How antibiotics cause antibiotic resistance

Jack A. Heinemann

Antimicrobial agents are approaching the end of their effectiveness. The prevailing drug development strategy is based on a presumption that results in resistance: that disease can be cured by exploitation of the vulnerabilities in microbial reproduction. Although some did predict the evolution of resistance to such drugs, the mechanisms by which genes conferring resistance have spread was not predicted. The author argues that the mechanism of spread is a consequence of the chemotherapeutics themselves acting on the evolution of pathogens, and that for future drugs to remain effective they must avoid such effects.

It is thus not the individual who forms language; it is the language which forms the individual.

– Alberto to Sophie in Sophie's World
[Gaarder, J. (1995) Phoenix House, London]

Medicine has been using antibiotics to wage 'war' on infectious disease since the introduction of penicillin in 1941. But no single drug is effective against all pathogenic bacteria and some pathogens are resistant to all clinically relevant drugs. Can any doubt remain as to who is winning this so-called war?[1,2]

War language has been more than a tool to communicate the biology of pathogenesis (for example, see Refs 3–5). The metaphor describes how we view pathogenesis; however, by calling the medical goal to eradicate infectious disease a war, we have misunderstood the nature of disease evolution and the drugs necessary to stop such evolution. The metaphor has guided a narrow search for agents that only prevent pathogens from reproducing. This class of drug has been highly successful but transient in nature (for example, see Ref. 2). The failure of such antimicrobial agents is primarily because of their poorly understood effects on 'sensitive' microorganisms. These effects are the promotion of inter- and intraspecies gene exchanges (particularly resistance and virulence genes) and induction of phenotypic resistance.

Recognizing that antibiotics select for the growth and dissemination of otherwise exceptionally rare resistant individual microorganisms has been important to begin to address the problem of antibiotic overuse[5]. To design a new generation of drugs and maximize their efficacy and longevity, however, will require much more than merely reducing their usage (Refs 6–8 and references therein). This review will collate a representative set of findings, not all of which are new, to present a new perspective on the current problem of antimicrobial drug design.

Seven effects of current antimicrobial agents that must be avoided in new agents will be used as illustrative rather than exhaustive examples to communicate two major points. First, that our knowledge of microbial physiology is too limited to enable curing diseases by using agents that stop microbial reproduction; and second, that the special nature of antimicrobial agents themselves promotes the evolution of resistance and possibly virulence (Fig. 1).

Resistance cannot be fully explained by antibiotics selecting resistant microorganisms, as they are in part the 'side-effects' of the evolution of subcellular entities that infect microorganisms and spread resistance genes[9]. The selection is not necessarily for resistant microorganisms but for vectors that carry resistance genes. Most resistance genes are mobile, moving from organism to organism as part of the horizontally mobile elements (HMEs) often

Jack A. Heinemann, Department of Plant and Microbial Sciences, Private Bag 4800, University of Canterbury, Christchurch, New Zealand. tel: +643 364 2926, fax: +643 364 2083, e-mail: j.heinemann@botn.canterbury.ac.nz

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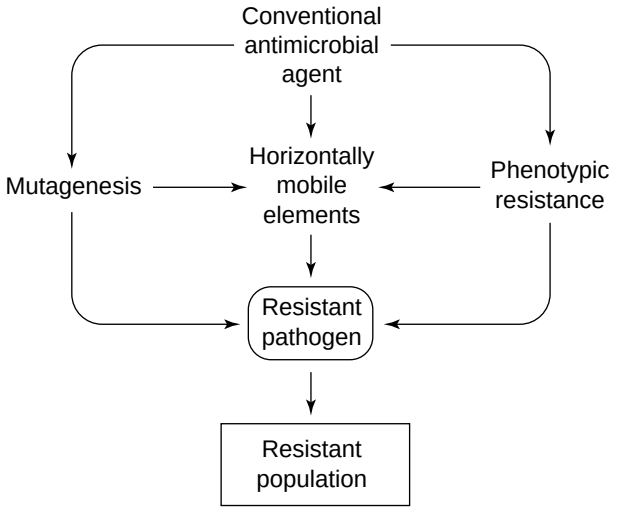

