INTERNATL MICROBIOL (1998) 1:265–270


REVIEW ARTICLE

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# Bacterial evolution and the cost of antibiotic resistance

Received 15 May 1998
Accepted 20 June 1998

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**Summary** Bacteria clearly benefit from the possession of an antibiotic resistance gene when the corresponding antibiotic is present. But do resistant bacteria suffer a cost of resistance (i.e., a reduction in fitness) when the antibiotic is absent? If so, then one strategy to control the spread of resistance would be to suspend the use of a particular antibiotic until resistant genotypes declined to low frequency. Numerous studies have indeed shown that resistant genotypes are less fit than their sensitive counterparts in the absence of antibiotic, indicating a cost of resistance. But there is an important caveat: these studies have put resistance genes into naive bacteria, which have no evolutionary history of association with the resistance genes. An important question, therefore, is whether bacteria can overcome the cost of resistance by evolving adaptations that counteract the harmful side-effects of resistance genes. In fact, several experiments (in vitro and in vivo) show that the cost of antibiotic resistance can be substantially diminished, even eliminated, by evolutionary changes in bacteria over rather short periods of time. As a consequence, it becomes increasingly difficult to eliminate resistant genotypes simply by suspending the use of antibiotics.

**Key words** Antibiotic resistance · Compensatory mutations · Evolution · Fitness · Plasmids

## Introduction

Antibiotic-resistant bacteria impose a substantial burden on the human population. In addition to morbidity and mortality caused by infections with resistant pathogens, society as a whole must pay for the development of new antibiotics to keep pace with continually evolving pathogens. It is clear, therefore, that there is a cost associated with antibiotic resistance from the perspective of human society. But is there any cost associated with antibiotic resistance from the perspective of a bacterium?

In an environment that contains an antibiotic, possession of a corresponding resistance gene is clearly beneficial to a bacterium. However, in the absence of antibiotic, resistant genotypes may have lower growth rates than their sensitive counterparts. Mutations that confer resistance do so by disrupting some normal physiological process in the cell, thereby causing detrimental side-effects. In the case of plasmid-encoded resistance functions, bacteria must synthesize additional nucleic acids and proteins; this synthesis imposes an energetic burden [7] and the products that are synthesized may interfere with the cell's physiology [12]. Resistant bacteria may therefore be inferior competitors to sensitive genotypes in the absence of antibiotics. If so, then a strategy for containing the spread of resistance would be to suspend the use of a particular antibiotic until resistant genotypes had declined to low frequency. (See [3] for a detailed analysis of this and related strategies.)

The efficacy of this strategy depends, in part, on the cost of antibiotic resistance to the bacteria. Assuming that some sensitive bacteria survive antibiotic treatment (or colonize after the treatment has ended), the amount of time necessary to reduce the abundance of resistant bacteria to a specified low level is

This paper is updated from one previously published in a Novartis Foundation symposium volume: Lenski RE (1997) The cost of antibiotic resistance—from the perspective of a bacterium. In: Chadwick DJ, Goode J (eds) Antibiotic Resistance: Origins, Evolution, Selection and Spread. Chichester: Wiley and Sons, pp 131–151

inversely proportional to the cost of resistance. For example, it would take ten times as long to eliminate a population of resistant bacteria when the cost of resistance is only 1% as compared to when the cost of resistance is 10% [10]. Thus, the efficacy of controlling the spread of antibiotic resistance by suspending the usage of an antibiotic is critically dependent on the relative fitness of resistant and sensitive genotypes in the absence of antibiotic.

In the following sections, I review several experiments that have measured the costs of antibiotic resistance from the perspective of bacteria. The major findings are two-fold. On the one hand, resistant bacteria are often inferior competitors to their sensitive counterparts in the absence of antibiotic. On the other hand, the costs of antibiotic resistance can evolve, and they tend to be reduced over time by natural selection. Unfortunately, this trend implies that it will become more and more difficult over time to control the spread of resistant strains.

## Results and Discussion

**Consensus findings indicate a cost to resistance** Many studies have shown that resistant genotypes are less fit than their sensitive progenitors when the two compete in an antibiotic-free medium. Some of these studies have demonstrated costs associated with carriage of plasmids and expression of plasmid-encoded resistance functions (e.g., [23]), whereas others have demonstrated side-effects of resistance mutations that impair growth (e.g., [8]).

