The Population Genetics of Antibiotic Resistance

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Mathematical models are used to ascertain the relationship between the incidence of antibiotic treatment and the frequency of resistant bacteria in the commensal flora of human hosts, as well as the rates at which these frequencies would decline following a cessation of antibiotic use. Recent studies of the population biology of plasmid-encoded and chromosomal antibiotic resistance are reviewed for estimates of the parameters of these models and to evaluate other factors contributing to the fate of antibiotic-resistant bacteria in human hosts. The implications of these theoretical and empirical results to the future of antibacterial chemotherapy are discussed.

Will the more prudent use of antibiotics reduce the frequency, rate of spread, and evolution of antibiotic resistance genes and plasmids in natural populations of bacteria? In the extreme case, a complete cessation of antibiotic use, the answer to this question is almost certainly "yes." However, even in this extreme case, it is not clear how long it will take before antibiotic-resistant pathogens are rare enough not to pose a problem.

Is it going to be a matter of years, decades, or millennia? Moreover, the complete termination of the clinical and prophylactic use of most antibiotics, including those for which resistance already poses a problem, is impractical and—in the absence of alternatives—unethical. Thus the issue of real concern is the relationship between the frequency of resistant bacteria and the incidence of treatment, and the direction and rates of change in that frequency under different regimens of antibiotic use.

In this report, we address these questions of the population genetics of antibiotic resistance. Using mathematical models, we examine the contributions of the different population processes that determine the frequencies of antibiotic resistance in commensal bacteria of treated hosts, how fast those frequencies will decline when the antibiotic stops being used, and how rapidly the frequency of resistance will increase when that antibiotic is employed again.

We review the findings of theoretical and experimental studies of the population biology of chromosomal and plasmid-borne antibiotic resistance that have considered (1) the cost (in terms of bacterial fitness) of antibiotic resistance, (2) the effect of horizontal transfer of R-plasmids on the maintenance of antibiotic resistance and on the rate of change in the frequencies of resistant bacteria, (3) the consequences of (co)evolution and the costs of R-plasmid carriage, and (4) the role of associated linkage selection on the persistence of antibiotic resistance.

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Theoretical Considerations

A Model of the Population Genetics of Antibiotic Resistance in the Commensal Flora

While antimicrobial therapy is generally directed at specific pathogens, the commensal bacteria of the gut, nasopharynx, and other habitats in the treated host are also affected by systemic and topical applications of antibiotics. The model we develop here considers the factors affecting the frequency of antibiotic resistance in the commensal bacteria of human hosts. These flora are generally complex assemblages of a number of different species of bacteria and sometimes many clones of the same species [1].

Antibiotic treatment imposes intense selection for bacteria in these communities carrying chromosomal and accessory element–borne genes that code for resistance to these chemotherapeutic agents. As a consequence of this antibiotic-mediated selection, the frequencies of the resistant bacteria rapidly increase and, commonly, the diversity of these commensal communities is markedly reduced and their structure (species composition) is often profoundly changed, sometimes with pathological consequences [2, 3].

The antibiotic-resistant, commensal bacteria ascending in treated hosts also pose two more-direct problems. In uncompromised as well as immune-compromised hosts, normally commensal bacteria such as *Escherichia coli, Haemophilus influenzae, Neisseria meningitidis, Streptococcus pneumoniae,* and *Staphylococcus aureus* are occasional and/or opportunistic pathogens. Second, these organisms carry antibiotic-resistance genes and accessory genetic elements that can be infectiously transmitted to more obligate pathogens, like *Shigella* species, *Salmonella* species, *Vibrio cholerae,* and *Neisseria gonorrhoeae.* Furthermore, levels of antibiotic resistance in the commensal bacteria can be used to monitor the general intensity of antibiotic-mediated selection in individual hosts and communities of hosts.

