# Resistance to Antimicrobial Chemotherapy: A Prescription for Research and Action

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**ABSTRACT: The growing problem of resistance to antimicrobial chemotherapy was discussed by participants at the February 1995 workshop at Emory University on population biology, evolution, and control of infectious diseases. They discussed the nature and source of this problem and identified areas of research in which information is lacking for the development of programs to control of the emergence and spread of resistant bacteria. Particular attention was given to theoretical (mathematical modeling) and empirical studies of the within and between-host population biology (epidemiology) and the evolution of microbial resistance to chemotherapeutic agents. Suggestions were made about the kinds of models and data needed, and the procedures that could be employed to stem the ascent and dissemination of resistant bacteria. This article summarizes the observations and recommendations made at the 1995 meeting and in the correspondence between participants that followed. It concludes with an update on the theoretical and empirical research on the between- and within-host population biology and evolution of resistance to antimicrobial chemotherapy most of which has been done since that meeting. KEY INDEXING TERMS: [Am J Med Sci 1998;315(2):87–94.]**

Microorganisms (bacteria, viruses, protozoa, and fungi) resistant to the chemotherapeutic agents used to control them may prove to be among the most significant sources of emerging and reemerging infectious diseases in the coming millennium. Like the majority if not all of the emerging diseases we have seen during these past three decades, the ascent of pathogenic and commensal microorganisms resistant to chemotherapeutic agents can be attributed to technology, in this case the use and overuse of chemotherapeutic agents. Not so clear, however, is whether technology can also provide a solution to the problem of emerging chemotherapy-resistant microparasites. How can we slow or reverse the rate at which resistant microbes evolve and spread? What must be done to better understand, control, and, ideally, preclude the appearance and proliferation of microbes resistant to chemotherapy? What are the alternatives to chemotherapy for the treatment of infections with microorganisms?

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### The Ideal and Real Worlds of Antimicrobial Chemotherapy

In the ideal world, before an antimicrobial agent is administered for treatment or prophylaxis, the target species of microbe would be identified and its antimicrobial resistance pattern would be known. The infection would be at a stage where it can be readily be controlled by chemotherapy, and the timing would be perfect. The antimicrobial agent would be very specific and there would virtually no effect of treatment on the commensal flora or side effects for the patient. While these microbes would have the potential to acquire resistance (by mutation or infectious transfer from other microbes)—even in the ideal world there is genetic variation, recombination, and evolution by natural selection—resistance would almost never preclude effective treatment. For most antimicrobial agents, the incidence of treatment would be so low and the cost of resistance to the microbe so great that the frequency of resistant strains would not increase. For the small minor-

ity of antimicrobial agents that fail because of a local build-up of resistance, there would always be effective alternatives. Finally, antimicrobial agents would cost little for consumers and still provide more-than-adequate profits for the producers.

Ours, however, is not the ideal world and, with respect to antibiotic use, it is quite far from ideal. Most antibiotic therapy is empiric. The target species of microbe and its resistance pattern is generally not known other than statistically (or by "experience") before antimicrobial agents are first administered. Commonly it is not even clear that a microparasite is the etiologic agent of the disease being treated or prevented. The stage of the infection is generally not known with any precision and in many cases, antimicrobial agent treatment may be ineffective because the infection has progressed too far or the microbes responsible are resistant or (at that stage of the infection) physiologically refractory to that antimicrobial. In some situations, even with antimicrobial agents to which the bacteria are susceptible, chemotherapy may not alter the course of the disease and may even be deleterious to the patient. Because of the limited direct information about the etiologic agent and its resistance pattern, the antimicrobial agents of choice often have broad spectrums and are commonly of the most recent origin and most expensive.[1] Consequently, the administration of antimicrobial agents often has profound effects on the ecology of the commensal flora—effects that may be severely deleterious or even toxic or lethal to the patient in their own right. While it may be that the carriage of the resistance genes and the accessory elements that bear those genes may engender a cost in the fitness of the target microbe, the magnitude of that cost may be low[2] or evolve to be low.[3,4] Indeed it is conceivable that those resistant genes and accessory elements may confer an advantage on their host, beyond that associated with resistance.[3] The net effect of all will be the maintenance or further increases in the incidence of resistant pathogenic and commensal microbes in treated host and the environment at large. Finally, in this all-too-real world, when an antimicrobial agent becomes ineffective because of resistance, alternative broad (or even narrow) spectrum agents may not exist or may not be produced for economic reasons.