In some other studies, there was no discernible cost to antibiotic resistance. Perhaps these results reflect a real absence of cost, or perhaps they simply indicate that the cost was too small to be seen given the experimental resolution. For example, Nguyen et al. [16] observed a large cost due to constitutive expression of plasmid-encoded tetracycline resistance in *Escherichia coli*, but they could not measure any significant cost for inducible resistance beyond the small cost of the plasmid vector itself. Evidently, repression of a resistance gene can be quite effective in avoiding the cost of resistance in an antibiotic-free environment.

I know of only one study in which a resistance function was shown to confer an immediate (see below) selective advantage in the absence of antibiotic. Blot et al. [2] showed that a Tn*5*-encoded bleomycin-resistance gene enhanced the survival of *E. coli* during prolonged starvation, even though no antibiotic was present. Bleomycin causes damage to DNA, and such damage may also occur during prolonged starvation. The gene product responsible for bleomycin resistance is thought to play some role in DNA repair, which may explain its beneficial effect during starvation.

**But there is a caveat** The preceding evidence indicates that resistant bacteria are often at a competitive disadvantage relative to their sensitive counterparts when there is no antibiotic present in the environment. However, there is an important qualification to this conclusion: These studies have been performed by putting an antibiotic-resistance gene (either a plasmid-encoded function or a chromosomal mutation) into a "naive" bacterium, one which has no evolutionary history of association with that resistance gene. An important question, therefore, is whether the cost of resistance can be reduced or even eliminated by allowing the bacterium to adapt to the resistance gene. I will now review several experimental studies that have addressed this issue.

**Evolutionary reductions in the cost of plasmid-encoded antibiotic resistance** Plasmid pACYC184 encodes resistance to two antibiotics, tetracycline and chloramphenicol. Bouma and Lenski [4] transformed a laboratory strain of *E. coli* with pACYC184, and they showed that the plasmid-bearing construct was competitively inferior relative to its plasmid-free counterpart in a minimal medium without antibiotic (Fig. 1A). The bacterial strain that they used had no history of association with pACYC184, and so they sought to determine if the cost of plasmid carriage could be reduced by evolutionary changes in either the host or the plasmid. To that end, Bouma and Lenski propagated the plasmid-host association for 500 generations (75 days) in the same medium, except supplemented with chloramphenicol. (The supplemental antibiotic prevented spontaneous plasmid-free segregants from out-competing the plasmid-bearing cells, which would have defeated the purpose of the experiment.)

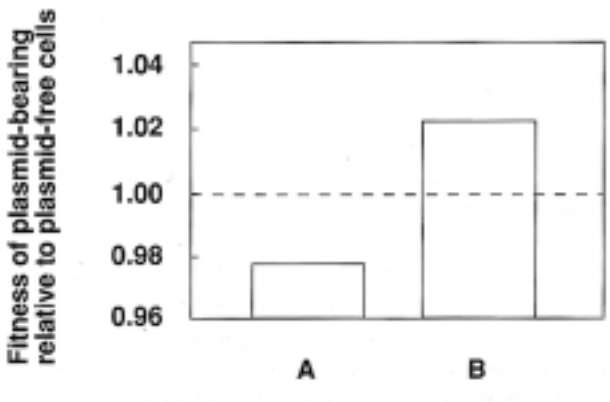


**Fig. 1** Effects of pACYC184 on the fitness of ancestral and evolved *Escherichia coli* hosts, in the absence of any antibiotic (summary of data from reference [13]). $B_0$ denotes the ancestral genotype, and $B_{500}$ is a genotype that evolved with pACYC184 for 500 generations. (A) Fitness of $B_0$/pACYC184 versus its plasmid-free counterpart, $B_0$. (B) Fitness of $B_{500}$/pACYC184 versus its plasmid-free counterpart, $B_{500}$. The values shown in (A) and (B) are the means of 30 replicate competition experiments. The costs and benefits of plasmid carriage for the ancestral and evolved genotypes, respectively, are both statistically significant

After 500 generations, Bouma and Lenski [4] isolated a spontaneous plasmid-free segregant of the evolved bacterial host. They then transformed each of the ancestral and evolved

hosts with both the ancestral and evolved plasmids, giving four genotypes: $B_0/P_0$, $B_0/P_{500}$, $B_{500}/P_0$, and $B_{500}/P_{500}$, where *B* and *P* denote the bacteria and plasmid, respectively, and subscripts 0 and 500 indicate the ancestral (naive) and evolved forms, respectively. Each of these genotypes was placed in competition with a genetically marked variant of $B_0/P_0$ in the same medium used during the 500 generations of experimental evolution. These competition experiments indicated that genetic adaptation had occurred in the bacterial chromosome, but not in the plasmid. That is, $B_{500}$ was competitively superior to $B_0$, and its advantage was unaffected by the plasmid's evolutionary status. These data demonstrated that the bacteria had adapted evolutionarily, but they did not show whether the bacteria had adapted to the plasmid, to the culture medium, or to some combination of the two.

