Do antibiotics maintain antibiotic resistance?

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Important human pathogens resistant to antibiotics result from the human use of antibiotics. Does this imply that reducing their usage or removing antibiotics from medicine and agriculture will restore the effectiveness of these drugs? The authors argue that resistance evolution and susceptibility evolution are not, in a sense, just different sides of the same coin. Resistance genes acquire new functions and the initial costs of resistance can evolve into advantages. Decreasing drug use might not replace a fundamental change in drug design to avoid the evolution of resistant, and encourage the evolution of susceptible, microorganisms.

Drug resistance in pathogens is the result of overuse of drugs in medicine and agriculture. A fundamental question is therefore whether prevention of overuse will ensure drug resistance becomes obsolete. That expectation, a prediction of what we call the 'reservoir hypothesis', is common in the literature[1,2]. However, several experimental observations suggest that this expectation might be naive[3–6]. Many effects of antibiotics on microbial physiology and ecology illustrate how difficult it can be to predict the return of susceptibility. The purpose of this article is not to catalog every effect of antibiotics, but to inform those who develop or apply new drugs, or those attempting to restore the efficacy of current drugs, about the factors that must be considered if anti-infectives are to be used to design desirable evolutionary outcomes rather than to provoke resistance.

The reservoir hypothesis

The apparently self-evident assumptions of the reservoir hypothesis are rarely stated (Fig. 1). In brief, it assumes that the evolution of resistance is explained by the effect of drugs acting on bacteria. It further assumes that some threshold quantity of antibiotics (used here to refer generally to antimicrobial agents) is required to both induce and then maintain resistance, because of the corollary that mutations in the genes targeted by drugs are not phenotypically neutral but uncompetitive in the absence of the drug. The threshold is the magic concentration of antibiotics in the environment sufficient to achieve a selection against even those microbes not causing disease. The drugs must be used on such a scale that very rare microbes, by nature resistant to the drug, begin to prosper and spread on the resources abandoned by their drug-sensitive contemporaries[6–14]. An emerging reservoir of drug-resistant microbes then adapt to the niche of previous pathogenic species or spread resistance genes to pathogens via the horizontally mobile elements (HMEs), such as viruses, plasmids and transposons. The clonal spread of resistant microbes and trafficking of resistance genes is consistent with this hypothesis[1,4,5,14–21]. The stability of resistance in relatively antibiotic-free environments is, however, a challenge to the hypothesis[3,13,22–26].

What impedes the retreat of resistance genes when antibiotics recede? The first part of this article will describe the mechanisms for maintaining resistance in the absence of overt selection, that is, when the genes are beneficial to the host or vector in other ways. The second part of the review will describe how some combinations of genes syn-

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thesize phenotypes that are qualitatively dissimilar to the effects of each gene alone.

Selection of multiple phenotypes

Genes and their products can have more than one function. Some functions might be irrelevant to the considerations of resistance, but of primary importance to the expectations of eliminating resistance. Biochemical, phenotypic and genetic cross-activities of resistance mechanisms and genes can maintain resistance to medicinal antibiotics. Some examples provided here illustrate qualities of more than one descriptive category.

Biochemical cross-resistance

In this section, seven examples are used to describe how a clinically relevant resistance could be maintained by selecting either:

- Single genes that encode another activity in addition to detoxifying an antibiotic, including detoxifying more than one toxin
- Overlapping biochemical activities encoded by different genes that neutralize a single toxin.