The ideal world will not and cannot be. The real world from whence the problems of microparasitic resistance to chemotherapy arose is well known,[5–9] as are the primary reasons for this problem.[1,10,11] Not so clear, however, is the prognosis for the continued efficacy of antimicrobial chemotherapy or what, in a realistic world, can and should be done about microbial resistance to chemotherapy. These not-so-clear points are the focus of this article.

**Box 1.**

The view that resistance is a problem of the *mis*use of antimicrobial agents, rather than just use, and that more prudent use of antimicrobials will be followed by rapid declines in the incidence of resistant microparasites, are appealing. These views are based on wishful thinking rather than fact or formal theory. Epidemiologic models can at least supply that theory; these models can also be used to design and evaluate, a priori, protocols for the use of antimicrobial agents in communities and hospitals, protocols that will minimize the rate of buildup and consequences of resistance to antimicrobial agents. Although better designed treatment protocols and the more prudent (reduced) use of antibiotics may not lead to rapid reductions in the frequency of resistance, they will certainly reduce the rate at which resistance is increasing and spreading to new lineages and species of bacteria.

## Mathematical Modeling

The evolution, ascent, and spread of microparasites resistant to chemotherapeutic agents is a problem of population and evolutionary biology, albeit one that has received little attention by practitioners of these subjects. The primary tool of theoretical population biology, mathematical modeling, provides one way to address questions of prognosis and intervention. Mathematical models can be used to:

1. Identify and quantitatively evaluate the relative contributions of the different genetic, environmental, and human practice factors that control the evolution, ascent, spread, and persistence of chemotherapy resistant microbes;
2. Predict the evolution of resistance and the direction and rates of change in the incidence of resistant microbes under different regimes of antimicrobial chemotherapy; and
3. Design and evaluate protocols for the application of antimicrobial agents that are effective in preventing or treating the disease and, at the same time, minimize the rates of (or better yet, preclude) the transmission of organisms to other hosts or to the environment at large.

**Two Kinds of Models.** For these endeavors, we see a need for two, ideally overlapping, classes of models: those that treat the evolution and spread of resistance in communities of hosts, and those that treat the changes in the incidence of resistant microparasites and commensals in an individual host during and following antimicrobial chemotherapy. Of particular concern for the former, epidemiologic, models is the relationship between the incidence and term of antimicrobial chemotherapy, the fitness costs associated with resistance, and other factors that determine the fate of resistant microbes in treated and untreated hosts. With these epidemiologic models, it should be possible to predict the direction and rates of change in the incidence of resistance under various regimens of antimicrobial chemotherapy

**Box 2.**

In the microparasites responsible for some diseases (eg, tuberculosis), resistance arises primarily by mutation. Thus, on first consideration it would seem that the simultaneous use of multiple chemotheraputic agents, as in short course chemotherapy,* would substantially prolong the term of effective treatment and completely preclude the evolution of resistance. The idea is that the pathogen would only be able to proliferate when it is simultaneously resistant to all of the chemotherapeutic agents employed. The likelihood of this occurring is on the order of the product of the frequencies of unsolected to resistance genes, probabilities on the order of $10^{-20}$ or lower when three or more antibiotics are used. Unfortunately, from the perspective of population genetics this problem is less straightforward. For example, even when the mutations for resistance to each antimicrobial agent are independent genetically and functionally—an implicit assumption in the above calculation—if the concentrations of these chemotheraputic compounds get too low or if there is tissue heterogeneity in their activity, selection could favor intermediates, microbes that are resistant to fewer than all the agents used for treatment. The net effect would be a far more likely and far more rapid evolution of multiple resistance.