To determine whether the bacteria had adapted to the plasmid, Bouma and Lenski [4] performed competition experiments in antibiotic-free medium between the evolved bacteria with and without the ancestral plasmid (i.e., $B_{500}/P_0$ versus $B_{500}$). If the bacteria had adapted specifically to the plasmid, then the cost of carriage should be lower in the evolved bacteria than in the ancestor. To their surprise, Bouma and Lenski found that the evolved plasmid-bearing bacteria had a competitive advantage relative to the evolved plasmid-free bacteria even in the absence of antibiotic (Fig. 1B). Thus, not only was the cost of plasmid carriage eliminated, but the plasmid actually benefited the bacteria that had evolved with the plasmid present.

Lenski et al. [13] sought to identify what aspect of the plasmid was beneficial to the evolved bacteria, but not to its naive ancestor. They constructed a series of plasmids in which they deleted either the chloramphenicol or tetracycline resistance functions from pACYC184. Resistance to chloramphenicol occurs by the enzymatic acetylation of the antibiotic, which renders it inactive. Resistance to tetracycline involves active efflux of the antibiotic using a trans-membrane protein. They showed that expression of chloramphenicol resistance imposed a significant cost for both the naive and evolved host bacteria. However, expression of the tetracycline resistance function was actually beneficial to the evolved bacteria (but not to the naive ancestor) in the absence of antibiotic. That is, an evolved host which carries a plasmid that expresses tetracycline resistance is competitively superior to its plasmid-free counterpart, whereas an evolved host which carries a plasmid that does not express tetracycline resistance is less fit than its plasmid-free counterpart (Table 1).

Although the physiological basis of this effect is not yet fully understood, this study shows clearly that the cost of plasmid-encoded antibiotic resistance can be reduced or even eliminated by natural selection. When pACYC184 was introduced into the naive bacterium, it was lost in the absence of antibiotic as spontaneous plasmid-free segregants competitively excluded the plasmid-bearing cells. But as a consequence of the evolutionary adaptation of the bacteria to the plasmid, plasmid-free segregants no longer have a competitive advantage and the plasmid-encoded antibiotic resistance is stably maintained.

In a conceptually similar study, Modi and Adams [15] examined the coevolution of *E. coli* and a derivative of plasmid pBR322 that encodes resistance to ampicillin and tetracycline. After about 800 generations, they found that the cost of plasmid carriage to the bacterial host had been significantly reduced, although it was not entirely eliminated in this case. They also showed that genetic changes in both the bacterial and plasmid genomes contributed to the reduced cost of plasmid carriage. As with the previous study, Modi and Adams' results indicate that it can become more difficult to eliminate resistance after bacteria and plasmids have had a history of association.

Turner et al [22] recently examined a somewhat different, but related, question: How does the cost of plasmid carriage depend on the plasmid's transmissibility? Starting with a conjugative plasmid isolated from nature, they derived ten plasmids during a 500-generation experiment. Five of these plasmids evolved higher rates of conjugative transmission than the ancestral plasmid, while five others evolved lower rates (including two that became unable to conjugate). The plasmids that had evolved higher conjugation rates became more costly to the bacteria than was the ancestral plasmid, whereas those that evolved lower conjugation rates were less costly. Evidently, the cost of plasmid carriage may increase or decrease, depending on the selective challenges that confront—and genetic solutions available to—a population of plasmids. Thus, a plasmid should tend to evolve a lower cost of carriage, all else equal, but selection for increased expression of a plasmid-encoded function (transmissibility, resistance, etc.) may sometimes oppose this tendency.