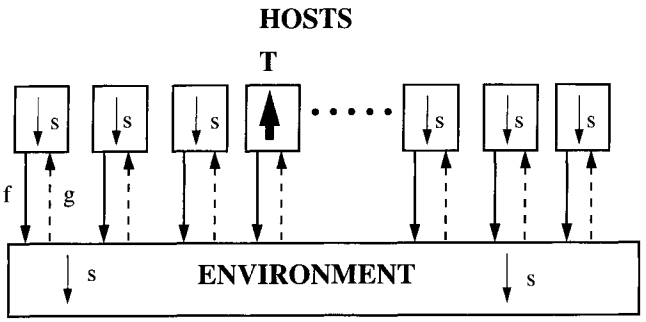

Figure 1. Model of the population genetics of antibiotic resistance in a commensal population of bacteria. The descending arrows in the hosts and the environment depict the decline in the frequency of resistant bacteria due to selection at a rate of s per generation. The ascending arrow in the treated host indicates a frequency of resistance of 1.0, and the average host experiences T courses of treatment per year. The solid and broken arrows connecting the hosts to the environment depict, respectively, the movement of bacteria from the hosts to the environment (so that a fraction f of the environmental bacteria are replaced by bacteria shed by hosts in each bacterial generation) and from the environment to the host (so that a fraction g of the bacteria in a host are replaced by bacteria from the environment in each bacterial generation).

Our model considers (1) a population of N hosts, each with its own commensal flora that includes the sentinel species of bacteria being monitored, and (2) a common environment into which all hosts shed part of their commensal flora and from which they also receive these bacteria (figure 1). Each host receives an average of T courses of treatment per year with the particular antibiotic under study. We assume that as long as antibiotic-resistant members of the sentinel species exist in the environment, they will be present at some (potentially low) frequency in all hosts. We further assume that once treatment occurs, the frequency of these resistant bacteria in a treated host goes to 100%.

Counterbalancing the positive selection for antibiotic resistance in treated hosts, we assume, is some cost in the fitness of resistant bacteria when the antibiotic is not present. Hence, in untreated hosts and in the environment, which we assume to be free of the treating antibiotic, the frequency of resistance is in a continuous state of decline. The rate of decline in the frequency of resistant bacteria in untreated hosts and the environment is governed by a selection coefficient s ($0 < s < 1$) per generation.

Finally, we assume that there is some interchange of bacteria between the hosts and the environment. In the model, this exchange is governed by two parameters: f, which is the fraction of the environmental bacteria replaced by bacteria from the hosts per unit time, and g, which is the fraction of the bacteria in each host replaced by bacteria from the environment in the same time interval. Since the hosts are treated while the environment is not, this interchange will result in a net flow of resistant bacteria into the environment and net a flow of susceptible bacteria into hosts.

To analyze the properties of this model, we used a Monte Carlo simulation. We simulated a population of 10,000 hosts, each of them exchanging bacteria with the environment at the rates f/10,000 and g, where these are, respectively, the fraction of the environment replaced by bacteria from each host and the fraction of each host's commensal bacteria replaced by the environment in each bacterial generation. Thus, a total fraction f of the environmental bacteria is replaced by host bacteria in one bacterial generation. Each generation, a fraction of the host population is chosen at random and treated with the antibiotic under consideration, and this fraction is determined so that the expected frequency of treatment for any given host is T treatments per year.

We assume that treatment instantaneously sends the frequency of resistant bacteria in a treated host to 100%. In all untreated hosts and in the environment, the total frequency of resistant bacteria is declining, with a selective coefficient of s per generation. We assume a bacterial generation time in the gut of 40 hours (219 generations/year) [4], so that resistant bacteria have a fitness of $1 - s$ per 40 hours (compared with a fitness of 1 for susceptible bacteria) in untreated hosts and the environment. In the absence of further information, we assume that selection operates at the same rate per unit of time in the environment.

These processes can be described by the following equations:

$$\Delta E = f\left(\sum_{i=1}^{N} \frac{H_i}{N} - E\right) - \frac{sE(1 - E)}{1 - sE};$$

and

$$\Delta H_i = 1 - H_i \text{ with probability } T/219;$$

and

$$\Delta H_i = g(E - H_i) - \frac{sH_i(1 - H_i)}{1 - sH_i}, \text{with probability } 1 - T/219$$

where t is the time in generations, H_i ($i = 1$ to N) represents the frequency of resistance in different hosts, and E represents the frequency of resistance in the environment. We started the model with negligibly small levels of resistance and ran the model until the average frequency of resistance in hosts and the frequency of resistance in the environment reached approximately steady states (some fluctuations are inevitable, given the stochastic nature of the simulations). Copies of the FORTRAN 77 program used for this simulation can be obtained from one of the authors (M. L.).

