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Rethinking the Composition of a Rational Antibiotic Arsenal for the 21st Century

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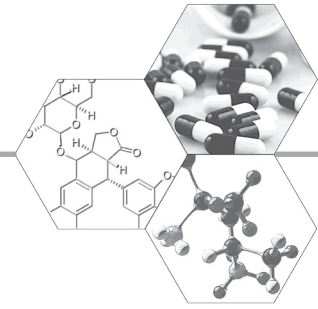

Rethinking the composition of a rational antibiotic arsenal for the 21st century

The importance of the human microbiome in health may be the single most valuable development in our conception of the microbial world since Pasteur's germ theory of the 1860s. Its implications for our understanding of health and pathogenesis are profound. Coupled with the revolution in diagnostics that we are now witnessing – a revolution that changes medicine from a science of symptoms to a science of causes – we cannot continue to develop antibiotics as we have for the past 80 years. Instead, we need to usher in a new conception of the role of antibiotics in treatment: away from single molecules that target broad phylogenetic spectra and towards targeted molecules that cripple the pathogen while leaving the rest of the microbiome largely intact.

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Beginning with the use of penicillin in World War II, we have witnessed a recurrent cycle: novel antibiotics are discovered, put into widespread use and soon rendered clinically ineffective by the inevitable rise of resistant pathogenic strains. For the first half-century of the age of antibiotics this pattern was viewed as an inconvenience, but not as a real threat. The microbial world appeared to offer up a virtually endless source of potential lead compounds. Coupled with progress in organic synthesis, which made the rapid modification of existing small-molecule scaffolds possible, new antibiotic leads seemed in vast supply and resistance was simply the inevitable price of doing business with rapidly evolving pathogens.

Skip ahead 50 years and the price we pay for resistance has become untenable. Pathogenic microbes, once easily controlled by antimicrobial drugs, now frequently fail to respond to many antibiotics [1,2]. Multiply resistant pathogenic strains have emerged in a broad range of species, including, among others, *Staphylococcus aureus*, *Mycobacterium tuberculosis, Pseudomonas aeruginosa, Salmonella enterica* and *Enterococcus faecium* [3–7]. This new situation is not merely an inconvenience – it has devastating societal, economic and human health impacts. Indeed, the entire treatment landscape for bacterial infections has been changed. Resistance significantly increases the length of hospitalization, complicates the treatment of other conditions, and may require the use of more toxic alternative treatments. More ominous still is the marked increase in mortality from both Gram-negative and -positive infections associated with 'inappropriate antibiotic treatments' where resistant pathogens fail to respond to the course of treatment [8]. The cost of treating infectious disease has increased drastically. In the USA alone, antibiotic-resistant infections are estimated to result in US$20 billion dollars in excess healthcare costs and $35 billion in societal costs [9,10].

The increased prevalence of antibiotic resistance is an expected outcome of evolution. Any population of organisms will exhibit heritable variability. In the case of bacterial populations, some proportion (often in the range of 1 in 1,000,000 cells) exhibits the ability to survive antibiotic exposure. When a person takes an antibiotic, the drug kills the defenseless bacteria, while simultaneously selecting those variants able to resist it. These renegade bacteria then multiply and rapidly increase in frequency. The antibiotic does not cause the resistance, but promotes its spread by creating a situation where an already existing resistant variant can flourish. In addition to this selection for resistant strains, we have now become aware of a second, equally deleterious consequence of antibiotic exposure: the disruption of the commensal human microbiome. The human body is a complex mosaic composed of nine parts microbial cells to one part human cells. The overwhelming majority of these commensal microbial partners, key players in maintaining human health, now become the collateral targets of any topical or ingested antibiotic. Under pressure from the antibiotic, many of these commensals evolve or acquire resistance – mechanisms that become part of the interspecific global trade in resistance determinants constantly underway in the bacterial world. Given our coarse approach to treating bacterial infections, the rise and spread of

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resistance should come as no surprise. What is, instead, surprising is that resistance is not more widespread.

Current approaches

Public and private approaches to deal with the now predictable evolution of bacterial resistance fall into two distinct categories [2,11], restricting the use of antibiotics and developing new ones. The first of these has resulted in an increase in the monitoring of antibiotic resistance, coupled with significant efforts to curb the indiscriminate use of antibiotics in order to extend their useful life span [2,12]. Efforts in this category include more stringent criteria prior to antibiotic prescription, more sensitive diagnostics prior to antibiotic use, increased emphasis on patient compliance, including use of full antibiotic courses, reduction and targeting of antibiotic use in food supplies (including animal feed and poultry processing) and an overall curb on the antibiotic load present in the environment [8,9,13]. The recent release of US FDA guidance on the judicious use of medically important antimicrobial drugs in food-producing animals suggests that the regulatory landscape may be poised to respond to this critical factor in resistance evolution [14]. We should be well beyond the point of questioning whether our use of antibiotics has selected for resistance.