* *Coombs et al., 1990.*[12]

and the effects of increasing or reducing the extent to which these agents are employed (Box 1).

We see two primary roles for within-host models of antimicrobial chemotherapy. One is to assess quantitatively the factors that contribute to the rate at which treated hosts disseminate resistant microbes into the community at large. The other is to explore quantitatively the factors that contribute to treatment failure due ascent of resistance (Box 2).

*Resistance in the Commensal Flora.* Mathematical models of the within- and between-host population genetics of resistance must not only consider the specific pathogens that are the targets of antimicrobial therapy and prophylaxis, but they must also consider resistance in commensal microorganisms in the treated hosts and in the environment at large. These usually commensal microbes are often not-quite-innocent bystanders in the chemotherapy drama. Some, like *Escherichia coli*, *Haemophilus influenzae*, *Streptococcus pneumoniae*, and *Neisseria meningitidis*, include strains that are occasional and opportunistic pathogens. Moreover, in the case of bacteria (but probably for other microorganisms as well), almost all of these commensals could serve as sources of resistance genes and accessory elements for other microbes, including pathogens. Changes in the commensal flora can also lead to pathogenesis in its own right (eg, antibiotic-associated colitis).[13] Finally, these commensals are bellwethers—they can be and are being used to estimate the incidence of resistance in individual hosts and communities of hosts.

For these reasons, we believe it would be useful to develop separate models for the population genetics of resistance for three classes of microbes: the persistent commensal flora, the pathogens responsible for acute infection that are cleared in short periods of time, and pathogens that persist over long periods.

## Empirical Studies

Mathematical modeling is a pleasant, ego-satisfying, safe, and relatively nonpolluting enterprise. However, for these models to be useful for the very practical problem of dealing with microbial resistance to chemotherapeutic agents, their construction and analysis have to be closely tied to empirical studies. We can of course make the case the other way as well; experiments and data-gathering, in the absence of theory, are often hard to interpret and virtually impossible to generalize upon. There is a need to coordinate the efforts of modelers and empiricists to develop realistic, useful models of the population genetics of resistance.

**Data Needed.** For the epidemiologic models, four kinds of data are needed:

1. Accurate estimates of the extent and nature of the use of antimicrobial agents. Included in these data should be estimates of the frequencies at which specific antimicrobial agents are used for different purposes, the doses employed and the nature and term of their use.
2. Retrospective and prospective estimates of the relationship between the application of antimicrobial agents and rates of change in the frequencies of resistant pathogens and commensals in treated hosts and communities of hosts. Of particular concern are the rates at which resistant forms ascend in the target pathogens and commensal flora of treated hosts and the frequencies these resistant microbes achieve. Also of concern is the effect of treatment on the rate of dissemination of the resistant microbes.
3. Retrospective and prospective estimates of the rates of change in the frequencies of resistant microbes in hosts and the in environment following the local or global termination of use of specific antimicrobials. At what rate do frequencies of resistant organisms decline? What levels do they reach? What are the reasons for these declines in the incidence of resistant organisms? Are the resistant bacteria being replaced by sensitive variants of the same clone or by other clones?
4. Determinations of the source of resistant microbes in the community. Is the resistance due to the de novo acquisition of resistance genes (or accessory elements) in treated hosts? Or are the resistant microbes (or their accessory ele-

ments) being transmitted between hosts and spread through the community of hosts?

5. Estimates of the effects of resistance on the fitness of bacteria within infected hosts and the environment and their ability to be transmitted to and colonize new hosts. In these considerations of the effects of resistance on the with- and between-host fitness of bacteria, it is necessary to consider the consequences of subsequent evolution on this fitness. Will the bacteria compensate for the fitness costs associated with resistance?

To obtain these data, it will be necessary to combine surveys of the genetic epidemiology of the microparasite using biochemical, molecular (DNA-based), or classical (serotype, biotype, phagetype) data with that on the frequency and pattern of antimicrobial resistance and the genes (and accessory elements) that code for this resistance.

