatures gathered from an array of antibiotics and organisms help to validate targets for established and novel antimicrobials, and to predict the mechanism of action for uncharacterized compounds, by comparison with databases of expression profiles. Most importantly, these compendia of transcriptome profiles have unexpectedly revealed that antimicrobials and the responses of the organism to antimicrobial stress do not always reflect interaction at a single target, but are instead consistent with multiple targets, mixed mechanisms of action and common stress responses. Thus, global expression profiling of bacterial responses to antimicrobials is challenging our current concepts of antimicrobial–target interaction and mechanism of action, and is hopefully revealing new strategies and areas for antimicrobial drug discovery.

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References

1 Fleming, A. (1929) On the antibacterial action of cultures of a penicillum, with special reference to their use in the isolation of *B. influenzae*. *Br. J. Exp. Pathol.* 10, 226–236
2 Overbye, K.M. and Barrett, J.F. (2005) Antibiotics: where did we go wrong? *Drug Discov. Today* 10, 45–52
3 Bax, R.P. *et al.* (1998) Antibiotic resistance–what can we do? *Nat. Med.* 4, 545–546
4 Coates, A. *et al.* (2002) The future challenges facing the development of new antimicrobial drugs. *Nat. Rev. Drug Discov.* 1, 895–910
5 Fleischmann, R.D. *et al.* (1995) Whole-genome random sequencing and assembly of *Haemophilus influenzae* Rd. *Science* 269, 496–512
6 Charlebois, R.L. and Doolittle, W.F. (2004) Computing prokaryotic gene ubiquity: rescuing the core from extinction. *Genome Res.* 14, 2469–2477
7 Gil, R. *et al.* (2004) Determination of the core of a minimal bacterial gene set. *Microbiol. Mol. Biol. Rev.* 68, 518–537
8 Jacobs, M.A. *et al.* (2003) Comprehensive transposon mutant library of *Pseudomonas aeruginosa*. *Proc. Natl. Acad. Sci. U. S. A.* 100, 14339–14344
9 Rosamond, J. and Allsop, A. (2000) Harnessing the power of the genome in the search for new antibiotics. *Science* 287, 1973–1976
10 Apfel, C.M. *et al.* (2001) Peptide deformylase as an antibacterial drug target: target validation and resistance development. *Antimicrob. Agents Chemother.* 45, 1058–1064
11 Payne, D.J. *et al.* (2001) Bacterial fatty-acid biosynthesis: a genomics-driven target for antibacterial drug discovery. *Drug Discov. Today* 6, 537–544
12 Bush, K. *et al.* (2004) Taking inventory: antibacterial agents currently at or beyond phase 1. *Curr. Opin. Microbiol.* 7, 466–476
13 Pucci, M.J. *et al.* (1991) Comparison of cefepime, cefpirome, and cefaclidine binding affinities for penicillin-binding proteins in *Escherichia coli* K-12 and *Pseudomonas aeruginosa* SC8329. *Antimicrob. Agents Chemother.* 35, 2312–2317
14 Young, K.D. (2001) Approaching the physiological functions of penicillin-binding proteins in *Escherichia coli*. *Biochimie* 83, 99–102
15 Satta, G. *et al.* (1995) Target for bacteriostatic and bactericidal activities of beta-lactam antibiotics against *Escherichia coli* resides in different penicillin-binding proteins. *Antimicrob. Agents Chemother.* 39, 812–818
16 Hancock, R.E.W. (1981) Aminoglycoside uptake and mode of action-with special reference to streptomycin and gentamicin. II. Effects of aminoglycosides on cells. *J. Antimicrob. Chemother.* 8, 429–445
17 Hancock, R.E.W. (1981) Aminoglycoside uptake and mode of action–with special reference to streptomycin and gentamicin. I. Antagonists and mutants. *J. Antimicrob. Chemother.* 8, 249–276
18 Friedrich, C.L. *et al.* (2001) Structure and mechanism of action of an indolicidin peptide derivative with improved activity against gram-positive bacteria. *J. Biol. Chem.* 276, 24015–24022
19 Patrzykat, A. *et al.* (2002) Sublethal concentrations of pleurocidin-derived antimicrobial peptides inhibit macromolecular synthesis in *Escherichia coli*. *Antimicrob. Agents Chemother.* 46, 605–614
20 Hancock, R.E.W. (1984) Alterations in outer membrane permeability. *Annu. Rev. Microbiol.* 38, 237–264
21 Marton, M.J. *et al.* (1998) Drug target validation and identification of secondary drug target effects using DNA microarrays. *Nat. Med.* 4, 1293–1301
22 Freiberg, C. *et al.* (2004) The impact of transcriptome and proteome analyses on antibiotic drug discovery. *Curr. Opin. Microbiol.* 7, 451–459
23 Shaw, K.J. and Morrow, B.J. (2003) Transcriptional profiling and drug discovery. *Curr. Opin. Pharmacol.* 3, 508–512
24 Gray, N.S. *et al.* (1998) Exploiting chemical libraries, structure, and genomics in the search for kinase inhibitors. *Science* 281, 533–538
25 Lockhart, D.J. *et al.* (1996) Expression monitoring by hybridization to high-density oligonucleotide arrays. *Nat. Biotechnol.* 14, 1675–1680
26 Schena, M. *et al.* (1995) Quantitative monitoring of gene expression patterns with a complementary DNA microarray. *Science* 270, 467–470
27 Hutter, B. *et al.* (2004) Prediction of mechanisms of action of antibacterial compounds by gene expression profiling. *Antimicrob. Agents Chemother.* 48, 2838–2844
28 Gmuender, H. *et al.* (2001) Gene expression changes triggered by exposure of *Haemophilus influenzae* to novobiocin or ciprofloxacin: combined transcription and translation analysis. *Genome Res.* 11, 28–42