Attention can now be turned to those measures that will extend the usefulness of the relatively few antibiotics that retain broad efficacy. While efforts directed at educating physicians, patients and the general public have increased our awareness of the problem of antibiotic resistance and have, in some cases, resulted in a reduction in the inappropriate use of antibiotics, they do not obviate the need to develop new ones. We stand at a critical juncture in this regard. One option is to continue to discover and develop new antibiotics as we have over the past half-century. That approach has involved the identification of compounds that exhibit high activity, broad range and low toxicity, while at the same time meeting a variety of criteria involving stability and deliverability. Within that framework, the prospect of antibiotic resistance is clearly seen as a major potential limitation on the utility and life span of any novel antibiotic. However, its emergence is usually treated as an unfortunate, undesirable, but unavoidable outcome of battling infectious agents.

A new approach

Today, both our faith in an endless supply of new clinically useful antimicrobials and our perspective on the challenges posed by antibiotic resistance have undergone radical transformation. The pipeline for new antibiotic leads has slowed to a trickle [15] and the incidence of pathogens resistant to virtually all currently employed antibiotics is on the rise [3,5,7]. For the first time since the discovery of antibiotics, we face the prospect of untreatable infections. This situation, alarming as it is, also presents an opportunity to radically rethink the composition of a rational antibiotic arsenal for the 21st century clinic. In addition to the established criteria for activity, efficacy and toxicity, we contend that two additional criteria must be taken into account in the design of new antimicrobial compounds:

- Resistibility: the frequency at which resistance to a novel antibiotic arises and the resulting fitness cost to the pathogen of that resistance;
- Specificity: the extent to which a novel antibiotic can be directed only against pathogenic strains while leaving the composition and structure of the commensal human microbiome undisturbed.

We argue that this second, more radical approach to antibiotic development is needed. Under this new paradigm, we acknowledge the lessons learned in the laboratory and in the clinic and incorporate the discoveries in genetics, genomics and microbial ecology into the search for and design of new antimicrobials. This proposed paradigm shift requires our dedicated focus on the infectious agent within the context of its occurrence in the human microbiome. It is no longer reasonable to spend a billion dollars developing a drug that will target the majority of beneficial bacteria, as well as the numerically insignificant (but clinically relevant) pathogens, thereby imposing massive and widespread selection for resistance, while concurrently depleting the diversity of our microbiome. Our microbiome is an essential factor in maintaining human health and efforts to treat disease must take this into account [16,17].

Fortunately, just as fungi and bacteria offered a diversity of solutions to fighting infectious disease in the early 20th century, in the form of penicillins, tetracyclines and so forth, they are similarly poised to provide a solution to our current dilemma. In the billions of years of their evolution, microbes have evolved an extensive palette of antimicrobials. This palette should be the starting point in our search for new, smart antimicrobials: a plethora of potential drug

candidates with compelling features is there to be found. The antimicrobials found in the microbial world exhibit a range of specificities and modes of action, from the exquisitely highly targeted killing by certain bacteriocins and bacteriophage, to the broad and indiscriminate action of bacterial lysozymes. Add to this list the exciting new candidates resulting from bioengineering efforts, such as RNA-based therapeutics, immunomodulating agents, phage lysins and antimicrobial peptides [18,19], and the number and range of potential drug candidates is encouraging.

Our drug-discovery task becomes even less daunting when we realize that until now we have essentially ignored some of the most successful solutions to the challenge of eliminating infectious agents. The magic bullets of the 20th century, that is, broad-spectrum antibiotics, embody a strategy rarely seen in the microbial world. Instead, most evolved antimicrobials act specifically against their closest competitors, those species consuming the same limited resources or inhabiting the same limited niche [20,21]. Employing a broad-spectrum, indiscriminate killing approach, as we have done by using traditional antibiotics, destroys the very own community of the microbe – and our essential microbiome. Contrary to the common perception of microbes growing as single species on a Petri plate, microbes in nature seldom live alone. Instead, they live in complex multispecies communities, with species dependent upon each other for their very survival. Our use of conventional antibiotics has essentially ignored this critical feature of microbial ecology, resulting in devastating collateral damage to the normal human microflora. We contend that the development of new drugs must take into account the potential for collateral damage. The good news is that there already exists an abundance of promising drug candidates that do just that.

