The biological cost of antibiotic resistance

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The frequency and rates of ascent and dissemination of antibiotic resistance in bacterial populations are anticipated to be directly related to the volume of antibiotic use and inversely related to the cost that resistance imposes on the fitness of bacteria. The data available from recent laboratory studies suggest that most, but not all, resistance-determining mutations and accessory elements engender some fitness cost, but those costs are likely to be ameliorated by subsequent evolution.

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Introduction

The use of antibiotics by humans can be seen as an evolutionary experiment of enormous magnitude, a window from which to view not-quite-natural selection operating in real time. Within 50 years, the number of species and strains of pathogenic and commensal bacteria resistant to antibiotics and the number of antibiotics to which they are resistant has increased virtually monotonically world-wide. Infections that had been readily treatable by chemotherapy may no longer be [1–4]. It is clear that the evolution and spread of resistance can be attributed to the use and overuse of antibiotics (also see Guillemot's review on antibiotic usage, this issue, pp 494–498). Not so clear is whether this situation can be reversed in a reasonable amount of time. That depends on factors which we may be able to control, such as the rate and pattern of antibiotic use, but also on factors over which we have no control, the biological cost resistance imposes on the fitness of bacteria [5•,6•], and the rate and degree to which natural selection will ameliorate these costs.

Measuring the biological costs of resistance

The fitness of the pathogens that are the targets of antibiotic therapy is a complex character with a number of interrelated elements. The most important of these are the relative rates at which antibiotic-sensitive and -resistant bacteria firstly, reproduce and die (compete) in infected hosts and the environment, secondly, are transmitted between hosts, and thirdly, are cleared from infected hosts [5•,6•]. At any given time, the magnitude of these elements will depend on the extent and pattern of antibiotic use.

These components of the biological cost of resistance can be measured in essentially three ways: first, retrospectively, by fitting mathematical and numerical models of the changes in frequencies of hosts infected with antibiotic-sensitive and -resistant strains of bacteria following known changes in the rates and patterns of antibiotic use in human populations; second, prospectively, by measuring the rates at which individual humans become infected with and cleared of sensitive and resistant bacteria; and third, experimentally, by estimating the relative rates of growth, survival, transmission and clearence of sensitive and resistant bacteria *in vitro* and *in vivo*. We are aware of only one study that formally estimated the biological cost of resistance by the first of these methods [6•], none by the second, and only a modest number by the third. Because of this bias and our own biases, we concentrate most of the rest of this 'opinion' on the results and interpretation of the modest number of experimental studies of the costs of antibiotic resistance and the adaptation to those costs.

Experimentally estimating the costs of resistance and studying the adaptation to those costs

Experimental studies of the biological cost of resistance have focused almost exclusively on the relative rates of growth, survival and competitive performance of antibiotic-sensitive and -resistant bacteria. In some studies, the costs of resistance were estimated from the exponential growth rates of sensitive and resistant bacteria in monocultures [7••,8•] but more commonly they have been measured by pairwise competition experiments. Mixtures of otherwise isogenic sensitive and resistant strains are inoculated into chemostats, batch cultures or laboratory animals, and the changes in their relative frequencies are followed by selective plating [7••,8•,9–12]. These pairwise competition experiments simultaneously estimate a number of components of the competitive fitness of sensitive and resistant bacteria: their lag periods, rates of exponential growth, resource utilization efficiencies, and their rates of mortality in the presence and absence of host defenses. By extensively replicating these pairwise competition experiments, it is possible to detect differences in fitness as small as 1% [13,14].

The rate, nature and consequences of evolution of the costs of resistance can be followed *in vitro* by maintaining resistant bacteria in chemostats, by serial passages *in vitro* or in experimental animals. By pairwise competition with the ancestral strains (or from the decline in the frequency of a low density, selectively neutral, indicator strain) it is possible to determine whether fitter mutants ascended to high frequency and isolate such mutants. One can then ascertain whether adaptation to the cost of resistance is accomplished by one of several possible events: first, reversion to wild type by back mutation or the loss of a resistance gene or accessory element; second, compensation by intracistronic or intercistronic suppression of the chromosomal resistance gene [7••,12]; or third, genetic changes in the accessory

element responsible for resistance and/or host adaptation to the carriage of this element [10,15,16].

The most general limitation of estimating the cost of resistance in these laboratory experiments is the interpretation of negative results. If no effect of resistance is observed in competition experiments performed under a variety of *in vitro* and *in vivo* conditions, it becomes increasingly likely that the costs of resistance are low or non-existent. It still remains possible, however, that there are realistic conditions where these costs are profound. On the other hand, if significant costs of resistance are observed in these experiments, it is reasonable to assume that in natural populations there are conditions where resistance would engender a burden on the fitness of a bacterium. Another limitation of the experimental studies of the cost of resistance performed to date is the focus on growth and competitive performance. Although one can estimate the transmission and clearance costs of drug resistance experimentally, to our knowledge, those experiments have not been performed (or not published).