Figure 1. *Effect of therapeutics on the evolution of resistance. Traditionally, the analysis of resistance has been on how antibiotics protect rare resistant individual bacteria until they grow into populations of resistant pathogens in the absence of competition from sensitive microorganisms. A full evaluation of resistance evolution and drug development must consider how resistance arises in individual pathogens. The specificity of antimicrobial agents can preserve non-target biochemical activities, some of which can be involved in inter-organism gene transfer. These pathways continue to function in the presence of the drugs just as well, sometimes even better, than in the absence of drugs. Other properties of chemotherapeutics may enhance gene transfer by transformation. Some drugs increase the basal mutation rate increasing the probability of exposed bacteria to develop genotypic resistance. This process may be relevant to populations of pathogens even if the genotypically resistant cell dies. If the mutation was on a horizontally mobile element within the cell it could still be spread by infectious exchange. By mimicking signals that induce alternative physiological states, some drugs induce phenotypic resistance to themselves and other drugs. By increasing the infection time, phenotypically resistant cells are more likely to acquire genotypic resistance by inter-organism gene transfer. They also may produce more offspring, increasing the probability of acquiring genotypic resistance through mutation.*

called conjugative plasmids, viruses, integrons and transposons, or may flow between organisms through processes of natural transformation[9–11]. The evolution of HMEs, because of their autonomous reproduction, can be very different from the cellular life carrying such elements[12]. The genes conferring resistance are either major components of HMEs or replace genes within the chromosome of the pathogen through recombination following transfer[8].

The seven examples of the effects of drugs on the evolution of resistance and virulence sort into two categories, called molecular redundancy and molecular infidelity. Assignment to a particular category is not absolute but depicts the tendency for a drug to either induce physiological states and/or consequences caused by other environmental factors, or affect the frequency of recombination or mutation.

Molecular redundancy

Antibiotics are ligands for their molecular targets, binding with the specificity of hormones to their receptors and nucleotide sequences to the proteins that regulate gene expression. Antibiotics may also be evolving functions similar to those associated with hormones and gene regulators.

Hormones and pheromones are signal molecules. By binding to receptors they effect a change in gene expression or physiology. The first two examples below illustrate how analogous antibiotic-mediated signals activate some HMEs and cause some pathogens to adjust their physiology.

Antibiotic-induced gene transfer

Transmission of some conjugative transposons is antibiotic-regulated. Tetracycline stimulates transmission of a transposon native to the Gram-negative *Bacteroides* by 100–1000-fold[13]. *Tn*925 transmission is also responsive to tetracycline. *Tn*925 is a conjugative transposon native to the Gram-positive bacteria and, like the *Bacteroides* transposon, encodes tetracycline resistance. Transposon transmission to antibiotic-sensitive bacteria was enhanced 5–100-fold following culture of the resistant host in tetracycline[14].

The *Bacteroides* transposon encodes two genes, *rteA* and *rteB*, homologous to known two-component regulatory genes[13]. Such regulators are characterized by a cell surface component that binds the signalling molecule and then activates an intracellular component. The second component effects a response, usually by inducing gene expression. RteA might be the environmental sensor as it contains two stretches of hydrophobic amino acids of sufficient length to span the membrane. RteB is homologous to several proteins known to interact with RNA polymerase, making it the likely cytoplasmic component.

Antibiotic-regulation of genes with functions unrelated to resistance provides striking evidence that the specificity of antibiotics can be co-opted by evolution.

Antibiotic-induced multiple-resistance

In the example above, an antibiotic induced the genes controlling horizontal gene transfer. In the examples to follow, the drugs induce resistance to other drugs.

Many environmental agents induce transcription of genes that confer pleiotropic phenotypes, including resistances to antibiotics. For example, salicylate induces transcription of the multiple-antibiotic-resistance (*mar*) operon (Box 1). Induction, or constitutive expression in operator (*marO*) or repressor (*marR*) mutants, confers cross-resistance upon cells to dissimilar agents by decreasing intracellular drug concentrations (Ref. 15 and references therein). Salicylate can elevate the frequency of simultaneous resistance to tetracycline, chloramphenicol and nalidixic acid 10^6-fold (Ref. 15 and references therein). The *marO* and *marR* mutants selected on tetracycline or chloramphenicol were 1000-times more likely to also acquire resistance to the structurally unrelated fluoroquinolones compared with populations exposed first to the fluoroquinolone itself[16].