Bacteriocins

The potent arsenal of drugs deployed by microbes is remarkable in its diversity. Let us turn to one of the most common superfamily of antimicrobials, the bacteriocins. Both in terms of abundance and diversity, bacteriocins contend for the prize as the primary mechanism of bacterial defense in nature [20–22]. Bacteriocins are loosely defined as biologically active peptides with a bacteriocidal mode of action [23–25], which, although variable among bacteriocin types, are all distinct from those of current chemotherapeutic agents [18]. The family includes a diversity of proteins in terms of size, microbial targets, modes of action and immunity mechanisms. Most, however, are highly specific in killing or inhibition activity, often active only against close relatives of the producing strains [23–26]. These potent toxins are produced by all major lineages of bacteria, and within a species tens or even hundreds of different kinds of bacteriocins can be identified [22]. Klaenhammer noted over 20 years ago that 99% of all bacteria may make at least one bacteriocin and the only reason more have not been isolated is that very few researchers have looked for them [27]. That statement remains fundamentally true today; we have only just begun to tap the diversity of this superfamily of potent toxins.

Bacteriocins exhibit numerous characteristics that underscore their viability as alternatives to conventional antibiotics [20,28]. First, bacteriocins active against all known human and animal pathogens already exist. As noted above, tens or even hundreds can be isolated from a single bacterial species. Furthermore, bacteriocins with a spectacular range of specificities also already exist, ranging from strain-specific moieties to those able to target all Gram-negative or -positive bacteria. A simple, rapid screen of a few hundred strains from a target pathogen, or its close relatives, will almost certainly reveal numerous compelling compounds [27,29–32]. Furthermore, the long, rich history of research on bacteriocin structure and function, and the resulting catalog of structures available in the literature, makes bioengineering-specific activities a straightforward task. The potency, specificity and stability of bacteriocins is manipulated by simply cutting and pasting the desired features from one bacteriocin onto another [33–35].

Bacteriocins boast a remarkable potency, many display single-hit kinetics: a single molecule entering the target pathogen will do the job [25,26]. They also act rapidly, inhibiting or killing within seconds of encountering a target cell, in sharp contrast to conventional antibiotics that often require actively growing cells. In fact, bacteriocin MIC values rival those of traditional antibiotics [36,37]. Equally compelling, bacteriocins are stable under a wide range of temperatures (from -20 to +65°C) and other environmental challenges, such as pH [38–40]. Numerous studies attest to the ability of bacteriocins to retain activity under a wide range of potential therapeutic conditions, including application on the skin and in the throat, bladder, bloodstream and intestines [18,38–47].

Industrial production methods have been developed for several bacteriocins [42,46]. Nisin, a bacteriocin produced by *Lactococcus lactis*, achieved generally recognized as safe (GRAS) status from the FDA in 1988 [41]. It has since been widely used as a food preservative and in numerous animal production applications, including prevention of *Salmonella* spp. colonization of chicken skins and surface-related infections such as mastitis in cows [18]. The industrial production methods of nisin and numerous additional bacteriocins of lactic acid bacteria are particularly well studied [45,47,48]. In fact, a global leader in the antimicrobial preservatives industry, Danisco A/S, recently acquired Aplin and Barrett of the UK and their primary production facility of nisin. The entry of large biopreservative manufacturers into the bacteriocin production market signals a key, dramatic shift in the industrial perception of the hurdles involved in peptide production.

A further crucial benefit of bacteriocins is the low or nonexistent toxicity to humans and other mammals [18,43,49–56]. Studies are mounting that demonstrate that bacteriocins have minimal impact on host cells, primarily due to their exquisitely targeted modes of action against bacteria (for a recent review see [49]). These studies underscore the low toxicity of bacteriocins and highlight the vast potential of bacteriocins as therapeutic agents [18,43,49–54]. The lack of bacteriocin toxicity is perhaps less remarkable when one considers that many, perhaps even most, members of our microbiome are producing them in and on our bodies.

Given the remarkable therapeutic properties of bacteriocins, it should come as no surprise that there is growing interest in their commercialization [18,57–59]. Several companies are already working on bringing bacteriocin-based approaches to the market. AvidBiotics (CA, USA) is currently exploring the use of bacteriocins from *Pseudomonas* spp., known as pyocins, in food safety, animal health and environmental management. Another company, Bacteriotix (MA, USA), is investigating the use of bacteriocins against urinary tract (UTIs) and skin infections. Several additional companies, such as Blis Technologies Ltd (New Zealand), are using these polypeptides in oral care products. Novacta Biosystems (UK) is also working on a bacteriocin treatment for the pathogen *Clostridium difficile*. These are just a few of the pioneering companies seeking to develop bacteriocins as a new tool in our antimicrobial toolbox.