Herein we report the average level of resistance in the entire population of hosts. However, if resistance is widely distributed among the population, this number may underestimate the extent of resistance, since even small numbers of resistant bacteria in a given individual may be important as donors of resistance genes and may occasionally become opportunistic pathogens. We are currently studying a related mathematical model to

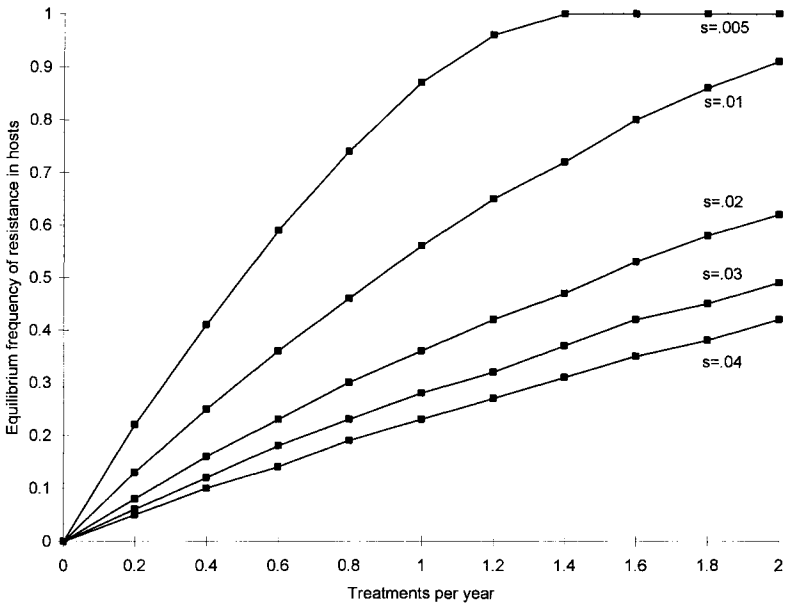

Figure 2. Simulation results: steady-state frequencies of antibiotic-resistant commensals as a function of the annual incidence of treatment and fitness cost (s) associated with resistance. In these runs, the proportion of bacteria in the environment replaced by enteric flora expelled by hosts, f, is 0.05 per generation. The proportion of the enteric flora that are replaced each generation by bacteria from the environment, g, is 0.005 per generation. In these simulations, the generation time in the hosts and in the environment is 40 hours.

determine the distribution of antibiotic resistance across the population of hosts.

The Anticipated Frequency of Resistant Bacteria

In our analysis of this model we consider the contribution of the different parameters to the frequency of resistant bacteria in randomly chosen hosts. While the choice of the parameter values used in this analysis are arbitrary in the sense of not being specific for any particular commensal species, we believe they are in a realistic range for *E. coli* in the enteric flora of human hosts.

As one would anticipate, for any given rates of exchange, f and g, the frequency of resistant bacteria in the host population is positively related to the rate of treatment, T, and negatively related to the cost of resistance, s (figure 2). In figure 2, $f = 0.05$ per generation and $g = 0.005$ per generation; if hosts are treated once a year with the selecting antibiotic and there is a 1% fitness cost associated with resistance ($s = 0.01$), then at steady-state the average frequency of resistance in treated hosts is ~0.56. If, in this case, the incidence of treatment is decreased to once every 2 years, the frequency of resistance drops to ~0.30. With once-a-year treatment and a 4% fitness cost, the average frequency of resistance in the host population would be ~0.23.

For any given cost of resistance and incidence of treatment, the frequency of resistant bacteria in a host is positively related to the number of bacteria from hosts that enter the environment, f, and negatively related to the rate at which the host's commensal flora is replaced by bacteria from the common environment. For example, with one treatment per year, a 1% fitness cost, an f value of 0.05, and a g value of 0.001, the steady-state frequency of resistance is 0.78. With the same treatment and cost, but with an f value of 0.01 and g value of 0.005, the steady-state frequency of resistance is 0.45.