For within-host modeling, two kinds of data are needed, both of which must be obtained experimentally. The first is pharmacokinetic, the concentrations of chemotherapeutic agents in treated hosts and the temporal changes and tissue heterogeneity in these concentrations. The second is microbial; included in this class of data are, for each chemotherapeutic agent: (1) the rates at which resistance arises by mutation or recombination (including accessory element transfer), (2) the intensity of selection for resistance imposed by that agent and how these intensities vary with concentration and among sites (eg, tissues); (3) the cost of resistance to the fitness of the microbe; and (4) the changes in the frequency of resistant microbes in the sites of their transmission. Also necessary for these models are estimates of the generation times of these microbes in infected hosts and information about positive and negative synergy and antagonism between chemotherapeutic agents when used simultaneously.

## Alternatives to Antimicrobial Chemotherapy

The results of the previously described studies of the population genetics of resistance are not in, and with few exceptions,[16–17] we do not have good estimates of the relationship between the incidence of use of antimicrobials and the directions and rates of change in the frequency of resistant microorganisms. Nevertheless, at this juncture based on what we *do* know, there is no reason to be optimistic. It is not clear that even if we could dramatically reduce the use of antimicrobial agents, there would be rapid declines in the frequencies of resistance to existing antimicrobials. The resistance that has already evolved may well be with us for some time to come. Now is the time to consider alternatives to classic antimicrobial chemotherapy to prevent and control infections. Some of these alternative procedures had been used in the past and were suppressed or eclipsed by the success of antimicrobial chemotherapy; others are now being developed.

**Prevention.** Clearly the most effective way to minimize the morbidity and mortality of microparasitic infections is either to prevent the colonization of the microbe in the first place or to minimize the size of the infecting inoculum. The procedures for doing this—essentially variations on themes of sanitation, hygiene, isolation of infected individuals, antisepsis, and vaccination—are well known and have proven effective. Indeed, these procedures, along with better nutrition and living conditions, are the primary reason, rather than chemotherapy, for the decline in infectious disease mortality and morbidity.[18] While there have been some impressive uses of modest technology to prevent microparasite infections (eg, insecticide-impregnated bed nets for malaria[19]), in general the rate of development and deployment of new methods to prevent infections has been modest. New vaccines are being sought, and there have been recent successes, such as the *Haemophilus* influenzae b vaccine.[20] However, the development, production, and distribution of new vaccines engenders scientific and economic problems that are not easy to surmount. Finally, the use of antimicrobial agents has, at least to some extent, had a negative impact on measures to prevent infection. In hospitals, the use of antibiotics for prophylaxis and treatment of nosocomial infections has almost certainly relaxed the reliance on the labor-intensive, time-consuming, and expensive antiseptic procedures employed in operating and recovery rooms.

**What Will Be and What Was.** To date, most of the effort and almost all of the success in treating microparasitic infections has been restricted to procedures that limit or prevent the proliferation of the population of infecting microbes. On the other hand, much of the morbidity and mortality associated with infections can be attributed to the host's immune defenses overresponding to the infecting microparasite[21] rather than just the proliferation of that microbe. Thus, one way to reduce the morbidity and mortality associated with at least some microparasitic infections is to control the host's response to the infecting microbe. There have been a number of efforts in this direction, particularly for the control of bacterial sepsis.[22,23]

Prior to the current era of antimicrobial chemotherapy, a number of procedures were employed to control microparasitic infections, including:

- Vaccine therapy: the use of antigens to limit infections; Pasteur's rabies treatment is not the sole example.[24,25]
- Serum therapy: passive immunization; it may have had problems, but for some infections like pneumococcal pneumonia it was[26,27]

- Phage therapy: the use of phage to control bacterial infections (Martin Arrowsmith wasn't the only one[28,29]); under some conditions, it can be effective[30–33]
- Interference therapy: the use of non-pathogenic microbes to prevent the pathogenic effects of other microbes.[34,35]