The use of bacteriocins will ultimately select for resistant strains, just as is the case with conventional antibiotics. However, because their therapeutic use would be directed at specific infections, the intensity of resistance selection is dramatically decreased. Even more compelling, by combining two or three bacteriocins, a cocktail can be produced that reduces the resistance frequency by several orders of magnitude, effectively eliminating resistance as an outcome (**Figure 1**). **Table 1** represents a second approach to resistance elimination, the use of a single bacteriocin with targeted substitutions that result in highly efficacious toxins with slightly altered specificity. Use of two or more of these variants results in a significantly reduced frequency of resistance [32].

Given that bacteriocins are ancient, widespread and in constant use by many bacterial species to displace competitors and invade novel environments, how have they remained a viable, highly effective means of bacterial defense? The answer is twofold. First, because these toxins target a minute fraction of a microbial community, the selection for mutations that confer resistance is not taking place in multiple species simultaneously, as is the case with broad-spectrum antibiotics. Second, bacteriocins occur in constantly changing and evolving combinations, thus, allowing the producer strains in nature to keep pace with emergent resistance in target strains [60]. It is precisely this strategy – target-specific, highly active antimicrobials supplied in changing combinations – that we argue should be emulated in our future therapeutic approach. We are convinced that this coupling will result in combinations that will be effective *in vivo* and greatly retard the emergence of resistance in target pathogens. We contend that the natural ecology of antibiotics has much to teach us – not only regarding potential lead compounds, but also about the rational therapeutic use of antibiotics. The time is right to assess the therapeutic potential of this highly diverse and abundant class of naturally occurring antimicrobials.

Bacteriophages

Bacteriophages represent a second family of compelling targeted drugs. Phage therapy involves the application of bacteriophages that, when encountering a specific pathogenic bacteria, can infect and kill them. However, before killing the cell, the phage directs the bacterial host to produce phage progeny, which are released during host lysis. Thus, phages are unique in

their ability to increase their numbers when in the presence of their bacterial targets [61]. Similar to bacteriocins, many phages are active against a single or relatively few bacterial strains or species. In fact, phage therapy is based upon the concept of cocktails, which can include numerous different phage types. Intestiphage, a product available in Georgia and Russia, contains a cocktail of phage that targets over 20 different pathogenic gastrointestinal bacteria [61]. The result of the narrow killing spectrum for phage and bacteriocins is a lower potential for side effects associated with dysbiosis, a negative impact on important normal bacterial flora.

Phages possess several additional features that make them compelling as therapeutic alternatives. First, is their extraordinary killing efficiency. As opposed to chemical antibiotics, only a single phage is needed to kill a single bacterium [62]. Furthermore, a small inoculum of phage, which then reproduces within the target pathogen, is often sufficient to kill even dense bacterial infections. This potential for phages to increase in density *in situ* could potentially reduce treatment costs and may improve product safety, since phages only increase in density when the target bacteria are present [63]. Phages tend to be bactericidal, in contrast with many conventional antibiotics [64]. Since they consist mostly of nucleic acids and proteins, phages are inherently nontoxic [63,65,66]. However, phages can interact with the immune system, at least potentially resulting in harmful immune responses, though there is little evidence that this actually is a concern during treatment [62,67–69]. Because phages infect and kill using mechanisms that differ from those of antibiotics, specific antibiotic resistance mechanisms do not translate into mechanisms of phage resistance. Phages consequently can be readily employed to treat antibiotic-resistant infections [62,67,68,70]. Phages have a demonstrated ability to clear biofilms, perhaps by lysing one bacterial layer at a time, or due to the display of biofilm exopolymer-degrading depolymerases [61]. The industrial costs of phage production are not out of line with the costs of pharmaceutical production, while the costs of discovery can be relatively low [63,66].

One area of concern with phage therapy is the potential host response to phage presence. However, numerous studies have revealed that phage therapy rarely, if ever, results in more than minor side effects [61,71–73]. Indeed, the immunological response of phage presence in animals has been studied for over a half a century and no substantial anaphylaxis has been reported [74–77]. Indeed, some studies have indicated a positive impact of phages on immune system functioning [68] and have explored potential phage antitumor properties [74]. A second area of concern relates to ability of some phages to modify host bacteria in ways that could make them more pathogenic, or to release host toxins during cell lysis. However, cell lysis and toxin release can also be induced by treatment with traditional antibiotics.