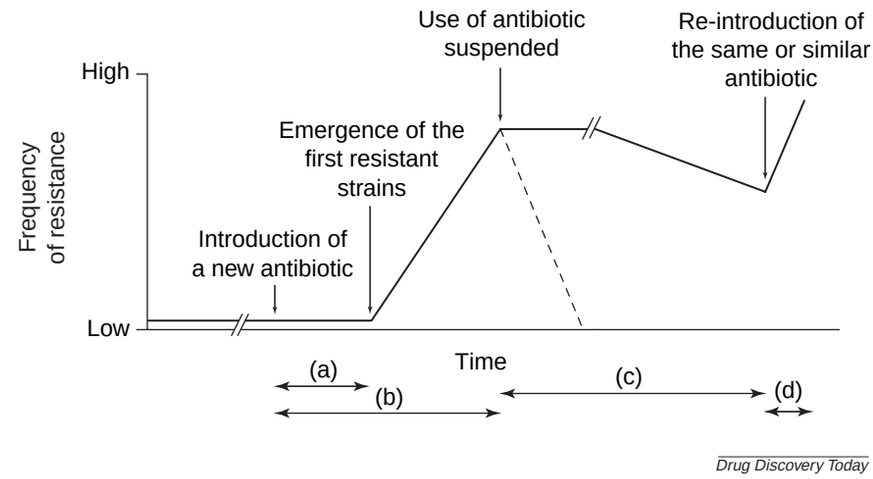

Figure 1. *The reservoir hypothesis. (a) Before human use of antibiotics, the two relevant microbial phenotypes, antibiotic-resistant and antibiotic-susceptible, in the world's reservoir of microorganisms were maintained in some hypothetical balance because of relative fitness, with the resistant types within some species being less common than the susceptible types (solid line). (b) Human use of antibiotics has created a selective pressure for the evolution of more resistant species and strains, illustrated by the rise in the frequency of resistance to one or more antibiotics (solid line). According to the reservoir hypothesis, selective pressure is offset by pre-existing selective pressures favouring susceptible microorganisms that compete with resistant microorganisms. (c) If antibiotic use where curtailed or stopped, the effect of the more competitive susceptible phenotypes should eventually drain the reservoir of resistant microbes (dashed line). (d) However, resistance might not fall to pre-antibiotic levels and, as similar types of drugs are re-introduced, resistance could return up to clinically relevant frequencies in a much shorter time (solid line).*

DNA damage repair. One example of a single gene that simultaneously neutralizes two antimicrobial agents, namely UV radiation and mitomycin-C, is *recA*. UV radiation is an example of a single antimicrobial agent that can select multiple genes with the common ability to detoxify a single antibiotic. The *Dictyostelium discoideum* DNA repair enzymes, *radA, radB* and *radD*, make it resistant to UV light and bleomycin[27]. Conversely, the resistance determinant, *ble,* ferried by the transposon Tn*5* (Ref. 28), promotes the growth of host cells in the absence of the antibiotic, possibly by contributing to the repair of DNA damaged by UV light and other mutagens[4]. Irrespective of whether bleomycin was the cause of *ble* evolution and distribution, bleomycin might no longer be required to maintain selection for *ble*.

Furthermore, the way resistance and other genes are discovered can impose a subtle underestimation of the range of functions expected of individual genes[29]. For example, *recA* was discovered at least six times, once as a recombinase, again as an inducer of the λ virus, then as a gene regulator, a DNA repair enzyme, a membrane binding protein and finally as a mitomycin-C resistance determinant[30]. Each of these activities had to be discovered because unknown activities could not be deduced from known activities. Similarly, any of these different activities could have arisen by selection. However, certain functions might not be subject to selection any longer, might have been selected historically and currently, or might be an attribute of the protein that has never been the target of any selection[31]! Antibiotics might have been the selective agents for some resistance genes but can be redundant for their maintenance.

Permeability and efflux. Reductions in membrane permeability[32] and the multidrug efflux pathways (for a review, see Ref. 4) illustrate the ability of a single mechanism to neutralize chemically different drugs. Efflux permeases pump out a remarkable variety of compounds with few or no common chemical properties, ranging from antibiotics