Among the genes controlled by the redox-responsive (*soxRS*) regulon of *Escherichia coli* are genes also under the control of *marRAB* (Refs 15,17–19). Likewise, the *acrAB* operon, which encodes an efflux pump, is induced in *marR* mutants and by stresses that activate *soxRS* (Ref. 17). Overexpression of the *robA* gene product, which is known to bind to the *E. coli oriC*, can also partially substitute for *MarA* and *SoxS* in resistance induction[20,21].

The overlap of the pathways increases the number of environmental signals that could maintain resistance phenotypes. Functional redundancy among transcriptional activators creates a network of environmental responders with a common repertoire of responses. Funnelling the detection of many dissimilar stresses to a common response phenotype perpetuates resistance in the absence of the therapeutics themselves.

Box 1. Flexibility of efflux-mediated resistance

Efflux-mediated resistance such as determined by *mar* and *acr* is a mechanism common to both microbial pathogens and recalcitrant human tumours[46]. Efflux pumps can evolve to recognize chemically unrelated substrates[47]. Such plasticity in substrate selection makes it difficult to predict which pump[48] might cause resistance and which compounds are likely to be compromised by changes in pumps.

Moreover, certain multidrug-resistance pumps are highly conserved in evolution. For example, the gene for the ABC transporter from *Lactococcus lactis* transforms mammalian cells to multidrug resistance[49]. The recent demonstration of gene transfer from intracellular pathogens to mammals[50], and the natural competence of some microorganisms (Ref. 10 and references therein), makes such versatility in resistance genes a cause for concern.

Antibiotic-induced phenotypic resistance

Organisms adapt their physiology to new environments following reception of a signal to switch physiological states. The signal usually comes from the new environment. Switching physiological states occurs uniformly when the new environment is not lethal to organisms in either physiological state; when the new environment is lethal to organisms in one physiological state, some may die before adapting.

Exposing *Pseudomonas aeruginosa*, for instance, to the aminoglycoside gentamicin for brief periods induces resistance to the drug among those few that survive initial exposure[22]. The resistance phenotype can also be induced by depriving the bacteria of Mg^{2+} (Ref. 23). Gentamicin-resistant cells concomitantly display resistance to other toxic agents such as netilmicin, tobramycin, amikacin, isepamicin, neomycin, kanamycin, streptomycin, polymyxin and EDTA (Ref. 22). Although the mechanism of physiological adaptation is not known, it is probably associated with a secondary effect that gentamicin has on the pathway for drug uptake.

Gentamicin creates a stress or signal that promotes a change to a physiological state that is also caused by Mg^{2+} starvation. Consequently, individual microorganisms become resistant to many classes of drugs. Moreover, the phenotype is inheritable, persisting for generations after gentamicin is removed or Mg^{2+} replenished. That this is a physiological rather than mutational adaptation is primarily inferred from the uniform appearance of the phenotype and then its uniform reversal in descendant generations after induction by either Mg^{2+} starvation or drug selection.

Physiological resistance, like the overlap of transcriptional regulators, reveals an unexpectedly plastic response of microorganisms to drugs. Persistence and reproduction in the presence of antimicrobial agents increases the probability of mutation to genotypic resistance or acquisition of a resistance gene through gene transfer.

Antibiotic-induced DNA escorts

Resistance and virulence genes transfer between microorganisms in patients and animals, and antibiotics are known to influence the frequencies of such events *in vitro*[13,24]. In the laboratory, antibiotics increase gene-transfer frequencies by reducing the effectiveness of the cell surface as a barrier to the release and uptake of genetic material or by

making the microorganism susceptible to fusion with other microorganisms and vesicles.

Wall-less, or protoplast, forms of microorganisms are prone to lysis and the release of DNA during osmotic stress. β-Lactams and bleomycin, a DNA-damaging antibiotic (Box 2), create protoplasts[25,26]. The reaction conditions and potential active intermediates in bleomycin-mediated wall degradation are similar to those of the free-radical oxidation mechanism of nucleic acid degradation by bleomycin, making both reactions relevant at therapeutic concentrations of the drug[26].

Antibiotics similarly influence DNA uptake by weakening the microbial cell wall and making cells more permeable to DNA, and/or by decreasing the concentration of periplasmic nucleases[27,28]. β-Lactam type drugs increase the frequency of interspecies DNA transmission between combinations of *E. coli*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Streptococcus faecalis* and *Bacillus anthracis*[29,30].