In the main, these procedures were not as effective as antimicrobial chemotherapy, and because of their specificity they are certainly more difficult and inconvenient to use. For these reasons and doubtless others, their development and application was eclipsed by antimicrobial chemotherapy. Nevertheless, from a perusal of this literature it is clear that under some conditions these methods were somewhat effective. Moreover, with what we now know about the mechanisms of pathogenesis, the immune system, and the genetics, molecular biology, physiology, and population biology of phage and with current biotechnology it may well be possible to substantially improve the efficacy of these older procedures. We certainly endorse quests for alternatives to contemporary antimicrobial chemotherapy. At the same time, however, it is essential that we also encourage the improvement of existing measures to reduce the transmission of microbes and the development of new ones. Even untreatable infections can be prevented by sanitation, vaccination, pasteurization, and other tried-and-true infection prevention measures.

### Technical Solutions Will Not Be Sufficient

We have no doubt that with a concerted effort, these and other effective alternatives to many current chemotherapeutic agents could be developed. However, it seems reasonable to expect that the majority if not all of the alternatives considered above will be substantially more specific than the broad-spectrum antibiotics currently favored for the treatment of bacterial infections. While this species and strain specificity can be a virtue from the perspective of the innocent commensals, it also makes the application of these alternative treatment protocols much more difficult than that of broad-spectrum antimicrobials. Effective treatment would require information about the species and possibly even genotype (strain) of the target microparasite. While this may not be difficult for epidemic, outbreak, or some nosocomial situations, it would be problematic for community-acquired infections with genetically and serologically variable species of microparasites. The use of very specific agents and procedures to treat community acquired infections would also require a broad education (and reeducation) program for clinicians.

The bright side is that with the use of molecular (genetic) procedures and a sufficient effort, the technology for this rapid identification could be developed for many microparasites. Also being developed is the technology to process and disseminate the vast amount of information needed for this kind of diagnostic program. The downside is economic, as it is so often the case. These more narrow-spectrum antimicrobial agents and procedures would have substantially more limited markets than broad-spectrum antimicrobials. Thus, the development, testing, licensing, production, and distribution of these alternatives would not be very attractive commercially. Government, universities and other nonprofit institutions would have to play a greater role in generating and possibly even producing and distributing these alternatives.

**Box 3.**

From the perspective of anything less than absolute selection (where only resistant microbes survive) $10^{-2}$ and $10^{-8}$ are not very different numbers. For example, with a 50% selective advantage per generation favoring resistance, if the initial frequency of resistant bacteria is $10^{-2}$, it will take about 9 generations before the frequency of resistance reaches 0.5. With an initial frequency of $10^{-8}$, and the same intensity of selection, it would take approximately 37 generations for half the bacteria in the population to be resistant. This is at least part of the reason for the failures of programs that cycle antimicrobial agents in hospitals; that is, unless resistant bacteria are totally eliminated or their frequencies are reduced to extremely low levels, when the same antimicrobial agent is used again, resistance will ascend very rapidly.

*Crow and Kimura*[14]

**Buying Time.** We believe that we can develop effective alternatives to current antimicrobial agents for many microparasitic infections that are now successfully treated with antimicrobial chemotherapy (and possibly others as well). Moreover, when for diseases caused by major (read: common) pathogens, the resistance problem approaches the stage where it is sufficiently grave and widespread to preclude effective treatment or prophylaxis with formerly effective antimicrobial agents, we expect (hope) there will be adequate motivation to overcome the economic and other nontechnical problems with producing and implementing these alternatives. Some pathogens are already at this critical stage, like vancomycin-resistant Enterococci.[36]

What will be needed for the development of these alternatives, in addition to the usual money and personnel, is time, possibly a lot of time. Although we do not yet have accurate estimates of the rates and extent to which resistance to antimicrobials is increasing among pathogens and commensals, the direction is already clear. If we maintain "business as usual" with respect to our overuse of antimicrobial agents, we can only expect the resistance problem to become increasingly acute, and more and more

pathogens will become appropriate fodder for the situation described in Box 3. It is unlikely that the resistance problem will rapidly wane, simply by being more prudent in our use of antimicrobial agents; on the other hand, it is certain that if we do *not* cut back our use of these agents, the resistance problem will worsen. Husbanding the antimicrobials in our current arsenal is only way we can buy time for the development and production of alternatives. In essence, we have to do the best we can to mimic the ideal world of antibiotic use described earlier in this article. Physicians and their patients, veterinarians, livestock breeders, and growers of high-value crops all have to learn to "say no to drugs."