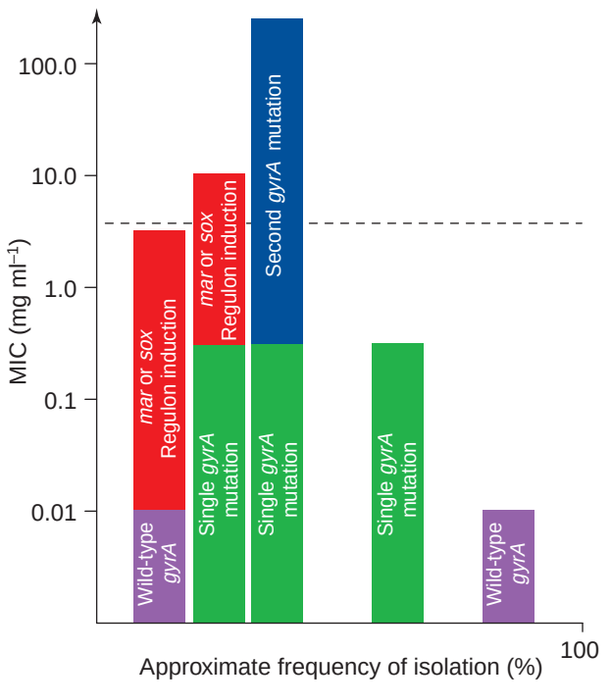

Figure 2. *Building on recessive and potentiating resistance mutations. DNA gyrase (gyr) is the primary target of quinolones, such as the fluoroquinolone ciprofloxacin. Escherichia coli is defined as resistant (all clinical isolates with phenotypes above the horizontal dashed line) when the MIC (minimal inhibitory concentration) of ciprofloxacin is ⩾4 g ml^{-1}. Mutations in gyrA that confer resistance are recessive and not always detected by clinical screens, although they can contribute to a significant increase in resistance. The coloured bars illustrate the effects of combinations of modest resistance mechanisms when assembled, like building blocks, in various combinations up to levels that could compromise chemotherapy (Refs 4 and 99 and Hullen, V. et al. Induction of the mar phenotype is a possible cause for the development of fluoroquinolone resistance in Escherichia coli. 38th Interscience Conference on Antimicrobial Agents and Chemotherapy. 24–27 September 1998, San Diego, CA, USA, abstract C-187).*

to amino acids[4,33]. Thus, aspirin, whose active ingredient (salycilic acid) induces the *mar* regulon (Fig. 2) of *Escherichia coli*, could be sufficient to maintain tetracycline resistance.

rRNA mutations. Multidrug resistance can be caused by modifications to, or mutations in, a single rRNA gene. Methylation of a base in the 23S rRNA results in complete cross-resistance to macrolides, lincosamides and streptogramin Type B (MLS), three structurally unrelated antibiotics[34]. The responsible methylase is often distributed by plasmids[35]. Significantly, a single point mutation at the corresponding site can also confer MLS resistance. Resistance to celesticetin, conferred by a point mutation in the 23S rRNA gene of *Sulfolobus acidocaldarius*, simultaneously confers resistance to chloramphenicol and carbomycin[36]. Even if the competitive fitness of some drug-resistant strains was reduced relative to susceptible progenitors in the absence of the drug[17], resistant strains only require one drug to maintain resistance to several drugs.

Multi-generational resistance. Single genes can detoxify drugs of different generations. The class A β-lactamases that hydrolyze both cephalosporins and penicillins are potent examples[37]. A similar phenomenon has been reported for chloramphenicol and its fluorinated derivative, florfenicol. Florfenicol is effective against strains made chloramphenicol-resistant by the cat (enzymatic modification) or *cmlA* (nonenzymatic resistance) genes[38]. The flo_{St} gene of the unusually virulent[39] *Salmonella typhimurium* strain, DT104, confers florfenicol- and chloramphenicol-resistance. The flo_{St} gene has more recently been reported in *E. coli*, making it probable that flo_{St} will soon appear in other pathogens. Whereas florfenicol might have been necessary for the flo_{St} gene to evolve, chloramphenicol could be sufficient to maintain it (Box 1).

