Shooting the messenger of antibiotic resistance: plasmid elimination as a potential counter-evolutionary tactic

'When facing a life-threatening, multi-resistant infection, even a toxic mixture of antibiotic and curing compounds might be preferable…'

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Mortality caused by bacterial infectious diseases decreased significantly with the introduction of antibiotics, but is again on the rise. Emerging pathogens, poverty and multi-drug resistance are contributing to this change. Many people die each year due to infections from antibiotic-resistant bacteria, and several billions of dollars are lost because of the need for additional therapies, extended hospitalization and other medical services, lost labor hours, and other complications. The pace at which new antibiotics are being developed is, so far, slower than the emergence and spread of resistance traits by horizontal gene transfer. We propose that drugs that act to eliminate or inhibit the replication of resistance-gene vectors (such as plasmids) might, under some circumstances, add years of life to current and pending antibiotics.

The origins of resistance

The evolution of antibiotic resistance is as much an effect of antibiotics acting on the environment and physiology of bacteria as it is a Darwinian phenomenon of the selection of pre-existing resistant strains [1]. Antibiotics can promote gene transfer by creating environments that concentrate resistance genes and by conditioning bacterial physiology to be more receptive to foreign DNA [2,3]. Mutations and the acquisition of new genes explain some resistance phenotypes [3], but they do not satisfactorily explain the pace, range and physiological diversity of resistance mechanisms [1]. How is it that resistance genes are found so commonly on plasmids and other horizontally mobile vectors, such as conjugative transposons and phages?

Horizontally mobile vectors transcend the boundaries of the organism in which the resistance gene initially appeared and exceed the limitations imposed on replication by clonal inheritance. Resistance was probably introduced into most taxa and individuals by horizontal gene transfer. There is no doubt that microbial participation in the broad network of organisms formed through the horizontal exchange of genes has driven the dispersal of resistance [4–7].

Why target plasmids?

Plasmids are ubiquitous in bacteria [8,9]. They might also cycle through other kinds of cellular organisms but, like viruses, do not accumulate because they do not replicate, cannot integrate into the genome or are actively degraded. They have long been characterized as parasitic or dispensable accessories – a way for bacteria to generate genetic diversity – that are not essential to survival in most environments. We have taken the view that neither of these categories precisely define the nature of plasmids [10]. Indeed, plasmids occupy a different 'evolutionary space' from those genes that primarily evolve in association with hosts adapting to environmental change [11,12]. The space, or reproductive niche, that plasmids occupy is not as sensitive to the effects of antibiotics as is the viability, or reproduction, of the host [3]. Understanding how plasmids evolve to carry resistance genes could enable us to develop the kinds of drugs that prevent plasmids from serving as the messengers of resistance [13,14].

We update the proposal [15] that – under very circumscribed conditions – agents that reduce plasmid stability, or expression of plasmid genes, can be clinically useful. Plasmids are profoundly important vectors of many forms of antibiotic resistance. Eliminating plasmids as a means to

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recover the usefulness of antibiotics has, consequently, occurred to others [15]. Some 30 years ago, experiments *in vitro* and *in vivo* [16] showed that common plasmid 'curing' agents could be used with antibiotics against resistant bacteria.

Challenges

Why return to this idea now? Four important obstacles extinguished previous anti-plasmid therapies: (i) the enormous variability of plasmids made it impossible to find a 'broad-spectrum' curing agent; (ii) most compounds known to cure plasmids, such as ethidium bromide (a mutagen) or SDS (sodium dodecylsulfate; a detergent), are toxic to humans; (iii) new conventional antibiotics were still being developed contemporaneously with these anti-plasmid ideas, reducing the need for anti-plasmid drugs; and (iv) plasmids are not the universal bearers of resistance genes and targeting them is not a panacea. But the science and circumstances have changed dramatically since these ideas were previously considered for commercial viability; most notably, the pace of the identification of new drugs and new drug targets is now much slower. As a result, reasons for rejecting this line of drug development might not be so obvious any longer.

Many antibiotics that are now used as last resorts are comparatively more toxic than the 'wonder drugs' of the mid-20th century. For instance, the 'occasional' seizures caused by imipenem have become reasonable side-effects only because these are often the last resort against multi-resistant bacteria. The difference in toxicity between some last-resort antibiotics and some potential anti-plasmid drugs has therefore lessened. When facing a life-threatening, multi-resistant infection, even a toxic mixture of antibiotic and curing compounds might be preferable to the outcome of a delayed or failed treatment, especially now when fewer new drugs are entering the market.