Rates of Decline in the Frequencies of Resistant Bacteria

In the above model, as long as hosts are treated, there will be a residual population of resistant bacteria. On the other hand, if we reduce the level of treatment, we should see a decline in the frequency of resistant bacteria, which would continue until the new equilibrium frequency is reached. In the best case—in which treatment ceases completely, there is a cost of resistance, and there is no other compensatory factor such as positive associated linkage selection—the frequency of resistant bacteria would decline to the level at which it is maintained only by recurrent mutation (or acquisition by horizontal transfer).

To get some idea of how long this would take, we consider a model of constant selection in a haploid population. Under these conditions, the amount of time, t, before the relative frequency of resistant bacteria goes from q_0 to q_t is given [5] by the formula

$$t \sim \frac{1}{s} \ln \frac{(1 - q_t)q_0}{(1 - q_0)q_t}.$$

For example, if $q_0 = 0.5$ and $q_t = 1 \times 10^{-8}$, with a 1% cost ($s = 0.01$), then t would equal 1,840 generations or, with a 40-hour generation time, about 8.4 years. With a 5% fitness cost, the same change in the frequency of resistant bacteria would take about 1.7 years.

While this may seem encouraging, it has to be emphasized that we are considering the best case, a complete termination of the use of the resistance-selecting antibiotic and no associated linkage selection. Moreover, even if the frequency of resistance became as low as 10^{-8}, with the kind of intense positive selection one would get with antibiotic use, the frequency of resistance would ascend quite rapidly if that antibiotic was used again.

For example, from the above formula the amount of time needed to go from a resistance frequency of 10^{-8} to 0.5, with a 10% net advantage due the presence of the selecting antibiotic, would be about 180 bacterial generations. Because of the disturbance of the established susceptible commensal population and reduced competition and increased resources, the generation time for the proliferating resistant bacteria is likely to be considerably less than the 40 hours for a steady-state flora.

Empirical Observations

Cost of Resistance

A number of investigators have presented evidence that in the absence of selection for the genes they are carrying, plasmids are likely to impose a cost in the fitness of their host bacteria [6–10]. A study one of us (B. R. L.) did a number of years ago [11] found that in chemostat culture, the carriage of the Inc FII plasmid R1 reduced the fitness of its *E. coli* K12 host by >5% per generation.

To explore the generality of this and other studies of the costs of plasmid carriage, another of us (L. S.) did a series of experiments with conjugative plasmids of six or seven different incompatability groups [12]. In these experiments, plasmid-bearing and plasmid-free cells of an almost isogenic *E. coli* K12 host were mixed at relative frequency of $\sim 10^{-4}$ to 1, and the changes in their densities were followed for a number of generations. These competing antibiotic-susceptible and antibiotic-resistant bacteria were maintained in serial-transfer rather than chemostat cultures and in surface as well as liquid communities (see [13] for the latter protocol).

In addition to following the changes in the densities of the original plasmid-bearing donors and plasmid-free recipients, L. S. also followed the changes in the concentration of new transconjugants. Five of these plasmids imposed a cost in the fitness of their hosts of 1%–6% per generation. For two plasmids, however, there was no evidence of a fitness cost in either liquid and/or surface culture.

Two of us (V. P. and S. S.) have examined the cost of chromosomal resistance by studying independent spontaneous antibiotic-resistant mutants of an acapsulate strain of *E. coli* 018:K1:H7 [14]. Of the four antibiotics considered, one of these resistant mutants, that to streptomycin, invariably reduced fitness (as measured by competition in serial-transfer culture), with costs of up to 20% per generation. While some of the mutants resistant to rifampin and spectinomycin were at a clear selective disadvantage under these culture conditions, resistance to nalidixic acid appeared to be selectively neutral.

Thus, taken at large, these experimental results are consistent with the conventional wisdom. In the absence of antibiotics, there are environmental conditions in which antibiotic resistance would be selected against. In some cases, however, the intensity of this selection can be quite weak.

Infectious Transfer—Maintaining R-Plasmids as Parasites

In the case of accessory element–encoded antibiotic resistance, there is a possibility that infectious transfer could retard or even offset the rate of decline in the frequency of resistant bacteria in the absence of antibiotics. This possibility is particularly problematic for conjugative plasmids [15], which, at least in theory, can be maintained by infectious transfer alone [16].