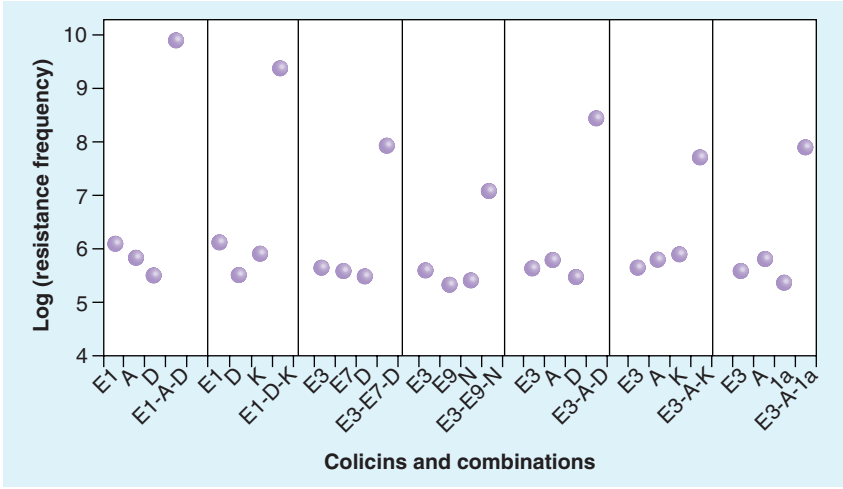

Figure 1. Colicin combinations reduce resistance frequency by several orders of magnitude.
Adapted with permission from [32].

Perhaps the most significant challenge to the development of phages as alternative therapeutic agents is simply the lack of familiarity of western medicine with them. This situation may be poised to change as several phage products have now been classified by the FDA as GRAS, registered by the Environmental Protection Agency, or approved for use by the United States Department of Agriculture [63,78]. It is important to note that therapeutic phages have been used to treat bacterial infections in eastern Europe for over 80 years [79]. As early as 1921, phage therapy was used to treat staphylococcal skin infections [62]. During World War II, bacteriophages were used to treat bacterial infections on the battlefields

Table 1. Targeted substitutions result in efficacious toxins with slightly altered specificity.

Colicin variant	Altered residues	Relative MIC
Colicin E9.0	None	1.0
Colicin E9.1	Leu293Arg	1.3
Colicin E9.2	Leu293His	0.7
Colicin E9.3	Arg291His	1.6
Colicin E9.4	Arg298His	2.5

by the former Soviet Union [80]. Early on, American and French pharmaceutical companies demonstrated interest in this approach and began to manufacture bacteriophage products. In the 1940s, the Eli Lilly Company (IN, USA) produced seven phage products for human use. Bacteriophages were primarily used for treating bacterial infections caused by *Staphylococcus, Streptococcus, Escherichia coli* and *Neisseria*. A variety of infections responded to bacteriophage therapy, including purulent infections of the skin and mucous membranes, upper respiratory tract infections, vaginitis and ear mastoid infections [79]. However, with the advent of antibiotics, commercial production of therapeutic phages quickly ceased in the USA.

Phages are currently being used therapeutically in the Republic of Georgia and Poland to treat bacterial infections that fail to respond to conventional antibiotics [61]. In the west, no therapies are currently authorized for use on humans. However, as mentioned above, the FDA recently gave its first official approval to the use of phage in food production with the approval of ListShield™ (a phage preparation targeted against *Listeria monocytogenes*) created by Intralytix (MD, USA) and the granting of GRAS status. Agricultural applications include the use of phages against *Campylobacter*, *Escherichia* and *Salmonella* in farm animals, *Lactococcus* and *Vibrio* pathogens in aquaculture, and *Erwinia* and Xanthomonas in plants of agricultural importance. Several companies seek to bring phage therapy into the western world, including PhageTech (Canada), Novolytics (UK) and GangaGen (India, USA and Canada). Phage therapy has been attempted for the treatment of numerous infections, including dysentery, gingivitis, UTIs, poly-microbial biofilms on chronic wounds, ulcers and infected surgical sites [81–83]. In 2007 a Phase I/II clinical trial was completed at the Royal National Throat, Nose and Ear Hospital (London, UK), employing bacteriophage to treat *P. aeruginosa* infections (otitis) [84]. Phase I clinical trials have now been completed in the Southwest Regional Wound Care Center (Texas, USA) for an approved cocktail of phages against bacteria, including *P. aeruginosa*, *S. aureus* and *Escherichia coli* [73].