Are plasmids underestimated as messengers? The fact that resistance is now frequently conferred by chromosomal genes belies the history of their creation and spread. These genes, or parts of these genes, were introduced into the chromosomes of the pathogens by gene transfer in many, if not all, cases [17–19].

These issues remain real challenges in the development of anti-plasmid therapies, but are not exclusive to this endeavor. If horizontal gene transfer were involved at some stage in most cases of resistance, particularly plasmid-mediated gene transfer, then this strategy could have merit.

Why might this strategy succeed?

Use of curing agents could be sufficient to remove those genes necessary for virulence and resistance because both types are frequently carried by plasmids. For example, the virulence of *Bacillus anthracis* (the causative agent of anthrax) is related to the number of virulence-plasmid copies a strain carries [20].

Exploiting the biochemical differences between plasmids and chromosomes might lead to a new sophistication in curing technology. Plasmids are cured by:

- intercalating compounds, such as ethidium bromide or acridine orange [21];
- agents that indirectly damage DNA, such as quinolones [22];
- compounds that affect the architecture of cell membranes, thereby disrupting plasmid partition, such as SDS or phenothiazines [23].

Curing agents can be applied at concentrations that do not kill bacteria but do destabilize plasmids, enabling continued chromosomal replication and gene expression. The chromosome seems to be less susceptible to these agents, and/or more readily repaired, than plasmids. For example, quinolone antibiotics [22], rifampicin [24], and the anti-cancer drugs bleomycin [25] and *cis*-dichlorodiamine platinum (II) [26] all eliminate several kinds of plasmids at sub-inhibitory concentrations.

Although currently available curing agents are toxic, they might not be equally toxic to bacteria and humans. The 'mild genotoxicity' of ascorbic acid, which is known to cause DNA damage and cure plasmids from bacteria [27], does not have an equivalently potent effect on DNA in eukaryotes [28]. There is hope for new curing agents that are relatively non-toxic to cellular life but lethal to plasmids, but many more curing agents need to be identified. Some other potentially useful compounds might be mined from the source of antibiotics – the microbial world – in the form of microcins and small antimicrobial peptides, such as those with activities similar to the quinolone antibiotics [29].

Co-technologies are the key

Epidemiology focused at the plasmid, strain and disease levels would make anti-plasmid therapies more effective. If plasmids carrying several resistance genes or carrying resistance and important virulence genes were eliminated during therapy, then more time and more options for treatment would be available to the clinician. Epidemiological plasmid-profiling surveillance will enable clinicians to plan for particular plasmids in strains causing diseases at any place or time. The epidemiology of such plasmids could be easily carried out using polymerase chain reaction (PCR)- or microarray-based surveys. Plasmids that represent clear public health threats, such as multi-resistance plasmids in enteric bacteria or vancomycin-resistance plasmids in enterococci, could be targeted.

Anti-plasmid therapeutics must be reserved for human medicine, and applied only if the disease is correctly diagnosed. Otherwise, we would again be promoting the emergence of resistance. New diagnosis technologies that are quicker and more accurate than those that sufficed in the past, must be developed as a co-technology for this strategy to avoid the undesirable resistance outcomes of the antibiotic era. An anti-plasmid strategy would be most effectively applied in the treatment of a severely ill patient known to have a susceptible plasmid–bacterium combination, or in curtailing an epidemic caused by a single type of plasmid within a hospital. Anti-plasmid therapy could also substitute for antibiotic therapy when a crucial virulence determinant is carried by the plasmid.

However, abuse of curing agents could have more severe consequences than the overuse of antibiotics. Plasmids selected to withstand the effect of such curing agents might be even more formidable as resistance and virulence vectors. Such enhanced plasmids might not only put current antimicrobial agents in jeopardy, but also compounds still in development, because resistance might develop and spread more readily with the aid of such plasmids. With these issues in mind, we believe there is value in re-tooling to shoot the messengers (the horizontally mobile elements such as plasmids), rather than relying solely on the old magic bullets (the antibiotics), to shoot the bacteria.

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