Novel Targets of Antimicrobial Therapies

SARAH E. MADDOCKS

Department of Biomedical Sciences, Cardiff School of Health Sciences, Cardiff Metropolitan University, Western Avenue, Llandaff, Wales, CF5 2YB

ABSTRACT Antibiotics are undoubtedly a pillar of modern medicine; their discovery in 1929 revolutionized the fight against infectious disease, instigating a worldwide decline in infection-associated mortality. Throughout the 1930s, 1940s, and 1950s the golden age of antibiotic discovery was underway with numerous new classes of antibiotics identified and brought to market. By 1962 all of our currently known families of antibiotics had been discovered, and it was a widely held belief, that humanity had conquered infectious disease. Despite varying bacterial cellular targets, most antibiotics targeted exponentially multiplying bacteria by interfering with integral processes such as peptidoglycan synthesis or ribosomal activity. The very nature of this targeted approach has driven the emergence of antibiotic-resistant bacteria.

Methods of antibiotic identification relied solely on scientific observation, and while chemical analogues such as amoxicillin, derived from penicillin, continued to be developed, they retained the same mechanisms of action and hence the same bacterial targets. Moreover, there are finite modifications that can ultimately be made to “old” classes of antibiotics. Consequently, only two new classes of antibiotics have been discovered in the past 40 years, and both entered the market early in the new millennium. The advent of the genomics revolution offered a new hope for the discovery of novel antimicrobial targets. Genomic strategies were utilized to identify potential antibacterial targets, namely those that, if inhibited, resulted in the death of the bacterium. Such targets were to be present in pathogenic strains of bacteria and absent from the human host; they could include metabolic pathways, receptor ligands, and virulence traits, to name a few. Despite the abundance of targets identified using this strategy, no new antibiotics have reached the marketplace as a result of the genomics approach. However, new antimicrobials with novel targets continue to be identified and contribute to the ongoing struggle against antimicrobial resistance that threatens to return humankind to a situation comparable to the preantibiotic era.

This article will describe and discuss some of the novel targets for emerging antimicrobial treatments, highlighting pivotal research on which our ability to continue to successfully treat bacterial infection relies.

INTRODUCTION: TRADITIONAL TREATMENTS AND CLASSICAL TARGETS

During the golden age of antibiotic discovery, from the 1930s through the 1960s, methods of antibiotic identification relied solely on scientific observation, and while chemical analogues such as amoxicillin, derived from penicillin, continued to be developed, they retained the same mechanisms of action and hence the same bacterial targets. Moreover, there are finite modifications that can ultimately be made to “old” classes of antibiotics. Consequently, only two new classes of antibiotics have been discovered in the past 40 years, and both entered the market early in the new millennium. The advent of the genomics revolution offered a new hope for the discovery of novel antimicrobial targets. Genomic strategies were utilized to identify potential antibacterial targets, namely those that, if inhibited, resulted in the death of the bacterium. Such targets were to be present in pathogenic strains of bacteria and absent from the human host; they could include metabolic pathways, receptor ligands, and virulence traits, to name a few. Despite the abundance of targets identified using this strategy, no new antibiotics have reached the marketplace as a result of the genomics approach. However, new antimicrobials with novel targets continue to be identified and contribute to the ongoing struggle against antimicrobial resistance that threatens to return humankind to a situation comparable to the preantibiotic era.

This article will describe and discuss some of the novel targets for emerging antimicrobial treatments, highlighting pivotal research on which our ability to continue to successfully treat bacterial infection relies.

Received: 23 April 2015, Accepted: 17 December 2015, Published: 8 April 2016

Editors: Indira T. Kudva, National Animal Disease Center, Agricultural Research Service, U.S. Department of Agriculture, Ames, IA; and Bryan H. Bellaire, Department of Veterinary Microbiology and Preventive Medicine, College of Veterinary Medicine, Iowa State University, Ames, IA


Correspondence: Sarah E. Maddocks, smaddocks@cardiffmet.ac.uk

© 2016 American Society for Microbiology. All rights reserved.
COMBINATION APPROACHES TO TACKLE MULTIDRUG-RESISTANT BACTERIA

Combination therapies are widely used in medicine and have proved crucial for the treatment of infectious diseases, including, for example, Mycobacterium tuberculosis, which is treated using four simultaneously administered antibiotics. Monotherapies are increasingly inadequate, and several strategies are currently employed that combine either different classes of antibiotics or antibiotics with targeted adjuvants. Above all, the principal aim of this approach is to reduce the minimum inhibitory concentration or to resensitize resistant organisms. Often this involves inhibition of different targets within the same synthetic or metabolic pathways, inhibition of the same target within different pathways, or inhibition of unrelated targets within different pathways. One such example is the commercially available antibiotic combination co-amoxiclav, which utilizes a combination of amoxicillin, a beta-lactam antibiotic, with the beta-lactamase inhibitor clavulanic acid, which renders beta-lactamase-producing microorganisms susceptible to the action of the penicillin-derived antibiotic (1).