Aminoglycoside modifications. Resistance to aminoglycosides arising from various phosphorylating, adenylating or acetylating enzymatic activities is caused by both the ability of single enzymes to modify multiple drugs and multiple enzymes with overlapping activities acting on the same drug[40]. Several different proteins that modify each potential target site in every clinically useful aminoglycoside have been identified. These enzymes modify vulnerable amino or hydroxyl groups on the aminoglycoside antibiotics that are likely to be important for target binding and drug uptake. In addition, individual proteins can inactivate more than one aminoglycoside[40] (Box 2).

Viruses and antibiotics. Single genes can confer resistance to both drugs and viruses or bacteriocins. Rifampicin-resistant *E. coli* are cross-resistant to phages T7, T4 and λ (Ref. 41) and streptomycin resistance can also confer resistance on λ and f2 (Ref. 42). Rifampicin-resistant *S. typhimurium* are resistant to phage MB78 (Ref. 43), while rifampicin-resistant *Bacteroides fragilis* are resistant to bacteriocins produced by other strains of *B. fragilis*[44]. Thus, these natural pathogens of our pathogens could maintain antibiotic-resistant strains.

Medicinal toxins. Single genes can simultaneously detoxify antibiotics prescribed to treat infections as well as other drugs that have unintended antimicrobial activities. The lat-

Box 1. Multigenerational resistance

Most 'new' antibiotics are chemical modifications of older drugs. Modifying the structure of a clinically proven antibiotic requires less effort and incurs less cost than developing an entirely new drug. Unfortunately, pathogens can readily broaden the activities of existing resistance determinants enough to include the modified drugs. The rapid introduction of new classes of β-lactam antibiotics (cephamycins, carbapenems, clavams, monobactams) between 1978 and 1986 was met by a doubling of the number of identified β-lactamases.

Modifications to existing resistance mechanisms are the result of either gene trafficking or mutation. Resistance to new β-lactam drugs was acquired through recruitment of new β-lactamase genes or mutations affecting the structure of the cell membrane and the activity of old β-lactamases[81]. Similarly, the region of *gyrA* where most of the mutations resulting in quinolone resistance have been found seems to be highly variable, even without evidence of selection[82]. Interestingly, the mutation rate of drug-resistant pathogens might be elevated by horizontally mobile elements (HMEs), which are also the carriers of multiple-resistance genes. Error-prone DNA repair mechanisms have long been carried by plasmids, even before the use of antibiotics[54]. Could new generations of older drugs be select for faster-evolving microorganisms by maintaining a linkage between resistance and mutagen-

ter, which includes psychotherapeutics, anesthetics, antihypertensives, diuretics and antihistaminics, might not have been developed to treat infectious diseases but they are, nevertheless, toxic to microbes[45].

'Unintentional' antibiotics can substitute for prescribed antibiotics for maintaining resistance. Bacteria expressing resistance to an antihistaminic drug, ambodryl, and a tranquillizer, promazine, were resistant to penicillin, streptomycin, chloramphenicol, tetracycline, kanamycin or some combinations of these drugs[45]. Although the mechanism of cross-resistance was not demonstrated, the range of compounds to which resistance was exhibited suggested a nonspecific permeability change. Permeability characteristics are determined by both chromosomal and HME genes, making these changes potentially mobile phenotypes[28,31,45,46]. Therapeutics for noninfectious diseases (Box 3) are often designed for extended use, maintaining resistance, and creating the opportunity for the evolution of multi-step, high-level resistance to the agents intended to treat infectious diseases.

Phenotypic cross-resistance

Drugs or other stresses can induce resistance phenotypes, a process distinct from selecting individuals with DNA mutations. Physiological resistance can be induced, maintained, or both, by either the drug or other agents, but is unstable and can lead to misleading laboratory assessments of susceptibility. Therefore, physiological resistance is difficult to study and even more difficult to control. The two varieties of the phenomenon presented here are overexpression and recessive resistance. A third example, an inheritable physiological resistance to gentamicin (Fig. 3), was recently reviewed[4].