29 Shaw, K.J. *et al.* (2003) Comparison of the changes in global gene expression of *Escherichia coli* induced by four bactericidal agents. *J. Mol. Microbiol. Biotechnol.* 5, 105–122

30 Ng, W.L. *et al.* (2003) Transcriptional regulation and signature patterns revealed by microarray analyses of *Streptococcus pneumoniae* R6 challenged with sublethal concentrations of translation inhibitors. *J. Bacteriol.* 185, 359–370

31 Sabina, J. *et al.* (2003) Interfering with different steps of protein synthesis explored by transcriptional profiling of *Escherichia coli* K-12. *J. Bacteriol.* 185, 6158–6170

32 Wilson, M. *et al.* (1999) Exploring drug-induced alterations in gene expression in *Mycobacterium tuberculosis* by microarray hybridization. *Proc. Natl. Acad. Sci. U. S. A.* 96, 12833–12838

33 Betts, J.C. *et al.* (2003) Signature gene expression profiles discriminate between isoniazid-, thiolactomycin-, and triclosan-treated *Mycobacterium tuberculosis*. *Antimicrob. Agents Chemother.* 47, 2903–2913

34 Rice, K.C. *et al.* (2005) Acetic acid induces expression of the *Staphylococcus aureus cidABC* and *lrgAB* murein hydrolase regulator operons. *J. Bacteriol.* 187, 813–821

35 Smulski, D.R. *et al.* (2001) Combined, functional genomic-biochemical approach to intermediary metabolism: interaction of acivicin, a glutamine amidotransferase inhibitor, with *Escherichia coli* K-12. *J. Bacteriol.* 183, 3353–3364

36 Phadtare, S. *et al.* (2002) DNA microarray analysis of the expression profile of *Escherichia coli* in response to treatment with 4,5-dihydroxy-2-cyclopenten-1-one. *J. Bacteriol.* 184, 6725–6729

37 Kaldalu, N. *et al.* (2004) Killing by ampicillin and ofloxacin induces overlapping changes in *Escherichia coli* transcription profile. *Antimicrob. Agents Chemother.* 48, 890–896

38 Hughes, T.R. *et al.* (2000) Functional discovery via a compendium of expression profiles. *Cell* 102, 109–126

39 Freiberg, C. *et al.* (2005) Discovering the mechanism of action of novel antibacterial agents through transcriptional profiling of conditional mutants. *Antimicrob. Agents Chemother.* 49, 749–759

40 Bandow, J.E. *et al.* (2003) Proteomic approach to understanding antibiotic action. *Antimicrob. Agents Chemother.* 47, 948–955

41 Utaida, S. *et al.* (2003) Genome-wide transcriptional profiling of the response of *Staphylococcus aureus* to cell-wall-active antibiotics reveals a cell-wall-stress stimulon. *Microbiology* 149, 2719–2732

42 Brazma, A. *et al.* (2001) Minimum information about a microarray experiment (MIAME)-toward standards for microarray data. *Nat. Genet.* 29, 365–371

43 Brazma, A. *et al.* (2003) ArrayExpress–a public repository for microarray gene expression data at the EBI. *Nucleic Acids Res.* 31, 68–71

44 Edgar, R. *et al.* (2002) Gene expression omnibus: NCBI gene expression and hybridization array data repository. *Nucleic Acids Res.* 30, 207–210

45 Yin, D. *et al.* (2004) Identification of antimicrobial targets using a comprehensive genomic approach. *Pharmacogenomics* 5, 101–113

46 Ji, Y. *et al.* (2004) Validation of antibacterial mechanism of action using regulated antisense RNA expression in *Staphylococcus aureus*. *FEMS Microbiol. Lett.* 231, 177–184

47 Goh, E.B. *et al.* (2002) Transcriptional modulation of bacterial gene expression by subinhibitory concentrations of antibiotics. *Proc. Natl. Acad. Sci. U. S. A.* 99, 17025–17030

48 Hutter, B. *et al.* (2004) Panel of *Bacillus subtilis* reporter strains indicative of various modes of action. *Antimicrob. Agents Chemother.* 48, 2588–2594