Other targeted approaches

Although bacteriocins and bacteriophages represent the largest families of targeted drugs currently being explored for use in treating infectious disease, other targeted approaches to dealing with infectious disease are rapidly catching on. These approaches include the use of nanoparticles designed to interact with specific pathogens [85,86], RNAi molecules that interact with specific sequences [87,88] and immunomodulatory interventions tailored against particular agents [89,90]. One of the leading contenders in the hunt for novel targeted therapeutics is a class of molecules that specifically block pathogen communication and, thus, inhibit pathogenicity rather than kill the cells. Quorum sensing (QS) is a system by which certain bacteria can monitor their own population density. They secrete specific autoinducer molecules, which, when concentrations reach critical threshold values, trigger specific response systems, causing the induction of sets of genes that are only expressed at high population density. Some of these genes enable the bacteria to form biofilms, making the cells virtually untouchable by conventional antibiotics. One of the first companies to pursue QS as a therapeutic focus was aptly named Quorum Sciences (IA, USA). The focus of the company survives through acquisition by Vertex Pharmaceuticals (MA, USA) and subsequent research has reported the discovery of novel specific inhibitors of the *P. aeruginosa* QS system. The researchers concluded that the novel QS inhibitors might be useful chemical tools, but not drug leads. However, the potential for targeted intervention of bacterial communication channels remains an intriguing avenue for future drug-development efforts.

Implementation hurdles

These compelling examples also bring to light hurdles that pharmaceutical companies face as they develop targeted antimicrobials. A bacteriocin or phage will not be sold as a single molecule; the most promising therapeutic formulations will almost certainly require a cocktail of bacteriocins or phages. The US regulatory system is designed to handle one-size-fits-all drugs, not individual tailored therapeutic combinations. Under existing regulations, the FDA would require every phage to go through a multi-year testing process – by which time the bug will almost certainly have evolved again. One possibility is that the FDA could revise its rules as it has for the influenza vaccine: although it is reformulated every year to maintain effectiveness, new versions do not need to repeat the entire testing process. Our experiences with HIV drug combinations and influenza vaccines have proven the power of having a nimble, responsive regulatory system.

Why have we not already capitalized on the existing diversity of these potent, targeted antimicrobials? One component of the answer is straightforward: until recently, physicians relied on symptom-based diagnosis and, thus, were often uncertain of the identity of the infecting agent(s). If the infection was bacterial-based, use of a broad-spectrum drug was almost always effective. However, high levels of pathogen resistance now require that prior to prescription we first identify an effective antibiotic; thus we have lost one key prior advantage to broad-spectrum antibiotics – speed and ease of use. Furthermore, the rapid development of molecular diagnostic methods now underway ushers in a different model for the treatment of infections, and frees us from our reliance on broad-spectrum antibiotics [91]. We will soon be in a position to rapidly identify the infecting culprit(s), determine resistance to available drugs and provide a therapeutic specifically designed for the situation at hand.

A targeted example

To illustrate the potential power of targeted drugs, we briefly consider the efficacy and utility of the use of targeted drugs in the treatment of UTIs and, in particular, catheter-acquired UTIs (CAUTIs). Most UTIs and CAUTIs are caused by *E. coli*, normally a commensal resident of the large intestine [92,93]. Other Gram-negative bacteria are sometimes involved and include *Klebsiella* spp., *Enterobacter* spp., *Serratia* spp., *Proteus mirabilis* and *P. aeruginosa* [93–95]. These infections are widespread in the human population, with over 150 million people infected worldwide each year [96–99]. Women are especially prone to UTIs: approximately one in five women develops a UTI during her lifetime, with many experiencing life-long recurrences of urinary tract disease [96,100–102]. In addition, CAUTIs are the most common form of nosocomial infection in acute care hospitals and such infections are almost universally present among patients with chronic in-dwelling catheters, both in the community and in long-term care facilities [103]. One study estimated that the cost of UTIs (including direct costs and indirect losses in productivity due to illness or hospitalization) reaches $1.6 billion per year in the USA alone, excluding the costs of catheter-based infections [100].

High levels of antibiotic resistance among the strains responsible for UTIs are creating a challenge to effective therapy [92,93,98,104–110]. Ampicillin and amoxicillin, formerly the cornerstones of UTI therapy, are no longer preferred because of high levels of resistance. Trimethoprim–sulfamethoxazole (TMP–SMX) was considered the drug of choice for uncomplicated UTIs due to its low cost and well-established efficacy [111,112]. However, levels of resistance to TMP–SMX in *E. coli* now, unfortunately, range from 18 to >30% suggesting that it will soon no longer be effective as a first-line therapeutic option [107,113,114]. Nitrofurantoin and fluoroquinolones, such as ciprofloxacin, are also sometimes used as alternatives to TMP–SMX for treatment of UTIs. Aminoglycosides, such as gentamicin, are used to treat severe infections. Their practical use, however, is limited due to high associated toxicity. A physician treating a UTI or CAUTI now faces a complex therapeutic landscape. Given that UTIs are the single most common nosocomial infection, the impact on resistance for this disease alone is incalculable and, perhaps, avoidable.