**Evolutionary reductions in the cost of chromosomal**

**Table 1** Expression of tetracycline resistance is beneficial to an *Escherichia coli* host that evolved with plasmid pACYC184 (summary of data from [13])

| Plasmid | Cm | Tc | Naive host | Evolved host |
|---|---|---|---|---|
| pACYC184 | R | R | – | + |
| pMP10 | S | R | 0 | ++ |
| pMP11 | S | R | 0 | ++ |
| pSCS1 | R | S | – | – |
| pSCS13 | R | S | – | – |

Abbreviations: Cm, chloramphenicol; Tc, tetracycline; R, resistant; S, sensitive. Plasmids pMP10 and pMP11 have deletions in the gene for chloramphenicol resistance. Plasmids pSCS1 and pSCS13 have deletions in the gene for tetracycline resistance. The last two columns indicate the effect of plasmid carriage on the fitness of naive and evolved bacterial hosts, in the absence of antibiotic. –, significant cost. 0, no significant effect. +, significant benefit. ++, significantly larger benefit.

**mutations that confer antibiotic resistance** The finding that the cost of antibiotic resistance can be reduced is not restricted to plasmid-encoded resistance. Schrag and Perrot [18] examined mutations in the *rpsL* gene of *E. coli* that confer resistance to streptomycin. In the absence of antibiotic, cells that carry these mutations are handicapped in competition with otherwise isogenic cells sensitive to streptomycin. The streptomycin resistant mutants have altered ribosomes and a lower rate of peptide-chain elongation, which may account for the cost of resistance. Schrag and Perrot also sought to determine if the cost of antibiotic resistance could be reduced by allowing the bacteria to evolve. After less than 200 generations of evolution in the absence of antibiotic, they found that the cost of resistance was substantially reduced. This cost-reduction was achieved without any significant change in the level of streptomycin resistance. Schrag and Perrot demonstrated that cost-reduction was achieved by secondary mutations outside the *rpsL* gene, and that these secondary mutations restored the rate of peptide-chain elongation to a level close to that of the sensitive progenitor (Fig. 2). More recently, Schrag et al. [19] also showed that, after these secondary mutations had achieved fixation in the population, sensitive genotypes were at a competitive disadvantage relative to resistant genotypes, even in the absence of streptomycin. Thus, there is no selective advantage to sensitive revertants once the secondary mutations that compensate for resistance have spread throughout the population (although sensitive bacteria without compensatory mutations are still the most fit type in this system and might invade from other populations). Evidently, evolving populations of bacteria can often compensate for the deleterious side-effects of resistance genes, including mutations as well as plasmid-encoded functions, and this compensation makes it more difficult or even impossible to restore antibiotic sensitivity by temporarily suspending the use of an antibiotic.

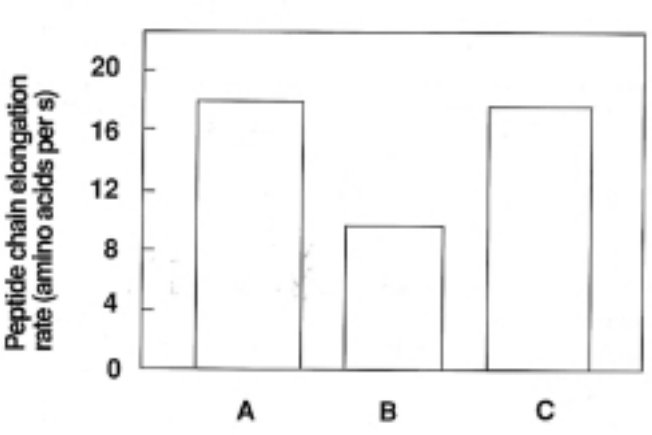


**Fig. 2** Rates of peptide chain elongation in *Escherichia coli* genotypes sensitive and resistant to streptomycin (summary of data from reference [18]). (A) Ancestral sensitive genotype. (B) Average ot two resistant genotypes. (C) Average of four resistant genotypes after 180 generations of evolutionary cost-reduction. The difference between (A) and (B) is statistically significant, whereas the difference between (A) and (C) is not

Cohan et al. [5] examined the cost of resistance to rifampicin in *Bacillus subtilis*. Mutations that confer resistance to rifampicin occur in the *rpoB* gene, which encodes the β subunit of the RNA polymerase, and these mutations tend to reduce the competitive fitness of the bacteria in the absence of antibiotic. However, Cohan et al. showed that the magnitude of this cost was variable depending on the specific mutation to rifampicin resistance as well as the particular strain of *B. subtilis* into which a mutation was transformed. (The cost of resistance did not correlate with the level of rifampicin resistance, which was high for all combinations of resistance alleles and genetic backgrounds.) This study therefore implies two mechanisms by which the cost of resistance can be ameliorated. First, selection among resistance alleles will favor those which impose the lowest costs. Second, selection among genetic backgrounds will favor those which are subject to the lowest costs. As a consequence of selection, the cost of resistance to rifampicin should become progressively reduced over time, as only the most fit combinations of resistance alleles and genetic backgrounds will prevail in competition.