Antibiotics combined with adjuvants in this manner have increased efficacy, but the adjuvant itself is generally not bactericidal; this approach reduces the onset of antimicrobial resistance but does not affect a new cellular target per se. Two-component sensor–regulator proteins are ubiquitous among prokaryotes but, despite their high degree of conservation, are not essential for viability. As such, they have become attractive targets for adjuvants, especially due to the predominant role many of these systems have in antimicrobial resistance. Cell wall biosynthesis in Staphylococcus aureus is in part regulated by the VraSR system, which coordinates the expression of D-alanyl-D-lactate, a peptide that is incorporated into peptidoglycan (2). Additionally, VraSR also mediates resistance to beta-lactams and glycopeptide antibiotics; as such, expression of this system is induced by exposure to beta-lactams, glycopeptide, and bacitracin. Null-mutations of vraSR result in greatly enhanced susceptibility to antibiotics that disrupt cell wall biosynthesis, similarly if vraSR expression is inhibited resistance is also lessened (3, 4).

Natural and synthetic two-component inhibitors exist, and the RWJ-family and its derivatives are the best characterized. These inhibitors are hydrophobic tyramines which exhibit a broad spectrum of activity against Gram-positive microorganisms and are themselves inherently bactericidal (5). Analogues vary in their ability to inhibit bacterial growth, a characteristic that has been correlated with an ability to “jam” two-component systems. Mechanistically, such inhibitors appear to function by impairing auto-phosphorylation of sensor kinases, sometimes completely abolishing this function. Compounds are thought to disrupt the four-helix bundles required for dimerization, driving them apart to expose hydrophobic residues that result in misfolding or aggregation, with a subsequent loss of function. Synthetic two-component inhibitors are also known for Gram-negative microorganisms; for Pseudomonas aeruginosa, inhibition of the AlgR1R2 system results in reduced expression of alginate biosynthesis genes, but inhibitions of two-component sensor-regulators are not directly antimicrobial (6). It is supposed that inhibitors of this nature could be used in conjunction with antibiotics to treat infected cystic fibrosis patients, with the aim of rendering P. aeruginosa more susceptible to antimicrobial treatment by disruption of the secreted, protective alginate layer. However, despite the promise of two-component-system targeting compounds, stepwise training experiments have demonstrated that microorganisms readily evolve resistance to them (5).

ANTIBIOFILM AND ANTIADHESIVE STRATEGIES

Communities of bacteria growing as a biofilm are afforded protection from environmental stresses, including antimicrobial treatments, by virtue of the thick, exopolysaccharide layer that surrounds the biofilm and impedes the diffusion of antimicrobial compounds. Colonization of the host requires bacterial adhesion prior to proliferation, and it is understood that a vast majority of pathogens exist within the host, at the site of infection, as a biofilm which is often comprised of more than one species. Prophylactic measures that impede microbial adhesion have the capacity to limit microbial colonization and thus prevent infection; antiadhesive compounds, if appropriately targeted, also have the potential to disrupt already established biofilms, being highly beneficial for the treatment of chronic infections.

Cationic antimicrobial peptides are well known for their broad-spectrum lytic activity and low propensity to induce resistance. Aggregating within the bacterial cell-leaflet, these small peptides mediate widespread disruption of the bacterial cell, but not all antimicrobial peptides have the same efficacy, and some exhibit poor lytic properties. A subgroup of this type of cationic antimicrobial peptide has potent antiadhesive activity, inhibiting biofilm formation as well as disrupting established microbial communities (7). Studies of an arche-
typical nine-amino-acid, antibiotic film antimicrobial peptide (1037) identified a consensus sequence (FRIRVRV) subsequently found to be well conserved among those antimicrobial peptides that were antiadhesive (8). Transcriptomics revealed that this peptide targeted expression of several genes associated with the expression of flagella, resulting in impaired swimming and swarming motility as well as biofilm formation. Interestingly, a preserved characteristic of antimicrobial peptides with antiadhesive activity is poor bactericidal efficacy. Subsequently, approaches have been implemented to synthesize hybrid peptides containing the peptide motif associated with antiadhesive activity that have good bactericidal activity but minimal hemolytic properties.