The authors and others have initiated investigations to explore the therapeutic potential of targeted treatments for UTIs [115–121]. One approach involves the use of bacteriocins. Many studies attest to the fact that bacteriocins able to kill or inhibit each of the primary UTI and CAUTI pathogens already exist [117–121]. **Figure 2** represents the activity of a small sample

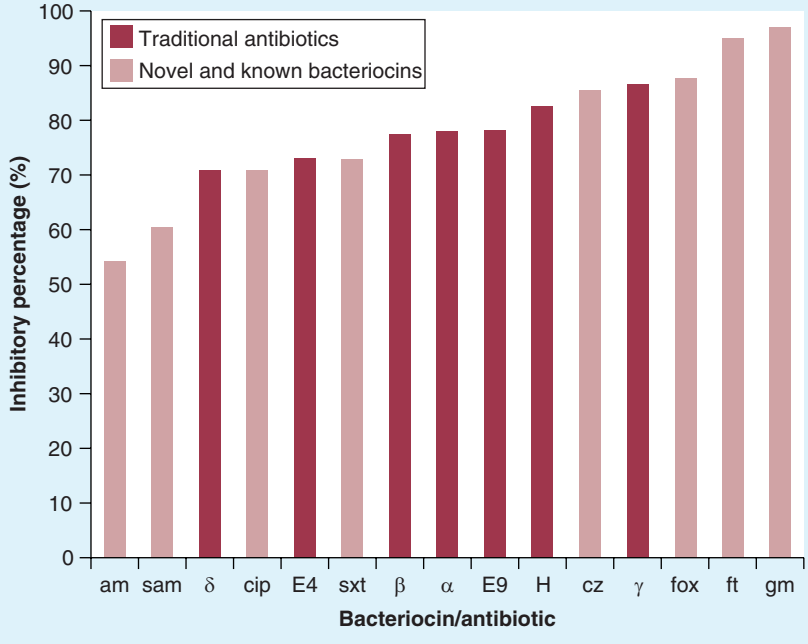

Figure 2. Inhibitory percentages of 96 uropathogenic *Escherichia coli* strains for both tradional antibiotics and novel and known bacteriocins.
Novel bacteriocins are labeled with Greek letters.
am: Ampicillin; cip: Ciprofloacin; cz: Cefazolin; fox: Cefoxitin; ft: Nitrofurantoin; gm: Gentamicin; sam: Ampicillin/sulbactam; sxt: Trimethoprim.
Adapted with permission from [32].

of bacteriocins and antibiotics against uropathogenic *E. coli* [32]. The bacteriocins are just as likely to inhibit the pathogens as our most potent antibiotics. Now, consider the fact that one can easily produce a sample of bacteriocins active against each uropathogen species, and the feasibility of designing therapeutics that specifically target CAUTI and UTI infections becomes strikingly clear. As anyone recently harboring a UTI will attest, there is no culturing prior to prescription, nor is the resistance of the pathogen determined. Those with the infection are simply given an antibiotic based upon the physician's prior treatment success, and hope for the best. Given that UTIs and CAUTIs are generally not fatal, are the leading causes for prescriptions in the USA and that current protocols do not require, or even permit, culturing and resistance determination, what are the arguments against developing a targeted therapeutic approach for these infections?

One particularly vexing aspect of CAUTI is that the bacteria form dense biofilms on catheter surfaces, which are virtually impossible to eliminate with conventional antibiotics. In contrast, bacteriocins not only inhibit the growth of biofilms, but some even break down existing biofilms [122–124]. Furthermore, if a bacteriocin is applied to the catheter and bladder immediately following catheter insertion, it is able to kill infecting cells before they have the opportunity to grow and attach to the catheter [124]. In contrast, an antibiotic wash is not only ineffective in killing the bacteria, it may even create a worse situation due to its toxicity to the bladder cells [125,126].

Bacteriocins have been reported to be effective *in vivo* in numerous experimental systems [18,51,55,127–134]. **Figure 3** represents the *in vivo* effect of a bacteriocin applied to an established mouse UTI. A relatively small dose of bacteriocin (2 μg) used as a bladder wash eliminated a well established UTI in four of the seven mice tested, and significantly reduced uropathogen frequency in two additional mice [32]. A further study revealed that simply coating a catheter with a bacteriocin-producing strain of bacteria prior to insertion eliminated subsequent biofilm formation [124]. For numerous additional *in vivo* examples see the recent review by Cotter *et al* [18].