Over-expression. Physiological resistance results from overexpression of otherwise weak resistance to antibiotics as varied as aminoglycosides and β-lactams. Susceptible bacteria overexpressing one or more housekeeping enzymes with weak aminoglycoside-modifying activity display low-level resistance to aminoglycosides[40]. When more than one of these enzymes binds and modifies the same aminoglycoside, the level of phenotypic resistance could be increased.

Box 2. Multiple mechanisms for multiple aminoglycoside resistances

Among the approximately 17 different classes of aminoglycoside-modifying enzymes are those that inactivate just two [e.g. gentamicin and fortimicin by class I (3)-acetyl-transferases] to those that inactivate as many as four [e.g. gentamicin, tobramycin, netilmicin and kanamycin by (6′)-acetyl-transferases or kanamycin, neomycin, amikacin and isepamicin by (3′)-phosphoryl-transferases] aminoglycosides[83]. Aminoglycoside resistance (AG^r) can also result from single, bifunctional enzymes, such as the (2′)-phosphoryl- and (6′)-acetyl-transferase that inactivates gentamicin, tobramycin, kanamycin, netilmicin, amikacin and isepamicin.

Although initially described in the gram-positive streptococci and enterococci, the bifunctional enzyme is the fusion product of two genes from gram-negative bacteria[84]. It was probably first 'assembled' in staphylococci and is now being spread by a plasmid found in the enterococci (Díaz-Mejía, J.J. *et al.* Enterococcus as an antibiotic resistance gene shuffler and mobilizer. *VII Congress of the European Society for Evolutionary Biology*. 23–28 August 1999, Barcelona, Spain, abstract II-83). Several enzymes determining AG^r in pathogenic bacteria have been acquired by horizontal gene transfer, as inferred from their likely ancestry in aminoglycoside-producing microorganisms[4]. Other remarkable examples of resistance genes being assembled from parts of different origins are the *vanA* glycopeptide-resistance[85] and penicillin-binding proteins[53].

Box 3. Unintentional benefits of medicinal antimicrobial agents could prolong their use

Subsidiary activities of medicinal antimicrobial agents enhance the pressures to use them. The list of useful attributes of the tetracyclines illustrates the point well[86]. Tetracyclines have the potential to control inflammation, inhibit tumor progression, bone resorption, angiogenesis and stroke through various activities, including the inhibition of protein kinase C and metalloproteinases. Their effects on interleukin 1β (IL -1β) levels protected mice against septic shock, lipopolysaccharide (LPS)-induced lethality and inflammation, and contributed to a neuroprotective effect in gerbils. Tetracyclines display 'chondroprotective' effects in inflammatory arthritides via the inhibition of nitric oxide (NO) synthase expression. As NO formation is increased in autoimmune diseases (such as osteoarthritis, rheumatoid arthritis, systemic lupus erythematosus, ulcerative colitis and Crohn's disease) and is associated with classical inflammatory symptoms (erythema and vascular leakiness), tetracycline might be an attractive therapeutic modulator of NO. The long and safe history of tetracycline would make it easy to adapt to these other clinical uses.

Similarly, overexpression of a plasmid-encoded and clavulanate-sensitive β-lactamase was sufficient to render the β-lactamase inhibitor, clavulanate, clinically impractical in some instances[47]. Significantly, tetracycline and antibiotics that target the cell wall are known to induce expression of genes conferring resistance to themselves and other antibiotics[5,48].

Prolonged growth of these strains, or their growth in niches and at times where antibiotic concentration is marginal, increases the likelihood of a mutation or horizontal gene transfer event that confers high-level resistance (Box 4).

Recessive resistance. Recessive mutations can also be a source of weak resistance and an important repository of resistance genes (Fig. 2). The phenotypes conferred can be amplified by transient or inheritable changes in copy number or expression (Box 4). Isolating rRNA mutations that confer drug resistance is facilitated when rRNA genes are on multicopy plasmids. Overexpression of mutant/recessive rRNA confers weak phenotypic resistance[49].