Protoplasts are also more susceptible to fusion with other cells or membranous vesicles (for example, Refs 31,32); such vesicles are produced naturally by Gram-negative bacteria (Ref. 28 and references therein). *P. aeruginosa* vesicle production is stimulated by the aminoglycoside gentamicin[33]. These vesicles sometimes contain plasmids and virulence factors[28,33].

Box 2. Activities of bleomycin

The many activities of bleomycin illustrate the important but under-characterized secondary effects of antibiotics. Bleomycin is known to kill cells by damaging DNA (Ref. 6). Resistance to bleomycin also increases resistance to ethyl methanesulphonate suggesting either an enhanced DNA repair mechanism or a common mechanism for removing these two toxins (Ref. 6 and references therein). Whatever the mechanism, resistance better adapts cells to even environments lacking bleomycin. Bleomycin also induces a multi-resistance phenotype in *Escherichia coli,* probably through transcriptional activation of linked resistance genes[51].

The secondary effects may be just as relevant as the primary effects for selecting resistance. Thus, they cannot be ignored when considering how to counter resistance. Expectations that withdrawing particular antibiotics from use will uniformly result in the cessation of resistance to that drug may be overly optimistic (Refs 7, 52 and references therein). The close genetic linkage of multiple resistance factors will resist loss of single genes on otherwise successful horizontal mobile elements and the pleiotropic benefits of resistance genes will slow the retreat of resistant pathogens or non-target microbial reservoirs of resistance determinants.

Polyethylene glycol is used in the laboratory to promote protoplast fusion, but that agent is dispensable *in situ*. For example, liposomes encapsulating the antibiotic tobramycin cleared a chronic pulmonary infection caused by *P. aeruginosa* better than 'free' antibiotic, presumably due to liposome fusion with the outer membrane of the pathogen[34] (Box 3).

Antibiotic-induced vectors

How is it that virulence and resistance genes seem to first accumulate on the HMEs rather than remaining on chromosomes? The answer may in part be due to an unfortunate irony in drug development. Antibiotics must be specific for certain molecules to avoid toxicity to the patient; but specificity has its disadvantages. While antibiotics effectively halt microbial reproduction, they rarely inhibit

Box 3. Antibiotics as anthropogenic pheromones?

The effect some agents, like aminoglycosides, have on *Pseudomonas aeruginosa* resembles natural examples of pheromone-mediated plasmid exchanges in Gram-positive bacteria[13,53] or the pheromone-coordinated cell fusion that occurs in the *Saccharomyces cerevisiae* reproductive cycle[32]. Although the molecular biology of these examples is dissimilar, the pheromones have the effect in each case of coordinating two cells for 'conjugation', in the one case resulting in plasmid exchange and in the other cell fusion. Pheromones halt the asexual cycles of two neighbouring haploid yeast cells permitting them to fuse into diploids at the same appropriate stage. Aminoglycosides resemble anthropogenically applied pheromones that impose temporal coordination of DNA exchange between microorganisms. The drug increases their receptivity to DNA-containing membrane vesicles produced by other microorganisms exposed to the same drug.

Simply lacing the environment with antibiotic resistance genes, with or without competence-stimulating antibiotics, may be sufficient to spread resistance to microbial populations through transformation[10]. DNA is surprisingly stable in animal guts and circulatory systems[54,55], and it has been detected for prolonged periods of time in the soil where naturally competent organisms are known to acquire DNA (Ref. 10). Resistance genes have been detected in commercial antibiotics, sometimes at concentrations that cause transformation of sensitive bacteria[56–58]. Human use of antimicrobial agents could be quantitatively, if not qualitatively, altering a natural process of genetic exchange.

Table 1. DNA transmission from antibiotic treated ('dead') bacteria[a]

Anti-microbial agent	Activity	Plasmid transmission out of dead bacteria	Plasmid transmission in and out of dead bacteria[b]
UV radiation	DNA damage	Yes	Yes
Mitomycin-C	DNA damage	Yes	Yes
Ciprofloxacin	Gyrase inhibitor	Yes	No
Streptomycin	Ribosome inhibitor	Yes	No
Tetracycline	Ribosome inhibitor	Yes	Nd
Rifampicin	RNA polymerase inhibitor	Yes	Nd

[a]Tests usually involve *Escherichia coli.*

[b]Nd, not determined.

the metabolism necessary for accepting and distributing genes on plasmids (Table 1). Such cells are called 'dead' vectors because the drug prevents the cells from dividing, either ever again (bactericidal agents) or in the presence of the drug (bacteriostatic agents). However, the cells remain active in the process of conjugation – a plasmid-mediated transfer of genes from bacteria to other bacteria and even to eukaryotes[9,35–38].