While experimental results suggest that the rates of transfer of most R-plasmids are too low to maintain them as pure parasites in the face of even modest costs associated with their carriage [15, 17, 18], there are exceptional plasmids that may be maintained by transfer alone [19]. Moreover, as indicated above, the cost of some plasmids may be negligible, and their long-term (if not indefinite) persistence by infectious transfer is a realistic possibility. Stated another way, in the absence of antibiotic-mediated selection, antibiotic-resistant bacteria could persist for extensive periods and their frequency could even increase because of infectious transfer.

Modification of the Cost of Resistance

While a resistance gene or accessory element may initially engender a cost in the fitness of the bacteria carrying that gene or element, natural selection would favor reductions in those costs. This could be achieved by selection for mutants at the resistance loci or mutant accessory elements that are less costly. This may not result in reversion to susceptibility or loss of the accessory elements. (Because of the nature of most chromosomal resistance—like modifications of ribosomal proteins, gyrases, or outer-membrane proteins—it is unlikely that deletions of single-copy resistance genes could occur without impairing the bacterium.)

Alternatively, the cost of resistance could be reduced by selection at other loci that modify the costs of resistance. In fact, there is evidence that the latter process does occur. Bouma and Lenski [9] examined the changes in relative fitness of an *E. coli* strain carrying a nonconjugative plasmid after 500 generations of serial passage in a medium selecting for the chloramphenicol resistance gene borne by the plasmid. Initially, the plasmid engendered a cost in host fitness. However, the bacteria isolated after 500 generations were not only more fit than their plasmid-bearing ancestors but also were more fit than plasmid-free segregants.

In essence, their increase in fitness could be attributed to an adaptation by the bacterial, chromosomal genes to the carriage

of the plasmid. The fitness of segregants of bacteria selected for the carriage of the plasmid was higher with carriage of naive plasmids than without carriage of such plasmids. Modi and Adams [10] also observed reductions in the cost of plasmid carriage, but in their case, the reduction in the cost of R-plasmid carriage involved changes in both the host and plasmid.

A recent study of streptomycin-resistant *rpsL* mutants of *E. coli* demonstrated that in the case of chromosomal resistance, fitness costs of resistance can also be rapidly reduced by natural selection without any reduction in the level of resistance. After 180 generations of growth in the absence of antibiotics, the fitness costs of resistance associated with two different *rpsL* mutations that initially imposed large costs (14% and 19% per generation) were reduced by more than one-half because of the ascent of compensatory mutations at a gene (genes?) other than *rpsL* [14].

Associated Linkage Selection

Many of the plasmids responsible for antibiotic resistance, R-plasmids, carry genes that code for resistance to more than one antibiotic [20]. From enteric bacteria one can readily isolate R-plasmids that determine resistance to five or more antibiotics. Consequently, even when a particular antibiotic is not used, the plasmid that encodes resistance to that antibiotic can be favored owing to the use of other antibiotics for which that plasmid also codes for resistance. In addition to this antibiotic-mediated associated linkage selection, R-plasmids often carry genes for other characteristics, like resistance to ultraviolet light, mercury, and other heavy metals; fermentation of carbon-energy sources; and virulence [21]. Associated linkage selection for these other plasmid-encoded characteristics could also contribute to the persistence of plasmid-borne antibiotic resistance, even when antibiotics are not used.

In fact, there is evidence that associated linkage selection for mercury resistance may indeed contribute to the persistence of some R-plasmids [22]. Summers and her colleagues [22] found that in the absence of recent antibiotic treatment, the enteric flora of humans with a high prevalence of resistance to mercury was significantly more likely to be resistant to two or more antibiotics than was the enteric flora of untreated humans that had no detectable resistance to mercury. To ascertain whether these mercury-resistance-determining R-plasmids were in fact selected for by the release of mercury from dental amalgam, Summers and her colleagues performed a prospective study with monkeys [22].

Their results support this interpretation. During the 5 weeks following the introduction of amalgam fillings, there was an increase in the frequency of mercury-resistant intestinal bacteria. This increase occurred again when the fillings were removed. In the periods immediately following installation and removal of these amalgams, there were corresponding increases in the concentration of mercury in the feces.