More comprehensive studies of so-called antibiotic film antimicrobial peptides have confirmed that at least one peptide (1018) mediates biofilm impairment by targeting molecules involved in the bacterial cellular stress response (9). This peptide does not impair the growth of planktonic bacteria but inhibits biofilm growth as well as disrupting established biofilms comprised of either Gram-positive or Gram-negative microorganisms, including P. aeruginosa, Klebsiella pneumonia, Acinetobacter baumannii, and S. aureus. Specifically, this peptide was found to target (p)ppGpp, a vital signal required for biofilm formation, and it is believed to target intracellular (p)ppGpp for degradation (9). This means that peptide 1018 must traverse the cytoplasmic membrane and gain access to the cytoplasm. It is hypothesized that this peptide might also mediate expression of (p)ppGpp through the enzymes SpoT and RelA, which are fundamental to the stringent response.

High-throughput screening of natural (including fungal and plant extracts) and chemically synthesized small molecules has begun to lead the way in identifying small molecules that interfere with bacterial adhesion and biofilm growth. With P. aeruginosa used as a test organism, over 65,000 compounds have been screened to identify those that exclusively inhibit biofilm formation without impairing the growth of planktonic cells. Biofilm growth and detachment assays reported 30 such compounds that fell into this remit but did not shed light on possible cellular targets. Parallel screening of a different library comprising over 70,000 small molecules, identified N-(4-chloro-phenyl)-0-2-[5-[4-(pyrrolidine-1-sulfonyl)-phenyl]-1,3,4-oxadiazol-2-yl sulfanyl]-acetamide) (abbreviated to AL1) as a strongly antiadhesive compound that suppressed the assembly of type I pili in uropathogenic Escherichia coli (10). Bacteria exposed to this chemical were found to be devoid of type I pili, and subsequently pilus-dependent adhesion was abrogated.

AL1 specifically targeted polymerization steps during the subunit incorporation cycle of the chaperone-usher pathway, specifically interaction between FimC and FimH; given the highly conserved nature of this pathway, it is likely that the effects observed for uropathogenic E. coli would be seen for other microorganisms. Activity of this molecule is believed not to be constrained only to de novo synthesis, because addition of AL1 to uropathogenic E. coli immediately disrupts adhesion, suggesting that it also targets preformed pili. Despite these advances in the discovery of antiadhesive/antibiofilm small molecules, it should be noted that currently, no approved drugs exist that specifically target biofilms.

Efflux pump inhibitors are a class of molecules whose major purpose is to impair the function of bacterial efflux pumps, which deal with removal of, for example, metabolic waste products, toxins, or antimicrobials (11). They have long been known to be efficacious against planktonic bacteria, and used in combination with other antibiotics, they can resensitize microorganisms to those antibiotics by preventing efflux from the cell. Such compounds do not possess antiadhesive properties but can impede biofilm development, and when a number of these molecules are administered together, they can completely abolish biofilm formation. Using E. coli as a model, it was documented that the expression of over 20 different efflux systems was highly upregulated in the biofilm mode of growth. Their role in the bacterial biofilm lifestyle remains incompletely understood, but gene expression analysis showed that many were involved in multidrug resistance, suggesting that to some extent these targets were no different than those observed for planktonic microorganisms (12). Therefore, these efflux pump inhibitors could be successfully used in combination with antibiotics to remove biofilm. Conversely, biofilm development was disrupted by efflux pump inhibitors alone, in the absence of additional antibiotics, suggesting an alternative role for them in maintaining a healthy biofilm. In addition, it has been hypothesized that the high expression of efflux systems within the biofilm serves as a waste-removal strategy to prevent metabolite accumulation, so impairing these systems could result in biofilm disruption as a consequence of the build-up of toxic waste products (13).