Bacteria harbouring plasmids that confer resistance to one or more drugs continue to transfer the plasmid to other cells long after the donating bacterium has been killed by several different antimicrobial agents[9,36–38]. Mitomycin-C and UV radiation at some doses irreversibly damage DNA, but bacteria killed by these agents can still receive plasmids and then redistribute them[9,39]. Even though some antimicrobial agents can inhibit conjugation under certain circumstances (for example, Ref. 40), other antimicrobial agents stimulate the frequency of conjugation by protecting the conjugation-specific metabolism from the actions of other antimicrobial agents (Fig. 2) or by reducing barriers to DNA exchange[29,30].

How could this metabolic resistance be relevant to the evolution of resistance in living microorganisms? It is true that resistance and virulence genes borne by HMEs may not protect the pathogen being targeted by the physician in the first instance. Nevertheless, the drug may convert the pathogen into a vector (undetectable by conventional microbiological assays) that may persist in the patient or local environment. Environments are in part a flux of organisms and antibiotic concentrations. If these 'stealthy'

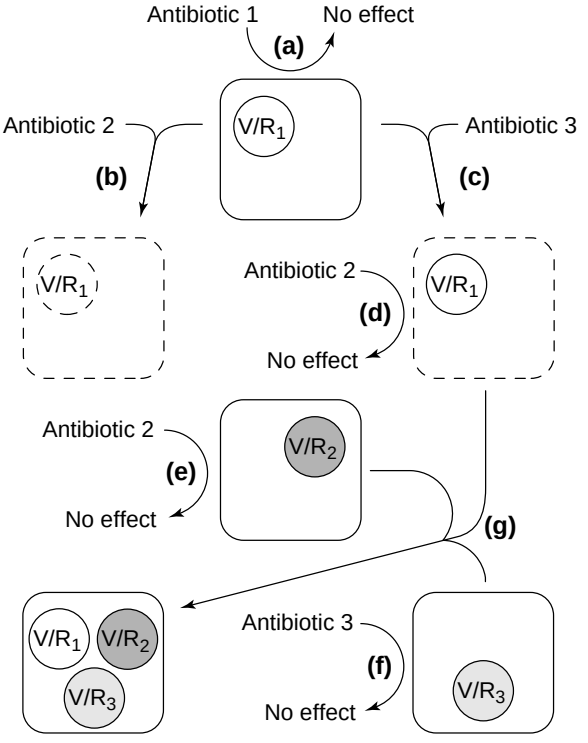

Figure 2. *Few antimicrobial agents simultaneously prevent the reproduction of chromosomes and horizontally mobile elements (HMEs). Most agents inhibit only chromosomes. Depending on the order in which a pathogenic organism encounters a series of assaults, the evolution of the HME it may contain could differ from the chromosome and cell itself. Illustrated is the particular case where a cell made pathogenic by a resistance (R,) plasmid-encoded virulence determinant (V) is 'killed' by application of either one of two alternative antibiotics to which the pathogen is genotypically sensitive. (a) Antibiotic 1 has no effect because of the plasmid-encoded resistance gene. (b) Antibiotic 2 renders the cell incapable of reproducing the chromosome and transmitting the plasmid. (c) The class of antibiotics represented by antibiotic 3 (see Table 1) effectively prevents vertical reproduction of the chromosome but (d) simultaneously makes horizontal transfer phenotypically resistant to antibiotic 2. Re-inoculation of the patient with organisms from the environmental reservoir, which usually must already be resistant to the medicating antibiotic [(e) antibiotic 2 or (f) antibiotic 3], could result in (g) living recombinants resistant to all three antibiotics. This and other scenarios describe plausible mechanisms for the unexpectedly high coincidence of multiple resistance and virulence traits.*

vectors persist long enough, they may transfer virulence and resistance determinants to other microorganisms that subsequently colonize the patient or environment, either because the colonizing microorganisms were already resistant to the first antibiotic or because the antibiotic has fallen below an efficacious concentration (Fig. 2).