Although bacteria are normally thought of as haploid, some loci are almost always diploid or polyploid, like repeated rRNA genes, or genes carried by plasmids. Mutations in rRNA that confer drug resistance, for example, might not result in phenotypic resistance if other copies of the gene are wild-type[49]. *Mycobacterium smegmatis* became resistant to various aminoglycosides when both rRNA loci were replaced with an altered 16S rRNA gene[50], whereas heterozygous *M. smegmatis* remained phenotypically sensitive to these drugs. Like recessive alleles in sexually reproducing species, recessive resistance genes (whose effects on phenotype are neutral to selection) could persist unnoticed long after the drugs are removed from clinics.

In what way could rRNA genes be exchanged or amplified by HMEs like plasmids? After all, rRNA genes are largely the defining characteristic of phylogenies; these genes must therefore not be on HMEs. The finding that at least one *Thermomonospora chromogena* rRNA operon was acquired from another species, probably *Thermobispora bispora*, challenges the presumption that some genes are not trafficked just because they are not routinely found on vectors[51].

Genetic cross-resistance

Antibiotic resistance determinants accumulate on HMEs (Refs 5,6,17,36,52). Multiple resistance genes on a single HME creates, in a sense, a genetic cross-resistance, because one HME can simultaneously adapt a bacterium to several unrelated antibiotics. One antibiotic at a time is all that is necessary to maintain the HME (Ref. 6).

Surprisingly, it is not only resistance determinants that are

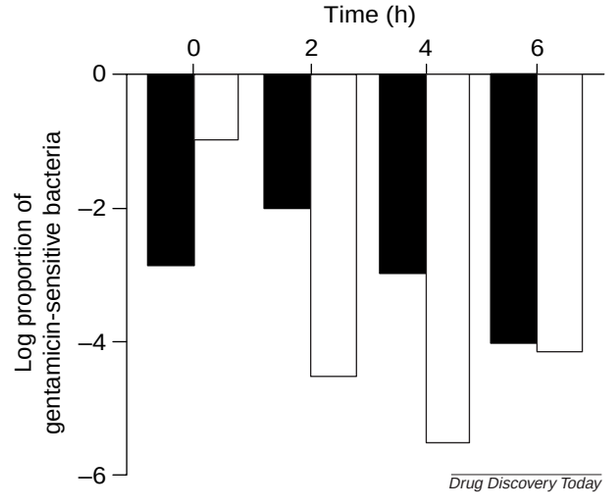

Figure 3. *Adaptive resistance. Pseudomonas aeruginosa that survive exposure to 20 μg ml^{-1} gentamicin for 1 h (open bars) produce offspring that are less sensitive to subsequent exposures of 4 μg ml^{-1} (approximately twofold the MIC) gentamicin for 90 min compared with control bacteria (closed bars). This difference lasts for up to 6 h (8 generations) after the drug is removed (data adapted from Ref. 100).*

Box 4. HME-induced fluctuations in gene expression

One example of Horizontally mobile elements (HME)-stimulated changes in gene expression is demonstrated by F plasmids integrated into certain regions of the *Escherichia coli* chromosome. Occasionally, genes near the integration site are overexpressed. For example, integration of F near the *argF* (ornithine transcarbamylase) locus, within Tn*2901*, increased the frequency of mutants able to utilize citrulline as a source of carbamyl phosphate (Car^+) by 20–100-fold relative to either F^+ or F^- strains[87]. The frequency was dependent on both F and Tn*2901*, and involved amplification of the 11 kb Tn*2901* or another, uncharacterized but F-dependent, regulatory change[88].

accumulating on HMEs. Because of unique aspects of the biology of genes that reproduce by moving between organisms[4,29,53], there is an emerging link between genes that confer resistance and other metabolic specializations[7,53,54] such as virulence[17,39], the ability to tolerate heavy metals, disinfectants and antiseptics[32,51,55,56], and the ability to degrade novel organics[57]. The following section will describe how genetic cross-resistance is also maintained by environmental toxins and the benefits of virulence.