Targeting iron metabolism within pathogenic microorganisms has been a long-debated strategy to attenuate virulence and impede microbial growth within the host (14). Evidence now suggests that this strategy could also be effective against biofilms and that by hijacking the iron-acquisition systems, biofilm bacteria can be
Maddocks

effectively killed and biofilm development blocked (15). Gallium is a redox-inactive metal that can replace iron in a number of biological iron compounds, including siderophores (15–17). Metallocomplexes comprised of the siderophore desferrioxamine and gallium (in place of iron) effectively target bacterial siderophore uptake systems to transport toxic gallium to the cytoplasm; in this methodology the siderophore is likened to a Trojan horse, tricking the pathogen into taking up a lethal metal ion (18). While the toxic effects of such metal ions on the bacterial cell are known, the observed impairment of biofilm development has not been thoroughly described outside the scope of a reduction in bacterial numbers, so putative targets in this respect remain unknown, and the process might be mediated purely by toxicity.

TARGETING PATHOGENICITY AND VIRULENCE

A significant factor associated with the development of infection is an organism’s ability to evade the immune system and damage the host. Targeting mechanisms of pathogenicity and virulence are attractive because they have the potential to render pathogens susceptible to host clearance mechanisms without killing them. Used alone or in combination with cidal therapies, antivirulence antimicrobials could have a place among traditional antimicrobial therapies, especially since current research suggests that resistance occurs less readily.

Numerous antivirulence strategies exist, but none as yet have entered the clinic. Such strategies are diverse in their approach and their mechanism of action and include small molecules that inhibit enzymatic activity, or biosynthesis of virulence factors, those that impede the expression of global regulators of known virulence traits, as well as inhibitors of quorum sensing (see below) (19). Small molecules have been utilized experimentally to inhibit the proteolytic activity of the lethal factor produced by Bacillus anthracis. In a mouse model the small molecule (2R)-2-[(4-fluoro-3-methylphenyl)sulfonylamino]-N-hydroxy-2-(tetrahydro-2H-pyran-4-yl)acetamide was found to offer a survival benefit (15). Gallium is a redox-inactive metal that can replace iron in a number of biological iron compounds, including siderophores (15–17). Metallocomplexes comprised of the siderophore desferrioxamine and gallium (in place of iron) effectively target bacterial siderophore uptake systems to transport toxic gallium to the cytoplasm; in this methodology the siderophore is likened to a Trojan horse, tricking the pathogen into taking up a lethal metal ion (18). While the toxic effects of such metal ions on the bacterial cell are known, the observed impairment of biofilm development has not been thoroughly described outside the scope of a reduction in bacterial numbers, so putative targets in this respect remain unknown, and the process might be mediated purely by toxicity.

Chemical inhibitors of K capsule biosynthesis in E. coli have demonstrated similar efficacy with regard to attenuation and subsequent clearance of infection following antibiotic treatments (21). Innate immunity is impaired by capsular polysaccharide that impedes opsonization and recognition by the complement system. Despite the molecular target remaining unknown, screens of small molecules have identified candidates that impair early stage capsule biosynthesis, rendering pathogens susceptible to C3 complement and sensitizing them to antibiotic treatment.

Alternative antivirulence strategies target receptor-mediated pathogenicity mechanisms, such as the interaction between Shiga-like toxins, produced by enterohemorrhagic E. coli and its receptor, Gb3, found on the surface of epithelial cells (22). Enterohemorrhagic E. coli can result in serious diarrheal disease, culminating in hemolytic uremic syndrome (HUS), and antibiotic treatment is not advised, meaning that current treatment for infection is supportive. In in vivo mouse infection models Shiga-like toxin inhibitors neutralize toxicity by preventing adhesion to epithelial cells, resulting in protection against doses that are known to be fatal. At present, the precise mechanism remains unknown, but this strategy offers a means of attenuating infection and preventing the onset of hemolytic uremic syndrome.

Perturbations in the environment in which infection ensues can also impact pathogenicity and virulence, as has been demonstrated by the use of the micronutrient zinc. At high concentrations zinc is toxigenic to most organisms, including bacteria, but at low levels it can disrupt aggregation and biofilm formation as well as the expression of numerous virulence-associated genes, without damage to the host. These effects are not dissimilar to those observed with other micronutrients such as iron (23). Enterohaemorrhagic E. coli has been used best to demonstrate these effects, where subinhibitory concentrations of zinc impaired both intracellular aggregation and the development of biofilm by this proliferatively aggregative organism. Moreover, gene expression analysis indicated that this process was likely mediated via significantly reduced expression of the virulence-associated transcriptional regulator aagR. Reduced gene expression was initially observed 3 hours posttreatment and was maintained following removal of treatment, with a 30% reduction in gene expression still evident (24).

