

Resistance is futile: the bacteriocin model for addressing the antibiotic resistance challenge

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Abstract

Pathogenic bacteria resistant to many or all antibiotics already exist. With the decline in microbiological research at pharmaceutical companies, the high rate at which resistance has evolved and spread has demanded a novel approach to addressing this critical human health issue. In the present paper, we propose a new paradigm in antibiotic discovery and development, one that applies ecological and evolutionary theory to design antimicrobial drugs that are more difficult and/or more costly to resist. In essence, we propose to simply adopt the strategies invented and applied by bacteria for hundreds of millions of years. Our research focuses on bacteriocins, powerful biological weapons, and their use as alternative therapeutics in human health.

The challenges of antibiotic resistance

The development and spread of bacterial antibiotic resistance has emerged as a major public health concern [1–4]. Pathogenic microbes, once easily controlled by antimicrobial drugs, now frequently fail to respond to many antibiotics. We are witnessing the emergence of multiply resistant pathogen strains in a broad range of species, including *Staphylococcus aureus*, *Mycobacterium tuberculosis*, *Pseudomonas aeruginosa*, *Salmonella enterica* and *Enterococcus faecium* [5,6]. Multiply-resistant bacterial infections significantly increase the length of hospitalization, complicate the treatment of other conditions and frequently require the use of more toxic alternative treatments. More ominous is the marked increase in mortality from both Gram-negative and Gram-positive infections associated with ‘inappropriate antibiotic treatments’, where resistant pathogens fail to respond to the course of treatment (<http://www.who.int/mediacentre/factsheets/fs194/en/>). Increased antibiotic resistance also has important economic consequences. For example, in the U.S.A. alone, antibiotic-resistant infections are estimated to be responsible for US\$20 billion in excess health care costs in addition to US\$35 billion in societal costs [8].

Public and private approaches to deal with the emergence of bacterial resistance fall into two distinct categories. The first of these is a dramatic increase in the monitoring of antibiotic resistance, coupled with significant efforts to curb the indiscriminate use of antibiotics in order to extend their useful lifespan [9,10]. Efforts in this category include more stringent criteria before antibiotic prescription, more sensitive diagnostics before 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 [11–13].

Although 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 resistance rates, they do not obviate the need to develop new antibiotics. 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 last half-century. That approach has involved the identification of compounds that exhibit high activity, broad range and low toxicity while meeting a variety of criteria involving stability and bioavailability. Within that framework, the prospect of antibiotic resistance is clearly seen as a major potential limitation on the utility and lifespan of any novel antibiotic, and the emergence of resistance treated as an unfortunate, undesirable, but unavoidable, cost of doing business with infectious agents.

Bacteriocins as human therapeutics

We contend that a second, more radical, approach to antibiotic development may be 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. The goal of the present paper is to explore the potential for a large family of naturally occurring toxins to serve as a new generation of target-specific ‘designer drugs’ which are both effective against particular pathogens and reduce the emergence and spread of antibiotic resistance.

Our entry point into this new strategy is a family of highly evolved, highly toxic and highly specific antibiotic proteins: the bacteriocins. In terms of both abundance and diversity, bacteriocins contend for the prize as the primary mechanism

Key words: antibiotic, bacteriocin, targeted therapeutic, urinary tract infection (UTI).

Abbreviations used: MIC, minimum inhibitory concentration; TMP-SMX, trimethoprim/sulfamethoxazole; UTI, urinary tract infection; CAUTI, catheter-acquired UTI.

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of bacterial defence in Nature [14,15]. They are highly specific in their killing action, often active only against close relatives of the producing strains [15]. An ancient family of proteins, routinely employed by microbes to displace competitors and invade novel environments, they remain a viable highly effective means of bacterial defence. Why have bacteriocins retained their efficacy in an environment in which they are constantly in use? The answer is two-fold. 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. Secondly, bacteriocins occur in constantly changing and evolving combinations, thus allowing the producer strains to keep pace with emergent resistance in the targets. It is precisely this strategy, i.e. targeted highly active antimicrobial agents supplied in changing combinations, that we emulate in our experimental therapeutic approach. We are convinced that this coupling will result in combinations that will be effective *in vivo*, exhibit virtually no toxicity to the host and greatly retard the emergence of resistance in the target pathogens. We argue that the natural ecology of antibiotics has much to teach us, not only about potential lead compounds, but also about the rational therapeutic use of antibiotics.