Environmental toxins. One of the most dramatic examples of environmental maintenance of antibiotic resistance is in a study by Timoney and coworkers[56]. They found ampicillin-resistant bacteria accumulating in ocean sediments on New York's bight. The complete absence of antibiotics was not demonstrated, but the concentration of ampicillin in the Atlantic Ocean would be extremely low. The frequency of heavy metal and ampicillin coresistance in this case was high enough to suggest genetic linkage of antibiotic and metal-resistance determinants on plasmids. This, together with a study by Calomiris and coworkers[58], demonstrated that the proportion of antibiotic-resistant organisms was increased in communities exposed to toxic metals, of the type leaching from pipes, but not in control sites. The close link between the various resistance genes creates a condition where either the heavy metal or the antibiotic is sufficient to maintain both resistance genotypes[52].

Virulence. Linkage with virulence determinants might also maintain resistance in medically relevant settings. As with resistance, virulence genes are carried by HMEs in many different microbes, from soil[59,60] to human flora[61–67]. Both the conjugative plasmids and bacterial viruses also have mechanisms to export proteins of pathogenic relevance[68]. *Bordetella pertussis* uses genes descended from the *tra* genes of conjugative plasmids to export its protein toxin[68]. The ancestral *tra* genes are necessary for DNA transmission and can facilitate protein transfer between bacteria[29]. *Salmonella* inject at least eight 'effector' proteins into host cells using the type III secretion apparatus. Both the apparatus and effector proteins were acquired by horizontal gene transfer[39]. A relatively rare effector, SopE, which facilitates bacterial invasion of mammalian cells, is carried by the multiply antibiotic-resistant strain, DT104 (Refs 38,65). The *sopE* gene is embedded in a viral genome, itself integrated into the DT104 chromosome, indicating that, as with the more common effector proteins and the transport apparatus itself, new effector virulence determinants are still evolving on HMEs.

Selection of synthetic phenotypes

Removing antibiotics is expected to restore a competitive advantage to susceptible microorganisms. As already discussed, that expectation is an extrapolation of the assumptions that mutations to resistance are not neutral in effect on the organism and that the observed function of genes is the reason that they exist. Another confounding violation of this reservoir expectation, the evolution of synthetic phenotypes, will now be discussed.

Synthetic phenotypes are difficult or impossible to anticipate. They are hard to measure outside the laboratory, making their role in maintaining environmental resistance untested. However, synthetic phenotypes are real and can maintain the competitive advantage of resistant strains that emerge under antibiotic selection. The three examples that follow illustrate synthetic resistance from the combined activity of multiple-resistance genes (Box 5), synthetic adaptations between HMEs and hosts that substitute for antibiotics to maintain resistance genes, and synthetic adaptations between virulence and resistance determinants.

Synthetic phenotypes from multiple resistance

Combinations of resistance mutations that create new phenotypes can be selected through the new phenotype rather than through drug resistance. The combination of the mutations *rpoB87* (rifampicin resistance) and *gyrA87* (nalidixic acid resistance), which provide protection against the DNA-damaging agent mitomycin-C (Ref. 69), could be maintained by mitomycin-C alone. Likewise, kirromycin could simultaneously maintain certain combinations of *rpsL* (streptomycin resistance) and recessive *tuf* mutations that together confer dominant kirromycin resistance[70].

Box 5. Synthetic susceptibility

Resistance phenotypes can also create new drug susceptibilities. For example, some tetracycline-resistant strains develop sensitivity to cadmium[89], aminoglycosides[90,91], and fusaric and quinaldic acids[92]. Resistance to spectinomycin is sometimes associated with hypersensitivity to fusidic acid[93]. Physiological resistance to gentamicin can lower intrinsic resistance to chloramphenicol and quinolones[94]. Frustratingly, the particular resistance or susceptibility phenotype is not always predictable[95–98].