Molecular infidelity

Various biochemical functions (for example, restriction enzymes) assess the status of DNA as either 'self' or 'foreign'. Usually restriction enzymes degrade DNA that is missing one or more of its methyl groups, which are added to certain bases at every occurrence in a particular sequence. Another way to assess DNA is to compare its sequence with other sequences in the same cell. Such comparisons are made by the homologous recombination enzymes before mixing together two similar sequences. Recognition processes are also vital for preventing mutation during DNA replication. A newly synthesized base pair must pass many DNA-polymerase tests before being immortalized. Pairings that violate H-bonding rules or helix geometry are readily aborted. Relaxing the stringency of these processes can result in faster evolution of resistance. The final two examples describe how antimicrobial agents directly and indirectly attenuate the fidelity of recognition processes, thereby elevating recombination and mutation rates.

Antibiotic-perpetuated stress

Stress can make cells more receptive to foreign DNA. For example, *Corynebacterium glutamicum* exposed to heat, ethanol, detergent and other protein-denaturing agents can receive DNA from other bacterial species 100–10,000-times more efficiently[41]. The antibiotic chloramphenicol enhanced the effects of stress on gene transfer. Probably, mild stress denatured enzymes that degrade foreign DNA and the antibiotic prevented resynthesis of the enzymes to prolong the bacterium's observed permissiveness towards foreign DNA (Ref. 41; Box 4 and Table 2).

Antibiotic-stimulated mutagenesis

Cells under various forms of stress have higher mutation rates[11]. Antibiotics that cause DNA damage, like mitomycin-C, can directly elevate the frequency of mutation[10]. Interestingly, antibiotics that affect translation fidelity also boost the mutation rate in bacteria[42]. Mutants arose more often during culture of wildtype strains of *E. coli* in the presence of streptomycin, which decreases translational fidelity, than during culture in the absence of streptomycin[42]. However, the antibiotic was not the mutagen *per se* because the mutation rate of strains resistant to streptomycin was independent of culture conditions.

A plausible mechanism for the increase in mutation rate is that mistranslated but functional DNA polymerases are produced by 'sloppy' ribosomes. Such mistake-prone polymerases may participate in DNA replication and then disappear when they are replaced by new polymerases made by ribosomes that have regained their stringency[43]. By stimulating the basal mutation rate, antibiotics increase the probability that a new resistance determinant will arise in a population (Box 5). Even more flexibility is achieved

Box 4. Antibiotics prolong the effects of stress

Only in *Corynebacterium glutamicum* have restriction enzymes been shown to be sensitive to the effects of physical or chemical stresses that elevate interspecies recombination. Nevertheless, the phenomenon of stress-elevated recombination is widely known[59,60]. Temperatures from 43–50°C increased recombination between *Escherichia coli* and *Salmonella typhimurium* as much as 1000-fold over recombination at the normal niche temperature of 37°C (Table 2).

Antibiotics can sustain these high gene-transfer frequencies even after the temperature has been reduced. The 50°C-inspired phenotype could be preserved at 37°C by adding either tetracycline or rifampicin to the culture medium before reducing the temperature. Although these bacteriostatic antibiotics reversibly inhibit gene expression, they have no apparent effect on DNA transfer[38] (Table 1). Therefore, *de novo* gene expression was required to restore an activity that discouraged gene transfer. These observations are also consistent with irreversible denaturation of a 'restriction' barrier to interspecies gene transmission at the elevated temperature. The restriction activity has not yet been identified, but whatever is its material basis the effects of stress were accentuated by antibiotics.

Table 2. Effect of temperature on gene transmission

Type of cross[a]	Mating temperature (°C)	Frequency of gene transmission[b]
Interspecies	37	$1 \times 10^{-6} \pm 4 \times 10^{-7}$
Interspecies	43	$3 \times 10^{-2} \pm 3 \times 10^{-3}$
Intraspecies	37	$1 \pm 5 \times 10^{-2}$
Intraspecies	43	$1 \pm 3 \times 10^{-1}$

[a]Interspecies crosses involved an *Escherichia coli* F′ (conferring ampicillin resistance) donor and an *Salmonella typhimurium* recipient. Intraspecies controls substituted an *E. coli* recipient (G. Reid and J.A. Heinemann, unpublished).