Intensity of Antibiotic-Mediated Selection

In our model of the population genetics of antibiotic resistance, we assumed that as a consequence of antibiotic treatment the frequency of bacteria resistant to that antibiotic will ascend to 100% in a very short amount of time (instantly in our model). Is this realistic? There is a plethora of evidence that antibiotic treatment will increase the frequency of resistance both in the species being treated and in nontargeted, commensal bacterial species. Indeed, that was the motivation for the World Health Organization meeting that spawned this collection of reports. However, we are unaware of any study reports offering estimates of the intensity of this selection in treated hosts and what frequencies these resistant bacteria achieve.

About 10 years ago, we did a pilot experiment to obtain some idea of the intensity of selection for resistance in the intestinal flora in association with oral antibiotic treatment in two human hosts (one of the authors, B. R. L., and a member of his family, M. R. L.). We estimated the density of *E. coli* (and other lactose-fermenting aerobes, but primarily *E. coli*) by plating on minimal lactose agar (with and without antibiotics) diluted samples of suspended fecal samples taken before, during, and after antibiotic treatment. One host took a course of ampicillin, and the other, B. R. L., a course of erythromycin and then of tetracycline. Estimates were made of the frequencies of bacteria resistant to tetracycline (300 μg/mL), kanamycin (250 μg/mL), ampicillin (250 μg/mL), and streptomycin (250 μg/mL).

Four observations are of particular importance. First, before antibiotic treatment, the frequency of ampicillin-resistant flora in M. R. L. and of tetracycline-resistant flora in B. R. L. were on the order of 10^{-2} or higher. B. R. L. had not taken antibiotics for at least 10 years before this encounter. Second, by the second stool sample taken after treatment commenced, the frequency of ampicillin-resistant coliforms in M. R. L. and of tetracycline-resistant coliforms in B. R. L. rose dramatically, and these dominated the bacterial population during most of the course of treatment. Third, the frequency of bacteria resistant to antibiotics other than those employed for treatment also increased, along with the frequency of those resistant to the selecting antibiotic. Fourth, for the month or so that samples were taken after treatment was terminated, the frequency of antibiotic-resistant bacteria waned but persisted at measurable levels.

The Frequency of Antibiotic-Resistant Coliforms in the Enteric Flora of Humans in the General Community

One prediction of this model is that as long as individuals in the population are intermittently treated with antibiotics and, in absence of treatment, the rate of loss of the resistant cell types is low to modest, there will be a substantial frequency of resistant *E. coli* in the enteric flora. Levy et al. [23] report a high frequency of antibiotic-resistant coliforms in the intestinal flora of humans, regardless of whether or not they have been

under recent antibiotic treatment. In part to ascertain the generality of their finding, and more to isolate R-plasmids, we have recently initiated a similar survey on a much more modest scale than the 600-subject study of Levy and his collaborators.

In our study we plated different dilutions of suspensions of fecal samples on antibiotic-free lactose minimal agar, to estimate the total cell (cfu) density, and on lactose minimal agar containing one of the following antibiotics: ampicillin (250 μg/mL), kanamycin (250 μg/mL), and tetracycline (300 μg/mL). So far we have restricted this survey to 13 people (some of the authors and their children and associates) in Georgia. None of these people were taking antibiotics during the sampling period. However, at least three of these hosts had been under antibiotic treatment within 3 months of this study.

The results obtained are presented in figure 3. Coliform bacteria resistant to one or more of these antibiotics were present in all 13 of these subjects. Resistance to ampicillin dominated, both in its incidence among hosts and in its frequency in individual hosts. Resistance to tetracycline was next in incidence and frequency, and resistance to kanamycin was a poor third. Included among the people with ampicillin-resistant coliform flora was a 10-month-old girl, who had never been treated with antibiotics. Virtually all of the coliform bacteria isolated from this infant's feces were resistant to ampicillin.