Synthetic intergenomic adaptations

HMEs and resistance genes can overcome any initial cost to fitness for carrying a plasmid (carriage cost) they impose on a host in the absence of antibiotics through co-adaptation. The resulting adaptation between the genome of the host and HME is caused by changes in either HMEs (such as transposons, phage and insertion sequences, and plasmids[71]) or chromosomes[4].

The effect is clearly demonstrated in a frequently cited study from the Lenski laboratory[72]. In the absence of chloramphenicol, the resistance-determining plasmid, pACYC184, decreased the growth-rate of *E. coli*. In the presence of chloramphenicol, however, host-plasmid pairs evolved a faster growth rate than their progenitors, which persisted in the absence of antibiotics[72]. The unselected tetracycline-resistance determinant (Tet^r) of pACYC184 was responsible for the accelerated growth-rate of the pairs. The co-evolved host received the same growth benefit from a different plasmid with the homologous Tet^r gene. Deletion of Tet^r from either plasmid restored the fitness cost. Bacteria and HME compelled to coexist by long-term selective pressures can generate more synthetic phenotypes than the sum of the initial genotypes. The resistance determinant, possessing biochemical or genetic activities not revealed by the antibiotic, can be maintained by selection in the absence of the relevant drug.

Synthetic phenotypes from resistance and virulence genes

The benefits of some combinations of virulence and resistance phenotypes overcome the initial fitness cost of resistance. For example, some streptomycin-resistance mutations decrease bacterial growth-rates, probably by reducing the speed of translation, and such mutants should be poor competitors of susceptible strains. An elegant continuous culture experiment by Schrag and Perrot[73] followed the evolution and competitiveness of several independent streptomycin-resistant variants (F_1) that arose from the same streptomycin-sensitive ancestor (F_0). F_1 peptide-chain elongation times were significantly greater than F_0 in the absence of the antibiotic. The expectation would be that in the absence of the antibiotic, spontaneous streptomycin-sensitive revertants (F_2) would eventually displace the slow-growing F_1 variant. After 180 generations, the cultures were still uniformly composed of individuals with F_1-resistance phenotypes but greatly reduced peptide-chain elongation times, some being equivalent to the F_0 phenotype. The F_2 phenotype was not produced by additional changes in *rpsL* genes, because F_2 clones had identical sequences to their F_1 ancestors.

Synthetic phenotypes have evolved in experimental animals infected by a multiple-drug resistant, but avirulent, strain of *S. typhimurium*. The drug-resistant strain was less virulent as a result of the resistance, but during the infection, secondary mutations restored virulence and competitiveness with susceptible strains without a loss of resistance[4].

Conclusions

The evolution of resistance is unlikely to be any more complex than the evolution of antibiotic susceptibility[4]. The return of susceptibility cannot be left to chance or to unsubstantiated evolutionary claims. Drug deployment and development must be informed by evolutionary lessons if evolution is to be engineered rather than just reacted upon.

The first generation of drugs that might treat infectious diseases without incurring the cost of resistance is being developed. For example, Balaban and coworkers[74] described an agent that inhibits density-dependent expression of virulence factors, while Maurelli and coworkers[64] express hope in the production of enterotoxin inhibitors. Vaccines against virulence determinants are becoming serious possibilities[75]. These drugs differ from their predecessors by neutralizing or penalizing the pathogen rather than generically killing the microbe.

New technologies are promising to identify new-generation drug targets and more rapidly assess the efficacy of new-generation drugs. High-throughput gene-expression analyses[76], such as DNA microarrays, could rapidly diagnose the effects of drugs that do not kill microorganisms but that alter the expression of virulence genes. Furthermore, genomic and protein function analyses are producing targets to inhibit[77], and to manipulate[78], pathogens.

Several correlated reductions in resistance rates and drug use[79] can be seen as anecdotal evidence that resistance will reduce with the 'wise' application of old drugs, or the intro-

duction of new drugs that act in a similar manner to current drugs[4]. However, the reasons for successful restoration of susceptibility, when it occurs, are difficult to determine[80], and reservoir predictions are far from guarantees of reduced resistance.

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