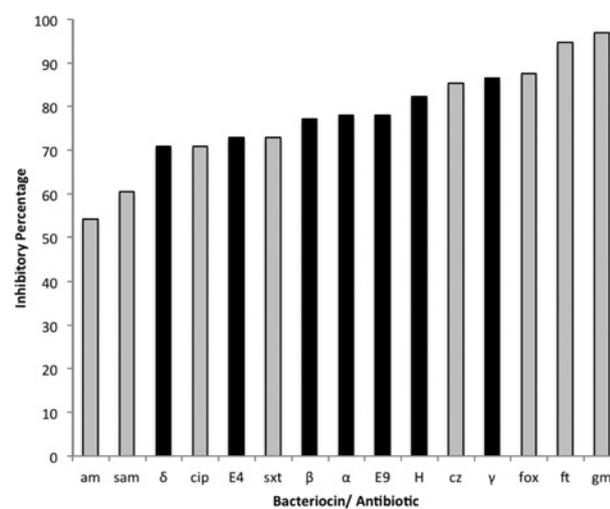
Why have we not already capitalized on this existing diversity of potential antimicrobials? The answer is simple. Until recently, physicians relied on symptom-based diagnosis and were thus often uncertain of the identity of the infecting agent(s). If the infection was bacteria-based, use of a broad-spectrum drug was almost always effective. Unfortunately, the overuse and abuse of these broad-spectrum antibiotics has significantly reduced their efficacy [16]. 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 [17]. The time is right to assess the therapeutic potential of this highly diverse and abundant class of naturally occurring antimicrobials.

The urinary tract infection model

To illustrate the potential power of employing bacteriocins as an alternative for broad-spectrum antibiotics, we explore the efficacy and utility of their use in the treatment of UTIs (urinary tract infections), and, in particular, CAUTIs (catheter-acquired UTIs). These infections are widespread in the human population, with over 150 million people worldwide infected each year [18–20]. Women are especially prone to UTIs: more than one in five women develops a UTI during her lifetime, with many experiencing lifelong recurrences of urinary tract disease [21–24]. In addition, catheter-associated UTIs 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 [25]. Recent studies estimate that the cost of UTIs (including direct costs and indirect losses in

Figure 1 | Inhibitory percentages of 96 uropathogenic *E. coli* strains for both traditional antibiotics (grey) and novel and known bacteriocins (black)

A standard bacteriocin assay was conducted using bacteriocin-producing strains tested against lawns of 96 uropathogenic *E. coli*. Inhibition was scored as a zone of clearing surrounding the producer colony. Resistance profiles for the 96 uropathogenic *E. coli* were provided by Cooley Dickinson Hospital (Northampton, MA, U.S.A.). Any strains listed as sensitive to or inhibited by each antibiotic were scored as inhibition. Novel bacteriocins are labelled with Greek letters. Antibiotic abbreviations: am, ampicillin; sam, ampicillin/sulbactam; cip, ciprofloxacin; sxt, TMP-SMX; cz, cefazolin; fox, ceftiofur; ft, nitrofurantoin; gm, gentamicin.



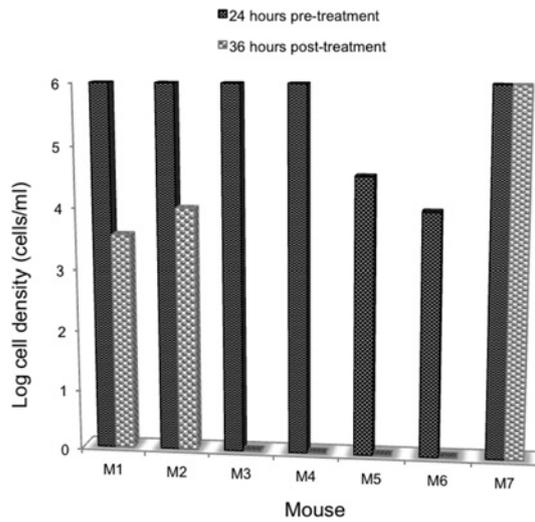
productivity due to illness or hospitalization) reaches US\$1.6 billion per year in the U.S.A. alone, excluding the costs of catheter-based infections [26].

Most UTIs are due to *Escherichia coli*, normally a commensal resident of the large intestine [27,28]. Other Gram-negative bacteria, including *Klebsiella* spp., *Enterobacter* spp., *Serratia* spp., *Proteus mirabilis*, *Ps. aeruginosa* and *Enterococcus* spp., are sometimes involved in UTIs [27,29,30]. *E. coli* is also the primary agent responsible for CAUTIs, with *Ps. aeruginosa*, *Providencia stuartii* and Staphylococci less frequently involved.

High levels of antibiotic resistance among the strains responsible for UTIs are creating a challenge to effective therapy [20,27,31–38]. Ampicillin and amoxicillin, formerly the cornerstones of UTI therapy, are no longer preferred because of high levels of resistance. TMP-SMX (trimethoprim/sulfamethoxazole) is considered the drug of choice for uncomplicated UTIs because of its low cost and well-established efficacy [39,40]. Levels of resistance to TMP-SMX in *E. coli* now unfortunately range from 18% (for community-acquired infection) to >30% (for hospital-acquired infection), suggesting that it will soon no longer be effective as a first-line therapeutic option [35,41,42]. Nitrofurantoin is also sometimes used in place of TMP-SMX or quinolones for treatment of UTIs. Aminoglycosides, the

Figure 2 | *In vivo* effect of a bacteriocin applied to an established mouse UTI

Mice were treated with 2 μg of a colicin bladder wash.



next line of defence, are highly effective for the treatment of infections caused by Gram-negative bacilli, including those strains causing UTIs. Their practical use, however, is limited due to high associated risk of nephro- and oro-toxicity. Fluoroquinolones also represent a significant advance in UTI therapy, but their cost and the importance of limiting their use, so as to prevent the emergence of resistant strains, excludes them as first-line therapy for uncomplicated UTIs, although they are the preferred drugs for complicated UTIs.