The versatility of antivirulence antimicrobials offers an abundance of possible avenues of scientific exploration and the development of novel antimicrobial treatments. However, the vastness of the field calls for a...
refined approach to avoid the emergence of an overwhelming situation in which the most promising or adaptable targets are overlooked. Large-scale analysis including phylogenetic profiling of proteomic clusters offers such clarity and has indeed successfully identified 17 potential candidates that are common to diverse human pathogens but which are uncommon in non-pathogenic microorganisms. Prospective antivirulence contenders identified using these methods include mgtB and mntH, both of which are involved in manganese transport, sodC (oxidative stress), the stringent response gene sspB, and the protoheme IX biosynthesis gene hemY, to name a few (25). The ubiquitous nature of these genes and their orthologues throughout the Gammaproteobacteria and Firmicutes, combined with their affiliation with virulence, makes them ideal. Consequently, these candidates could form the basis of a preliminary antivirulence design for therapies that could be efficacious against a large variety of pathogens, or several subgroups of similar organisms, much like current antibiotics (i.e., broad- and narrow-spectrum antibiotics).

**PREVENTING MICROBIAL COMMUNICATION**

One of the most intently studied antivirulence strategies involves jamming microbial communication. This approach has been pursued as a means of attenuating virulence, adhesion, and bacterial community development without impairing growth and survival, thus providing less selective pressure toward resistance. Quorum sensing occurs between bacteria of the same species and members of diverse species; thousands of natural quorum sensing inhibitory compounds have been screened and numerous synthetic analogues produced that have the potential to impair microbial communication. Three major quorum sensing targets have so far been utilized; these include inhibition of quorum sensing molecule synthesis, arrest of molecule–receptor interaction, and degradation of quorum sensing molecules (26).

The majority of quorum sensing inhibitors (QSIs) that impair synthesis of quorum sensing molecule systems has been developed using *P. aeruginosa* as a model microorganism. These include signal agonists that target LasR or MvfR. MvfR is a global quorum sensing regulator known to be nonessential for growth. Eight compounds that bind directly to the MvfR protein have been shown to impair quorum sensing and attenuate virulence in a mouse infection model, without reducing bacterial load (27). These compounds share structural similarity, and each possesses a benzamide–benzimidazole backbone. This provides the host with an advantage, making infection more likely to clear, or allows successful co-treatment with other bactericidal antimicrobials.

Anti–quorum sensing stratagems that target acyl-homoserine lactones produced primarily by Gram-negative microorganisms, as well as peptide quorum sensing molecules produced by Gram-positive microorganisms, have proved effective at impairing the expression of virulence factors *in vitro* as well as attenuating pathogenicity *in vivo*. Moreover, the autoinducer 3 system, known to mediate interspecies signaling, can similarly be impaired; for example, strategies to impede phosphorylation of QseC could potentially impede signaling between species as diverse as *E. coli*, *Salmonella* spp., and *Francisella tularensis*, to name a few. QseC is conserved in over 25 bacterial species, making it a diverse target for anti–quorum sensing approaches (28).

Molecule–receptor interaction is imperative for the bacterial response to the presence of quorum sensing molecules; targeting this aspect of quorum sensing relies on natural or synthetic signaling-molecule analogues that preferentially bind to receptors without stimulating an intracellular response signal (29). These general non-responsive signal–receptor complexes block subsequent binding by quorum sensing molecules. LuxR-type receptor proteins have been well studied as potential candidates for this type of interference, and crystal structure analysis is likely to prove imperative for applicable inhibitor design. However, screening of natural compounds has revealed a number of efficacious analogues capable of inhibiting signaling in this manner (30).

Quorum sensing signal degradation is generally reliant on enzymes such as lactonases, acylases, or oxidoreductases (31). Lactonases tend to have a broad specificity for acyl homoserine lactones by virtue of the highly conserved lactone ring within these molecules. Acylases exhibit substrate specificity based on the chain length of the acyl moiety and tend not to be as broadly acting as lactonases. The least is known about the oxidoreductases, which work via oxidation of reduction of the acyl side chain.