Is there a cost to resistance?

It is convenient to separately consider the costs of resistance encoded by chromosomal mutations, where resistance is achieved primarily by the modification of target molecules [17–21], and that determined by accessory elements, where resistance is generally due to enzymes that inactivate the antibiotic or pumps that remove it from the cell [17–23]. Resistance encoded by accessory elements may also give rise to costs associated with the replication and maintenance of the elements themselves.

In the majority of studies performed, resistance caused by target alterations has been found to engender some cost to fitness (Table 1), but mutants with no measurable costs have also been observed. One example of a 'no cost' resistance mutation is the 42nd codon AAA (Lys)→AGA (Arg) substitution of the *rpsL* gene, responsible for resistance to high concentrations of streptomycin in *S. typhimurium* and other enteric bacteria [7••,8•]. Other substitutions at the same position cause severe reductions in fitness both *in vitro* and in mice [7••,12], whereas these *rpsL* AGA mutations appear to be selectively neutral and may even confer a slight advantage over wild type [7••].

Also apparent from these studies is that the fitness cost associated with chromosomal resistance depends on growth conditions. For example, resistant mutants that show no cost in laboratory medium may have large costs in laboratory mice, and conversely, mutants that show no cost in mice may have substantial costs *in vitro* (J Björkman, D Hughes, DI Andersson, unpublished data). This conditionality supports the view that experimental estimates of the cost of resistance should be done under a variety of culture conditions *in vitro* as well as in experimental animals.

A number of studies have reported fitness burdens associated with the carriage of resistance-encoding (R) plasmids [9–11,24–27] At this juncture, however, it is not clear how common these costs are and how they vary among plasmid–host combinations from natural populations. All of the quantitative studies estimating the fitness burden of R-plasmids of which we are aware have been done *in vitro* and most commonly with cloning and other plasmids that have been genetically altered or maintained in the laboratory for extensive periods.

Reversion, compensatory evolution and amelioration of fitness costs

Although occasionally, in the absence of antibiotics, drug-sensitive revertants have evolved in most cases, adaptation to the costs of chromosomal resistance *in vitro* and *in vivo* has been through compensatory mutations (Table 2). In the majority, but not all cases, the second site mutations compensating for the cost of resistance have been identified. These occur by additional (or alternative) mutations at the same locus as the resistance gene, intragenic suppression, or at other loci, extragenic suppression. The physiological mechanisms by which compensatory mutations restore fitness have been determined for a few cases. For example, compensation for the cost of streptomycin

Table 1

The biological cost of resistance conferred by target-altering chromosomal mutations.

Bacteria	Resistance	Mutation	Cost	Assay system	Reference
S. typhimurium	Streptomycin	*rpsL*	Yes/no	Mice, *in vitro*	[7••,8•]
	Rifampicin	*rpoB*	Yes	Mice, *in vitro*	[7••]
	Nalidixic acid	*gyrA*	Yes	Mice, *in vitro*	[7••]
	Fusidic acid	*fusA*	Yes/no	Mice, *in vitro*	[34](a)
E. coli	Streptomycin	*rpsL*	Yes/no	*In vitro*	[12,32]
	Rifampicin	*rpoB*	Yes/no	*In vitro*	(b)
M. tuberculosis/bovis	Isoniazid	*katG*	Yes	Mice	[35–37]
S. aureus	Fusidic acid	*fus*	Yes/no	Rats, *in vitro*	(a)

[a]J Björkman *et al*., unpublished data. [b]BR Levin *et al*., unpublished data.

Table 2

Compensatory evolution and amelioration of fitness losses caused by chromosomal mutations.

Bacteria	Resistance mutation (resistance)	Compensatory mutation (resistance in compensated mutant)	Selection for compensation in	Reference
S. typhimurium	*rpsL* (streptomycin)	Intragenic, *rpsL* (maintained)	Mice	[7••]
	rpsL (streptomcyin)	Extragenic, *rpsD/E*, (maintained)	Laboratory medium	[7••,8•]
	gyrA (nalidixic acid)	Intragenic, *gyrA* (maintained)	Mice	[7••]
	rpoB (rifampicin)	Intragenic, *rpoB* (maintained)	Mice	[7••]
	fusA (fusidic acid)	True reversion, *fusA* (lost)	Mice	(a)
	fusA (fusidic acid)	Intragenic, *fusA* (often maintained)	Laboratory medium	[34]
S. aureus	*fus* (fusidic acid)	Intragenic, *fu*, (maintained/lost)	Laboratory medium	(a)
E. coli	*rpsL* (streptomcyin)	Extragenic, *rpsD/E* (maintained)	Laboratory medium	[12,32]
	rpoB (rifampicin)	Intragenic, *rpoB* (maintained)	Laboratory medium	(b)
M. tuberculosis	*katG* (isoniazid)	Extragenic, *ahpC* (maintained)	Humans	[33]

[a]J Björkman *et al*., unpublished data. [b]BR Levin *et al*., unpublished data.

resistance in *rpsL* mutants is achieved by second site mutations restoring the efficacy and rate of translation to wild-type or nearly wild-type levels [8•,12].