[b]Frequencies are in recombinant phenotypes (transconjugants) per limiting parental phenotype.

if the mutant gene is on an HME because these have the potential to be disseminated independently of the survival of the host cell (Table 1).

Conclusions

Currently used antimicrobial agents are united in two common properties:

- They differentiate the reproduction of chromosomes from the reproduction of HMEs
- They do not work for long

Antimicrobial agents satisfied an immediate and now resurgent need to treat the critically ill. Screening for agents of low mammalian- and high microbial-toxicity, rather than designing agents that can be expected to have a sustained efficacy, still dominates drug discovery programs. Clearly, not enough is known about the effects of these biochemicals to support the medical confidence that current strategies of drug discovery can either keep pace with resistance or eradicate infectious disease.

The mechanisms by which resistance arises are not obvious outcome of our current views of evolution. There is no a priori reason to expect that a selection for resistant microorganisms would result in a proliferation of HMEs that confer resistance. Indeed, what has been revealed by human application of antimicrobials is that the secondary effects of these agents on gene transfer are of primary importance to how resistance evolves.

How collections of genes are organized into either HMEs or chromosomes is determined by the effect differences in reproduction have on the success of these replicons. Antimicrobial agents discriminate between the reproductive pathways of different replicons, telling us how resistance genes associate with mobile genetic elements. Determining the rules governing gene organization will produce predictions of which drugs will clear infections without inciting the forces that lead to resistance. Thus, we may one day predict when the organizations called viruses, for example, will evolve to be virulent or benign – a prediction of low reliability in present models of evolution[44].

How do we design drugs with longer therapeutic lives? Drugs that subvert the pathogenic phenotype without disrupting microbial reproduction should avoid the selection for resistance and certainly produce no reward to the HMEs that might carry resistance genes. Amabile-Ceuvas and his group[23] are looking to drugs that might destabilize the HMEs themselves, while others target pathogen-specific determinants rather than pathogen reproduction (for example, Ref. 45). The new drugs must at least avoid the physiological complications described herein. Of course, caution in developing and dispensing new agents is essential[5]. However, the true answer to the question will not be found within the context of this metaphorical war on disease. A new metaphor must be used that will concentrate our enthusiasm for discovery onto research in microbial physiology and evolution as our best hope for developing future chemotherapies.

Box 5. Impact of transient mutators

Transcriptional or translational errors could be responsible for generating up to 10% of the single, and >95% of the double, mutations in cells with reversibly high mutation rates[43]. Mutations derive from errors in mRNA or protein synthesis if those errors produce altered DNA-metabolizing enzymes, such as DNA polymerase, from wild-type genes. The mutation frequency will fall when the enzymes responsible for the mutation frequency become diluted by turnover or cell division.

Mutations in genes necessary for methyl-directed mismatch repair (MMR) can also produce mutators[61–63]. MMR may be responsible for directing the repair of most base mismatches whether they result from an inattentive polymerase, through DNA damage or strand slippage[61]. MMR in *Escherichia coli* uses the hemimethylated state of newly replicated DNA to distinguish between the parental (methylated) and daughter strands, replacing the base in the daughter to match the parent. In all organisms, the essential requirement for such systems is the distinguishing between template and mutant strands for base replacement.

Incurring DNA damage when strands are indistinguishable could make cells transient mutators[61]. Damage repair in cells with two fully methylated DNA strands would be randomly templated (RT), leading to a 50% probability of immortalizing the incorrect base. Intriguingly, it is possible that antimicrobial agents could create metabolic states that would favour RT-MMR initiated mutations, and some agents could even incite the DNA damage itself.

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In short…

Abgenix (Fremont, CA, USA) and **AVI BioPharma** (Portland, OR, USA) have signed a research license and option agreement to develop fully human antibodies to human chorionic gonadotrophin (hCG) for the treatment of cancer. AVI BioPharma's clinical evaluation of hCG indicates that it may be a target with significant potential as a cancer treatment. The company will use Abgenix XenoMouse™ technology to generate product candidates. Denis R. Burger, president and CEO of AVI BioPharma, stated, 'We look forward to applying Abgenix's human antibody technology to our hCG development program'. AVI BioPharma's goal is to use hCG technology to treat all cancer patients, including those with weakened immune systems.