Anti–quorum sensing treatments have the most potential to be used in combination with either other antimicrobials or surface antiseptics or disinfectants, due to their inherent tendency to disrupt biofilm formation. For example, biofilms of *P. aeruginosa* treated with a QSI appeared more susceptible to dispersal using a simple detergent such as sodium dodecyl sulfate (32). Despite their promise as novel new antimicrobial targets, QSIs also have their limitations. Primarily, they can interact
with one another. QSIs that target LuxR result in transcriptional feedback which generates a nonlinear response to increases in inhibitor concentration (33). Moreover, competitive LuxR inhibitors can weakly activate LuxR, alone or in combination with other QSIs, leading to increased bacterial virulence. These models have focused only on Lux systems to date, but these types of transcriptional feedback effects could occur for any QSI that acts by interfering with transcriptional regulators.

Quorum sensing molecules can have a direct effect upon the host. The quorum sensing signal molecule of P. aeruginosa, N-(3-oxododecanoyl)-L-homoserine lactone (OdHL), modulates inflammation and immune responses in mammals by acting as a PPARγ inhibitor, preventing NF-κB gene expression and ultimately suppressing STAT3 activity (34). Consequently, the innate proinflammatory immune response is dampened, thus promoting a shift toward infection. QSIs, particularly LasR inhibitors, which have cross-reactivity with OdHL, administered appropriately as a prophylactic measure could prevent this process and promote immune clearance. As more work is undertaken to elucidate a place for QSIs in antimicrobial treatment, it is clear that an appropriate application must be considered, be this as part of a cotreatment or as a prophylactic measure; it is imperative that QSIs are administered at the right time and at the right dose to increase microbial susceptibility to the host immune response and other antibacterial agents.

**NANO-FORMULATED ANTIBIOTICS**

Nanomedicine, or nanotechnology, is a relatively new application in the field of medicine that exploits a novel means of delivery of antimicrobial compounds to microorganisms and may also target novel components of the bacterial cell. Nanoparticles are broadly defined as particles with at least one dimension that are smaller than 100 nm; their surface area and biological and chemical activity can be modified for a preferred application. Antimicrobials formulated as nanoparticles characteristically exhibit higher antimicrobial activity due in part to their polycationic or polyanionic nature, facilitating better interaction with the bacterial membrane; moreover there is a correlation between the size of a nanoparticle and its inherent antimicrobial activity, with smaller particles having the best efficacy (35).

To date, the majority of antimicrobial nanoparticles have been primarily metal-ion-based, with zinc and silver receiving the most attention (36). Metals are well documented for possessing antimicrobial activity, but those that have so far been developed as nanoparticles are more effective antimicrobials and are associated with a lower likelihood of the emergence of resistance when compared to their chemical counterparts (37). It is currently hypothesized that the antibacterial activity of nanoparticulate zinc oxide is facilitated by its electrostatic attraction to the negatively charged bacterial membrane, whereupon it likely results in altered permeability and eventual disruption of the cell envelope and subsequent death. However, silver nanoparticles are believed to mediate bacterial death via the production of reactive oxygen species such as hydroxyl radicals or superoxide, which catalyze extensive lipid peroxidation (38). Likely additional targets for metal-ion formulated nanoparticles include metal ion transporters and impairment of efflux pumps. The relative lack of specificity of these compounds makes them efficacious against a broad spectrum of pathogenic bacteria.

More recently, nanoparticulate formulations of common disinfectants such as chlorhexidine have been produced which can be deposited onto surfaces such as glass, titanium, and ethylene vinyl acetate (39–41). These particles exhibit antibiofilm activity and appear to be more efficacious than non-nano-formulated chlorhexidine against some of the most notorious health care– and wound-associated pathogens including P. aeruginosa and methicillin-resistant S. aureus. While the mechanism of action of chlorhexidine as a membrane-disrupting chemical is well described, it is thought that additional targets might be involved or that the novel delivery mechanism itself might predispose organisms that ordinarily show good tolerance to chlorhexidine to its bactericidal properties.

Therefore, nano-formulated antibiotics deviate from traditional antibiotics that are chemically synthesized or biosynthesized and have a broad range of bacterial cell targets, which is advantageous with regard to the development of resistance. As such, they do not affect novel targets per se (that are currently known), but their novelty lies in their chemistry and delivery. By using nanotechnology, it might be possible in the future to reintroduce redundant antimicrobials or enable antimicrobials to be better internalized by bacteria.