Clearly, it will not be business as usual if a pharmaceutical company pursues such 'unconventional' therapeutics, such as bacteriocins or phages. Existing business models simply will not apply. However, we are inching closer to the precipice where bacterial infections will, once again, become a leading cause of death in the USA. Perhaps when that critical place is reached, and we are forced to admit that we have lost the race against bacterial pathogens, we will finally turn our existing antibiotic development paradigm on its head and engage in a more refined approach to targeting bacterial infections.

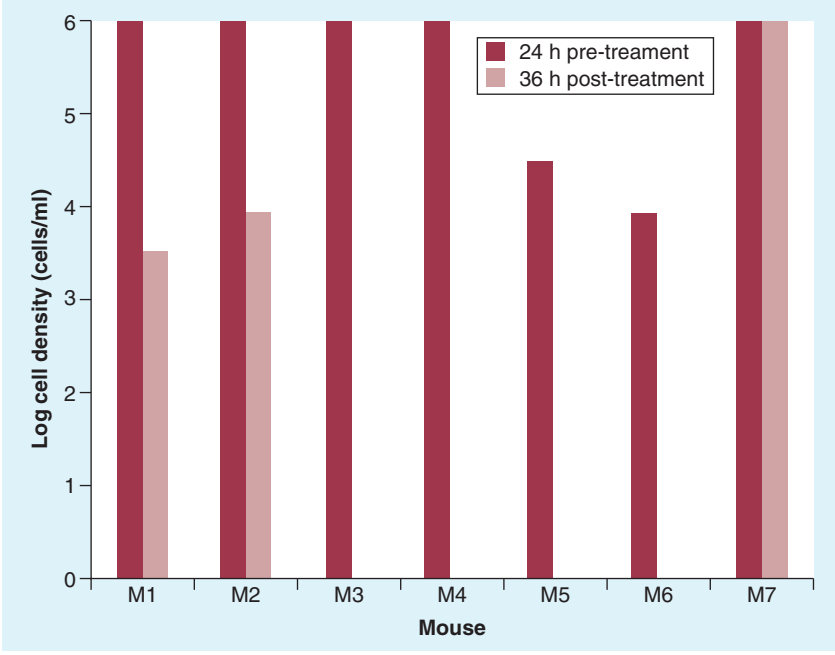

Figure 3. *In vivo* effect of a bacteriocin applied to an established mouse urinary tract infection. Mice were treated with 2 μg of a colicin bladder wash. Adapted with permission from [32].

Future perspective

In this short perspective we can only lightly touch on the compelling features of targeted antibacterials that make them such an attractive candidate for therapeutic development. The features include their targeted specificity, limited impact on the normal healthy microflora of the patient, significant reduction in the selective pressures for resistance emergence, a long history of use in food preservation, mounting evidence of their limited toxicity to mammalian cells, ease of production and their stable nature. Their potential as therapeutic agents remains largely untapped. However, all of the early signs suggest that these potent, naturally occurring families of toxins provide a compelling, ecologically sound alternative for the treatment of infectious disease.

This new ecologically based conception of the importance of the human microbiome in health may be the single most significant development in our perception of the microbial world since

Pasteur's germ theory of the 1860s. Its implications for our understanding of health and pathogenesis are profound. Coupled with the revolution in diagnostics that we are now witnessing – a revolution that changes medicine from a science of symptoms to a science of causes – we cannot continue to develop antibiotics as we have for the past 80 years. Instead, we need to usher in a new view of the role of antibiotics in treatment: away from single molecules that target broad phylogenetic spectra and towards targeted molecules that cripple the pathogen while leaving the rest of the microbiome largely intact. Similarly, while it is certain that resistance will always evolve in the face of selection, we can begin to incorporate the lessons learned in the design and deployment of treatments that delay the onset of resistance mechanisms. We cannot, and need not, continue to do the same thing and expect different outcomes. Instead, the time has come for a new smarter and more agile approach to the delicate balance between pathogens and their hosts.

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Executive summary

- For the first time since the discovery of antibiotics, we face the prospect of untreatable infections.
- With no magic bullets on the horizon, we have the opportunity to radically rethink how we identify and develop novel antimicrobials.
- We contend that resistibility and specificity should become primary design criteria, to both limit the spread of resistance and reduce the collateral damage on the human microbiome.
- Two large families of naturally occurring antimicrobials, the bacteriocins and bacteriophages, offer compelling targeted alternatives to conventional antibiotics.
- Such a dramatic shift in drug-development strategies brings to light numerous challenging regulatory issues and requires companies to explore new business models.
- However, we contend that the time has come for a new smarter and more agile approach to the delicate balance between pathogens and their hosts.

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