Why, in the absence of antibiotics, evolution ameliorating the cost of resistance is more common than reversion to drug sensitivity has been attributed to two processes: compensatory mutations being more common than true reversion (which is commonly restricted to single nucleotide substitution), and the population bottlenecks associated with serial passage [28•]. Moreover, once the compensatory mutants are fixed in the population, revertants are unlikely to ascend because in the genetic background of the compensatory mutant they may be at a substantial disadvantage [8•,12]. Also see Borman *et al*. [29] for the same phenomenon for the adaptation of HIV resistance to a protease inhibitor.

The degree of restoration of fitness by the compensatory mutations varies greatly, and in some cases restoration appears complete, whereas in others it is only partial [7••,8•,12]. An unexpected finding is that the types of compensatory mutations obtained depend on the conditions under which they were selected. One example is the streptomycin resistant *rpsL* mutants in *S. typhimurium*. When selected in mice, compensatory mutations are only intragenic. In contrast, when selection for restored fitness is done in laboratory medium, compensation occurs by extragenic suppressor mutations [7••,8•].

The few experimental studies that have been done on plasmid–host coevolution suggest that compensatory evolution may also play a prominent role in the adaptation to accessory-element-encoded resistance [10,11,15,16,24,26]. Although R-plasmids could be lost by vegetative segregation, two processes work against this. One is post segregational killing [30] by which R plasmids, like R1 and RK2, have mechanisms that kill bacteria that lose these elements. The other is, of course, intermittent antibiotic-mediated selection for one or more of the resistance-encoding genes borne by these elements.

Reality

Experimental studies of the costs of resistance, and adaptation to those costs make a number of predictions that can be tested (and rejected) by examining the resistance genes and accessory elements found in bacteria isolated from humans and domestic animals to see if the same mutations ascend. The results of the few tests of this type done to date suggest that chromosomal mutations responsible for acquired resistance in pathogenic bacteria are likely to be the same as those mutations observed to have little or no cost experimentally. For example, Böttger *et al* [31•] found that the *rpsL* mutations responsible for resistance to streptomycin in clinical isolates of *Mycobacterium tuberculosis*, were the same as those that had no cost in experiments done in *S. typhimurium* and *Escherichia coli*. Not so clear from this study, is whether these 'no cost' streptomycin-resistance genes were the primary mutations, or evolved to compensate for a more costly original mutation as has also been observed experimentally [7••,8•,12,32]. To test for compensatory evolution, it will be necessary to either see the second site compensatory mutations along with the original mutations observed *in vitro*, or follow the progression from the first appearance of primary resistance in a patient.

To our knowledge, the only evidence for compensatory evolution occurring in bacteria isolated from patients comes from a retrospective study of isoniazid-resistant *M. tuberculosis* [33]. These bacteria become resistant by virtue of knock-out mutations in the *katG* gene, which cause a loss of catalase activity and avirulence [34–36]. The majority of clinical isolates with the *katG* mutation also contain a promoter-up mutation in the *ahpC* gene, which causes an increase in the level of alkyl hydroxyperoxidase reductase (AhpC). Even though no direct causality has been established, it is likely that the overproduction of AhpC due to promoter-up mutations compensates for the

lack of catalase in the isoniazid resistant *katG* mutants and restores virulence [33].

Implications

Although it has been long thought that antibiotic-resistance genes and accessory elements would engender a cost in the fitness of bacteria, the actual evidence for this being the case is, at best, modest and that which has been gathered recently does not paint a rosy picture for the future of the resistance problem. Resistance mutations, such as those found in the bacteria from patients treated with antibiotics, have virtually no cost when measured by competition experiments *in vitro* or in experimental animals. Moreover, in those cases where resistance mutations and accessory elements engender a cost, subsequent evolution in the absence of antibiotics commonly results in the amelioration of those costs rather than reversion to drug sensitivity. If these laboratory observations reflect the situation for bacteria in hospital and community acquired infections, even low levels of antibiotic use could be sufficient for the ascent and long-term persistence of resistance [5•,6•].

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