**INTERFERING WITH RNA**

Small RNA (sRNA) profiling of pathogens has enabled the rapid identification of potential targets for antimicrobial therapeutics. At between 50 to 500 nucleotides in length, these are naturally occurring genetic regulatory elements that govern posttranslational expression
of a wide variety of bacterial genes (42). sRNAs are involved in the bacterial response to “challenging” conditions, including methicillin-resistant S. aureus using RNA sequencing technology have identified an sRNA profile for this organism following exposure to antibiotics, which has provided a framework to investigate the potential of sRNA-targeted or sRNA-mediated antimicrobial therapies.

The postantibiotic treatment sRNA profile for methicillin-resistant S. aureus revealed 195 sRNAs, some of which had a role in general metabolic processes and others which constituted a critical component of the antibiotic resistance response. Some of these novel ribo-targets were associated with expression of gyrA and mecA, both of which are involved in the expression of antibiotic resistance traits and provide an opportunity to explore the benefits of manipulating sRNA targets to resestisize microorganisms to antimicrobial treatment (43).

Similar studies with multidrug-resistant isolates of Pseudomonas putida treated with rifampicin, tetracycline, ciprofloxacin, ampicillin, kanamycin, spectinomycin, and gentamicin identified 138 new sRNA targets that were related to genes encoding antibiotic resistance traits. Crucially, the observed relationship between mRNA and sRNA expression emphasizes the importance of such targets, which are thought to play a critical role in fine-tuning the antimicrobial response (44).

A different strategy also making use of sRNA-based technologies has utilized phage delivery systems to silence or knock down antibiotic-resistant phenotypes in E. coli. In this case sRNAs were specifically designed to impair the translation of mRNA transcribed from either kanamycin-resistance or chloramphenicol-resistance cassettes and successfully restored sensitivity to both of these antibiotics within populations that were demonstrated previously to be resistant to treatment with kanamycin or chloramphenicol (45). While proof of principle was applied only to E. coli, this strategy has the potential to be applied to numerous multidrug-resistant pathogens for which there are known phage.

This shotgun approach to sRNA antisense screening has the potential to reveal large numbers of novel antimicrobial targets and has proved successful for S. aureus, E. coli, and P. aeruginosa. Despite this, therapies that utilize or target sRNAs remain in the early stages of development. However, much like other targeted strategies, nonessential genes such as those that encode virulence factors could be targeted to attenuate pathogens without providing the selective pressure that favors resistance.

TOXICOGENOMICS

There are a plethora of novel antimicrobial targets with remarkable potential as targets for antimicrobial therapies. Strategies to best exploit them continue to be developed, but numerous barriers lie in the way. Finding a balance between the therapeutic dose to have efficacy against pathogens and toxicity within the host is a challenge that can be addressed by traditional toxicology and toxicogenomics. Toxicogenomics assesses combined information regarding gene and protein activity within a particular cell, tissue, or organism (46). Transcriptomic and proteomic approaches are employed to gather this type of data to elucidate mechanisms involved in the presentation of toxicity, and in this manner molecular expression profiles can be obtained following exposure to a given toxin. This can predict genetic susceptibility.

The application of toxicogenomics for the screening of new antimicrobial compounds is clear, but it also has the potential to be used to identify mechanisms of toxicity for novel antimicrobials in bacteria and indeed to facilitate the rapid identification of potential new targets that could be exploited (47). Systematic strategies to enable effectual antimicrobial candidates remain a challenge, and the toxicogenomic approach can identify developable contenders early on while eliminating ineffective ones. Furthermore, this kind of approach could provide a profile of targets that could form the basis of a library of antimicrobial targets for an informed assessment of new antimicrobial candidates.

SUMMARY AND CONCLUDING REMARKS

Novel targeted antimicrobial therapies are critical to triumph in the relentless race against the evolution of antimicrobial resistance. A directed approach to recognize specific targets is shrewd, but it remains imperative to ensure that consideration is given to appropriate targets that are not likely to encourage rapid emergence of resistance. Comprehensive, inclusive methodologies are paramount to establish a pool of suitable antimicrobial targets that provide little evolutionary pressure for survival and if used appropriately can extend the lifetime for which they are effective. Combination therapies are attractive and are often significantly more effective, offering an antimicrobial approach akin to the hurdle technologies used by the food industry. Importantly, a sustained commitment to identify and augment such targeted approaches, in whatever form they may take, should not lose momentum against a backdrop of an impending antimicrobial crisis.
Maddocks

REFERENCES