We have initiated investigations to explore the therapeutic potential of bacteriocins in the treatment of UTIs. Figure 1 shows the frequency of inhibition of 96 uropathogenic strains of *E. coli* with both classical antibiotics and novel and known

bacteriocins. It is clear from these data that bacteriocins are as effective at inhibiting uropathogens as our most potent broad-spectrum drugs. Not shown are the data on MICs (minimum inhibitory concentrations) for these same drugs, which reveal that bacteriocins possess MICs as low as for the traditional antibiotics. One particularly vexing aspect of CAUTIs is that the uropathogens form dense biofilms on the catheter surfaces, which are virtually impossible to eliminate with classical antibiotics. In contrast, bacteriocins not only inhibit the growth of biofilms, but some even break down existing biofilms [43,44].

Bacteriocins have been shown to be effective *in vivo* in several experimental systems. Figure 2 shows the *in vivo* effect of a bacteriocin applied to an established mouse UTI. A relatively small dose of bacteriocin ($\sim 2 \mu\text{g}$) 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. A further study revealed that simply coating a catheter with a bacteriocin-producing strain of bacteria before insertion eliminated subsequent biofilm formation [44].

The use of bacteriocins will ultimately select for resistant strains, just as is the case with classical antibiotics. However, because their therapeutic use would be directed at specific infections, the intensity of resistance selection would be 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, significantly reducing the probability of the emergence of resistance as an outcome (Figure 3). Table 1 shows a second approach to combating resistance emergence: the use of a single bacteriocin with targeted substitutions that result in highly efficacious toxins with slightly altered specificity. The use of two or more of these variants results in a significantly reduced frequency of resistance.

Figure 3 | Colicin combinations reduce resistance frequency by several orders of magnitude

Crude lysates of colicins (equivalent units) were applied to a sensitive strain and resistance rates were measured

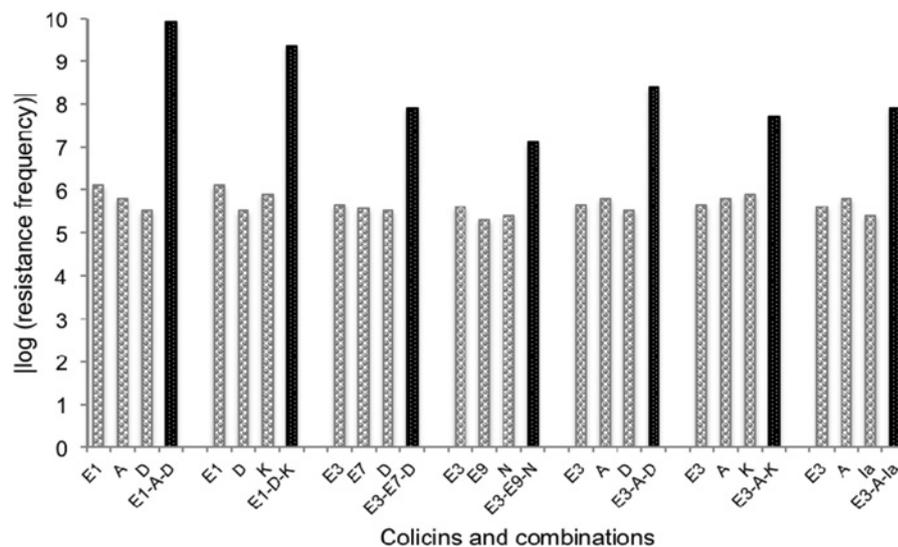


Table 1 | Targeted substitutions in the receptor recognition region result in efficacious toxins with slightly altered receptor recognition specificity

Colicin variant	Mutation	Relative MIC
E9.0	None	1.0
E9.1	L293R	1.3
E9.2	L293H	0.7
E9.3	R291H	1.6
E9.4	R298H	2.5

Conclusions

In the present article, we can only begin to touch on the compelling features of bacteriocins that make them such an attractive candidate for therapeutic development. Some of these features include: targeted specificity, limited impact on normal healthy microflora of the patient, significant reduction in the selective pressures for resistance, a history of effective use in food preservation, mounting evidence of limited toxicity to mammalian cells, ease of production and their stable durable nature. There is much to be explored in the development of bacteriocins as therapeutic agents. However, all of the early signs suggest that this potent family of toxins can provide a compelling alternative approach to enable the production of an ecologically sound alternative for the treatment of infectious disease.

Antibiotic resistance is becoming a global health crisis, and there is no magic bullet on the horizon. The traditional paradigm for antibiotic discovery, development and therapy is not capable of responding to the rapid evolution and shifting ecology of our most virulent pathogens. We must act now to search for alternative solutions to this fundamental challenge to human health. We propose to develop a drug-development programme, based on sound ecological principles, which begins with lead compounds honed by three billion years of evolution. Our approach provides a bold challenge to the existing broad-spectrum drug discovery and development paradigm. If we are to outsmart germs at the game they play so well, we must attempt something radically different.

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