### Update: October 1997

In early 1995 when we held this workshop (and for some time before[37]), there was already a great deal of concern about the clinical and public health problems associated with increasing frequencies of microbes resistant to antibiotics and other chemotherapeutic agents. In the less than three years since, this resistance problem has become perceptibly worse. The frequencies and diversity of resistant strains have, with few exceptions,[38] increased, and no antibiotics with new targets have been put on the market. These few years have also witnessed some significant landmarks in the era of antimicrobial chemotherapy, perhaps the most dramatic of which has been successful combination (multidrug) chemotherapy for HIV/AIDS,[39] and the first reported sightings of the long-feared vancomycin-resistant *Staphylococcus aureus*, albeit fortunately only low-level resistance.[40] There has also been heightened awareness of this problem by healthcare workers and more and better information about how to deal with the problem of resistance.[40a]

There has also been a marked increase in interest in the problems of drug resistance by investigators trained in population and evolutionary biology and a number of collaborations between people working in this area and medical microbiologists. This workshop in fact served as the catalyst for some this research and these collaborations. These studies have addressed and provided answers to the some of the questions about the population biology and evolution of resistance considered in this article.

Some of these investigations have examined the relationship between the rate of antibiotic use and the frequency of resistance in the normally commensal and occasionally (and opportunistically) pathogenic bacteria such as *S pneumoniae*, *E coli*, and *Moraxella catarrhalis*.[41-43] Other theoretical studies have used "compartment" models to follow the epidemiology of resistance in directly transmitted pathogenic bacteria that can be cleared by antibiotics,[44] to evaluate the efficacy of antibiotic treatment on the epidemiology of tuberculosis,[45] and the epidemiologic consequence of cycling and simultaneously employing multiple antibiotics.[46]

The models employed for commensal bacteria[41] suggest that even if the rate of antibiotic use is modest (eg, one treatment every second year), if the fitness cost resistance imposes on a bacterium is relatively low (eg, a 1% reduction in growth rate), the expected equilibrium frequency of resistance would be substantial, on the order of 30%. The equilibrium frequency of resistance would of course be lower if the fitness costs associated with the resistance-encoding genes and accessory elements are greater. Experimental studies with bacteria and HIV suggest, however, that even when drug resistance severely reduces the fitness of bacteria and viruses, subsequent evolution, even in the absence of drugs, is likely to reduce that burden by compensatory mutations rather than by reversion to susceptibility.[47,48] These in vitro experiments also suggest that the compensatory mutations that ascend in these situations establish a genetic background which virtually precludes reversion to sensitivity in that lineage.[47,49] Taken at large, it is hard to generate an optimistic interpretation of the results of these theoretical and in vitro experimental studies of the population genetics of resistance. They suggest that even with the more prudent use of antimicrobial agents, the frequency of resistance will not decline very quickly, if at all.

In addition to studies of the epidemiology of drug resistance, a number of investigators have used mathematical models to explore the within-host population dynamics of antimicrobial chemotherapy. One of the goals of this research is to assess the conditions under which resistance is anticipated to evolve when single and multiple chemotherapeutic agents are used for treatment. These theoretical studies have considered bacterial resistance to antibiotics and combine elements of pharmacokinetics and pharmacodynamics of drug treatment and drug action with the population dynamics of an infecting population of bacteria.[50-53] Analogous studies have been employed for HIV resistance to reverse transcriptase and protease inhibitors.[54-59] A particularly attractive feature of most of these studies of the within-host dynamics of antimicrobial chemotherapy is the (uncharacteristic) reality of the models used. In constructing these models and analyzing their properties, these investigators have paid close attention to the results of the experimental work being done on bacterial and HIV infections and antimicrobial chemotherapy.

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