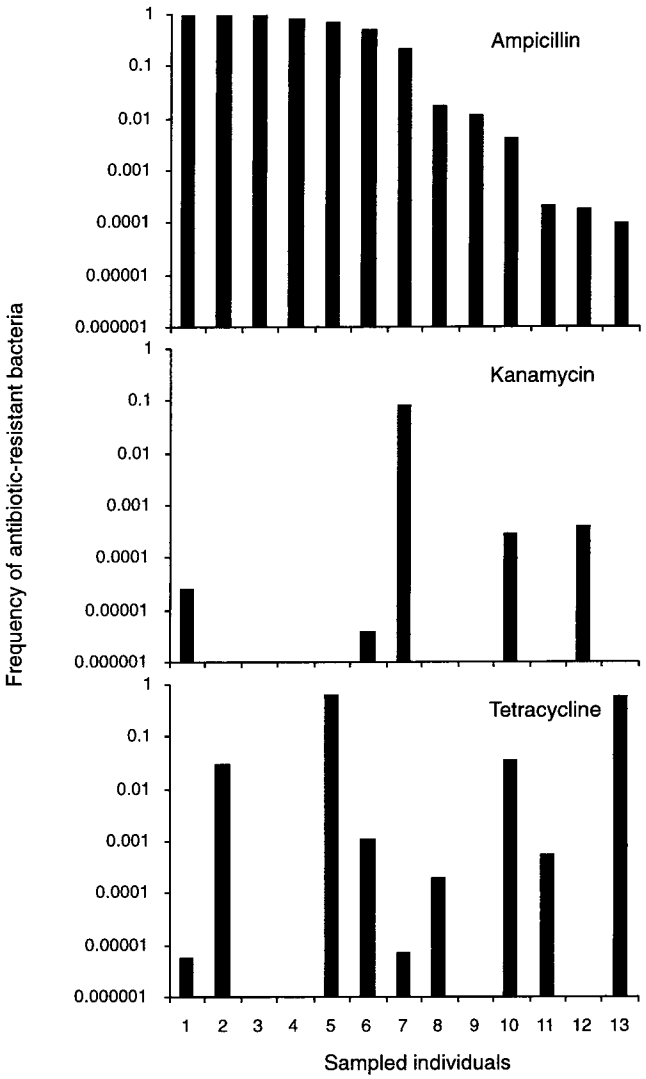

Figure 3. Frequency of antibiotic-resistant bacteria in the coliform flora of 13 human hosts. Fecal samples were suspended in 2 mL of saline. The total cell densities per mL of suspension were estimated from data about colony counts on minimal lactose agar. The fraction of this total cell suspension resistant to ampicillin (250 μg/mL), kanamycin (250 μg/mL), and tetracycline (300 μg/mL) was estimated by the plating of different dilutions on agar containing these antibiotics at the noted concentrations. One sample was obtained from each host.

Discussion

Overview

The results of our analysis of our model of the epidemiology of antibiotic resistance in the commensal flora of human hosts indicate that as long as antibiotic-resistant bacteria exist and a fraction of hosts are treated with antibiotics, resistant bacteria will persist, even when resistance engenders a cost in fitness. The frequency of antibiotic-resistant cells in the bacterial population is directly related to the rate at which hosts are treated and the rate at which bacteria are transmitted from their human host to the common environment. The frequency of resistant bacteria in this model is inversely related to the cost of resistance and the rate at which host flora is replaced by bacteria from the antibiotic-free environment.

With the complete cessation of use of an antibiotic for which there are resistant bacteria, as long as there is a cost associated with resistance, the frequency of these bacteria resistant to that antibiotic will decline to low levels. Even with very modest fitness costs (on the order of 1% per generation), these low levels of resistance could be achieved within a decade or less. On the other side, even when the frequencies of resistance are quite low (on the order of 10^{-8} or less), if resistant bacteria are present in that bacterial population, then once the selecting antibiotic is used again to any extent (as it may be in a hospital setting), these resistant bacteria will ascend to high frequencies very rapidly (within days or months).

While experimental results suggest that both chromosomal and plasmid-borne antibiotic resistance are likely to engender a cost in fitness of bacteria, these costs can be quite modest ($<1\%$ per generation) or even undetectable in competition experiments performed in vitro. Moreover, the results of (co)evolution studies indicate that at least for plasmid-borne antibiotic resistance, as time proceeds, the cost of carrying these resistance plasmids will decline. The host cells and/or their plasmids adapt to each other.