**Do similar phenomena occur in nature?** The experiments reviewed above, showing the cost of resistance and its evolutionary reduction, were all performed in the laboratory and in vitro. Björkman et al. [1] recently extended these findings to an in vivo system. They demonstrated that mutants of *Salmonella typhimurium* resistant to streptomycin, rifampicin, or nalidixic acid were usually avirulent in mice, indicating that this pathogen suffers a profound cost of antibiotic resistance. But as was the case in vitro, the bacteria evolved compensatory mutations that reduced the cost of resistance and restored their virulence, even while they kept their resistance to the antibiotics.

And there is every reason to believe that similar phenomena occur in nature. For example, *Neisseria gonorrhoeae* was for many years universally susceptible to ampicillin. In 1976, resistant strains were first detected that had a plasmid-encoded β-lactamase, probably derived from an enteric bacterium. According to Roberts et al. [17], the resistance plasmids were initially very unstable, but within a few months they had become much more stable. A plausible explanation for this increased stability is an evolutionary reduction in the cost of antibiotic resistance, because plasmid instability is strongly affected by even small differences in the growth rate of plasmid-bearing cells and plasmid-free segregants [6, 11]. If some other antibiotic had been used to treat gonorrhea when resistance to ampicillin was first detected, and while the plasmid was still unstable, then resistance might have disappeared from the population; ampicillin could then have been used for many more years. But once the plasmid was stabilized, it became difficult or impossible to restore sensitivity. Evolutionary reductions in the cost of resistance have also been proposed to explain the

surprising tenacity of resistance to tetracycline and streptomycin [9, 20, 21]. The role of cost-reduction in extending the persistence of antibiotic-resistance genes in nature deserves further careful study.

**Evolutionary cost-reduction is not restricted to antibiotic resistance** Proliferation of antibiotic-resistant bacteria provides a dramatic example of evolution because of its rapidity as well as its medical importance. However, the evolution of resistance, the costs associated with resistance functions, and the subsequent reduction of costs occur in other circumstances as well. McKenzie et al. [14] describe a compelling example of all these phenomena in an insect pest. For about ten years, the insecticide diazinon was successfully used to control the Australian sheep blowfly, *Lucilia cuprina*. A mutation that conferred resistance eventually appeared and diazinon-resistant flies subsequently became widespread. At first, resistant flies developed more slowly and had reduced survival relative to their sensitive progenitors in the absence of insecticide, indicating a cost of resistance. After a few more years of continued usage of diazinon, however, a second mutation appeared in the fly population that eliminated the cost of resistance. Diazinon-resistant flies that had this second mutation were as fit as sensitive flies, even in the absence of diazinon. The opportunity to control the spread of resistance by temporarily suspending the use of diazinon was lost.

In summary, evolutionary cost-reduction is a simple and general manifestation of the tendency for organisms to undergo genetic adaptation by natural selection. Just as organisms may adapt to overcome adverse aspects of their external environment (e.g., by becoming resistant to antibiotics), so too may they adapt to overcome adverse aspects of their internal physiology (e.g., by reducing any harmful side-effects of resistance).

## Conclusions

(i) The sensitivity of bacterial pathogens to antibiotics can be viewed as a natural resource, one which has tremendous value to the human population. Unfortunately, much of this resource has already been depleted in the last half-century, as a consequence of the evolution of pathogens that are resistant to antibiotics.
(ii) In principle, antibiotic sensitivity is a renewable resource. If there is a cost of resistance to bacteria, then sensitivity may be renewed by temporarily suspending the use of an antibiotic to which resistance has emerged, thereby allowing sensitive genotypes to competitively displace their resistant counterparts.
(iii) However, after evolving resistance to antibiotics, bacteria may then adapt to the deleterious side-effects and other costs of resistance genes. Therefore, with continued use of an antibiotic after the emergence of resistant genotypes, it may become increasingly difficult to renew sensitivity.

**Acknowledgments** I thank S. Mochales and S. Levy for organizing stimulating meetings, which encouraged me to think more about bacterial evolution in the context of antibiotic resistance. Thanks also to the colleagues who discussed ideas with me and contributed to the research reviewed here.

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