Although theoretical and experimental studies of the population dynamics of antibiotic-resistance plasmids and transposons have concluded that these accessory elements are unlikely to be maintained by infectious transfer alone, there may be excep-

tions. Under any conditions, the infectious transfer of these elements will retard the rate at which they would be lost due to selection against their carriage. Finally, because multiple genes are carried on many antibiotic-resistance transposons and plasmids, even when specific antibiotic-resistance genes are no longer under positive selection, these genes could be maintained for extensive periods of time by associated linkage selection favoring other loci.

The results of surveys of the incidence of antibiotic-resistant *E. coli* in the enteric flora are at least qualitatively consistent with the predictions of this model of the population genetics of resistance in commensal populations of bacteria. Antibiotic-resistant *E. coli* (and other aerobic gram-negative coliform bacteria) can be isolated at substantial frequencies from the feces of humans who have not recently received antibiotic treatment and even from those who have never been treated with antibiotics.

Thus, the picture one gets from putting together these theoretical predictions and experimental observations is not optimistic. These results suggest that even if we become considerably more prudent in our use of antibiotics, as long as we continue to use these antimicrobial agents at all, the resistance genes (and accessory elements) that are currently present in our commensal flora will continue to persist at substantial frequencies. Moreover, unless we can completely eliminate these resistance genes from a population, even when their frequencies are quite low, they will quickly achieve problematic frequencies once the selecting antibiotic is used commonly.

Caveats and Direction of Future Work

Of course, this conclusion can be seen as only one opinion, and an opinion founded on simple models and empirical results based on limited amounts of data, mostly from studies with *E. coli* and other enteric microbes. We certainly see these models as only first steps in the development of a formal (mathematical) theory of the population genetics (or, more broadly, the population biology) of antibiotic resistance—a pretentious-sounding but, we believe, very necessary enterprise.

We are now in the process of constructing and analyzing the properties of additional models of the population genetics of antibiotic resistance. One of these new models is for acute and potentially cleared infections with bacteria that are the target of antibiotic treatment. We are also extending our models of antibiotic resistance in the commensal flora to consider the distribution of frequencies of antibiotic-resistant flora among hosts, rather than just the mean, as considered here.

In our analysis of these models, consideration is being given to the rate of decline in the incidence of resistance following reductions in the frequency of treatment, rather than to the steady-state frequency for particular parameter sets, as in the above model. In addition to the goal of predicting the steady-state frequencies and changes in the frequency of antibiotic-resistant pathogens and commensals, we expect (or, more accurately, *hope*) that these models or extensions of them will also be useful for developing treatment protocols that will be effective and at the same time will minimize the rate of buildup of resistance.

The construction of mathematical models and analysis of their properties are enjoyable, satisfying, and nonpolluting endeavors. However, the utility of these models for prediction or design depends on their reality and generality and the accuracy and generality of the estimates of their parameters. At this juncture, we believe a fair amount of additional empirical research (experiments as well as survey studies) is needed to properly evaluate these different kinds of models and to obtain realistic estimates of their parameters and ranges of these parameters.

Conclusion

We expect that the precise relationship between the frequencies of resistant bacteria and the rates of antibiotic use will vary among different models of the population genetics of resistance, and among different species of pathogens and commensals. Nevertheless, we doubt that the qualitative conclusions will be very different. The frequency of antibiotic-resistant bacteria and the direction and rates of change in these frequencies are intimately tied to the extent to which antibiotics are used. The more sagacious use of antibiotics would almost certainly be reflected in declines in the frequency of antibiotic resistance in populations of commensal and pathogenic bacteria. However, without a complete termination of the use of an antibiotic, we anticipate that, in general, the rates at which these frequencies decline will be, at best, modest.

Moreover, as long as the resistance genes and plasmids are present in even very low frequencies, they can rapidly ascend to problematic levels once the selecting antibiotic becomes commonly employed again. At this point, it seems unlikely that the antibiotics with waning efficacy because of resistance will be replaced at an adequate rate to compensate for their loss from our arsenal [24]. Technology is losing the arms race with evolution.

For these reasons we believe it is appropriate as well as prudent not to anticipate returning to the antibiotic-susceptibility days of yesteryear in reasonable amounts of time. Now is the time to develop alternatives to traditional antibiotics for the prevention and treatment of bacterial infections.

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