Antimicrobial Use and Resistance

Michael A. Rubin, MD, PhD, and Matthew H. Samore, MD

Address
Division of Infectious Diseases, Department of Internal Medicine, University of Utah School of Medicine, 300 North 1900 East, Salt Lake City, UT 84132, USA.
E-mail: Michael.Rubin@hsc.utah.edu; Matthew.Samore@hsc.utah.edu

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As antimicrobial use continues to rise, we are experiencing a concomitant rise in the prevalence of antimicrobial resistance. The precise relationship between use and resistance, however, has been challenging to define. Although the selection pressure exerted by antibiotic therapy appears to be the primary force promoting resistance, it is clear that the pathway to resistance is different for various organisms and antimicrobial agents. By understanding the mechanisms by which resistance emerges and spreads, it should be possible to design intervention strategies to slow or halt the process. This review summarizes some of our current understandings about the development and transmission of antibiotic-resistant bacteria, some of the control measures designed to interrupt the process, and how mathematical modeling can help us to better understand these complex pathways.

Introduction

The discovery and development of antibiotics, which were first employed in medical practice in the 1940s, stands as one of the major accomplishments of medicine. Antibiotics, combined with vaccination programs and improvements in sanitation, contributed to the remarkable decline in deaths from infectious diseases during the past century [1]. However, the emergence of antimicrobial drug resistance, particularly among nosocomial pathogens, now appears to be threatening these medical advances. The problem has become so critical that antimicrobial resistance is now being identified as the most pressing infectious disease threat to public health by a number of leading organizations [2••]. Concern is heightened now more than ever, particularly with the recent report of the first clinical isolate of *Staphylococcus aureus* that is fully resistant to vancomycin [3••].

Evidence strongly suggests that antimicrobial use is a potent selective force for the emergence of drug-resistant organisms, and that the collective effect of antimicrobial use over the years has been to increase the overall prevalence of drug resistance. Nevertheless, clearly defining the link between antimicrobial use and antimicrobial resistance has been challenging. The pathway from antimicrobial use to the development and spread of antimicrobial resistance is complex and multifaceted, and if we are to have any impact on this problem it is essential that we gain a thorough understanding of the many processes involved. The goal of this review is to summarize our current understanding of the mechanisms leading to the emergence and spread of antimicrobial resistance, at both the organism and population levels; to examine how this understanding has led to interventions aimed at reducing the development and transmission of resistant organisms in the hospital setting; and to describe how mathematical modeling can aid in our understanding of these complex processes.

Mechanisms by Which Antimicrobials Select for Resistance

Most would agree with the broad concept that antimicrobial treatment exerts selection pressure that promotes antimicrobial resistance. This causal relationship is supported not only by a plausible biologic basis, but also by a long history of consistent observations. For instance, many reports over the years have documented the emergence of resistance to new antibiotic classes shortly following their introduction [4–6]. As well, throughout the latter half of the 20th century, countless studies recognized the association between antimicrobial consumption and the frequency of antimicrobial resistance [7–9]. As antimicrobial use has risen over the past few decades, we have certainly seen a concomitant rise in the prevalence of antimicrobial resistance, particularly among nosocomial pathogens.

Still, the precise relationship between an antibiotic and microbial resistance to that antibiotic is not altogether as straightforward as it may seem. In fact, in several cases it has been difficult to demonstrate that treatment with an antibiotic places a patient at an increased risk for the acquisition of an organism resistant to that antibiotic [10,11,12•]. For many antimicrobials and pathogens, the pathways by which exposure to the antimicrobial agent leads to the emergence, acquisition, and/or spread of a resistant organism can be quite varied and indirect (Table 1). Some of these pathways are described below, though such concepts have been explored in considerably more detail elsewhere [13•].

Organism-level antibiotic effects

In general, acquired antimicrobial resistance in microorganisms is mediated by modification of existing genes or acquisition of new genetic material. At one end of the

Table 1. Postulated roles of antimicrobial agents in promoting or selecting for resistant microorganisms

Organism/phenotype-level effects
Killing or inhibition of target pathogen with susceptible phenotype
Killing of commensal microorganisms
Failure to inhibit or kill existing subpopulations of resistant mutants
Intermediate levels of resistance
Fully resistant organisms
Failure to inhibit or kill new types of resistance organisms generated by recombinant events
Induction of previously unexpressed resistance elements
Stress-induced alteration of mutation rate
Individual/patient-level effects
Expansion of resistant subpopulations
Transient carriage
Persistent colonization
Treatment failure
Super-infection
Increased risk for acquisition of new strains
Increased load of resistant colonizers
Group/population-level effects
Increased transmission of resistant organisms
Higher probability that contact results in transmission
Decreased colonization resistance
Increased shedding by carriers
Higher frequency of contact as prevalence increases
Decreased transmission of susceptible organisms

spectrum is *Mycobacterium tuberculosis*, in which resistance to most antimycobacterial drugs is conferred by a single point mutation [14]. In the absence of antimicrobial selection pressure, bacterial cells with these mutations typically have no survival advantage or possess a decrement in fitness compared with wild-type organisms and are therefore unlikely to compete successfully against the dominant susceptible population. Exposure to an antimicrobial agent is the key selective force that promotes expansion of these mutant, resistant subpopulations. Emergence of resistance may thus occur during treatment of an infection in the individual host, depending on the density of organisms and the activity of the antimicrobial agent(s) used.

At the opposite end of the spectrum is methicillin resistance in *S. aureus* and vancomycin resistance in *Enterococcus*. For these resistance types, resistance is mediated by a set of transferable genes associated with transposable elements or plasmids. In these cases, as with *M. tuberculosis*, antimicrobial exposure acts as a selective force, but unlike the previous example, resistance does not arise de novo in an individual patient. Pre-existing susceptible strains need to acquire new genetic material to become resistant, an event that may occur at a rate that is exceedingly low, depending on the genetic requirements for resistance and frequency of gene transfer. Antimicrobials still promote dissemination of resistance but through actions on colonization resistance and suppression of susceptible populations. Bacteria exhibiting this mechanism of resistance often are colonizers rather than obligate pathogens. Furthermore, their exposure to antibiotics typically occurs during therapy for an unrelated infection, and thus their persistence and/or rise following therapy is not necessarily an indication of treatment failure [13•].

Situated between these two ends of the spectrum are many types of resistance that emerge as a sequence of multiple mutational events. In the case of fluoroquinolone resistance in *Streptococcus pneumoniae*, resistance occurs in stepwise fashion as a result of multiple mutation events in the genes coding for DNA gyrase and topoisomerase IV [15,16]. Typically, an initial mutation event occurs similar to that described above for *M. tuberculosis*, which results in low-level resistance to fluoroquinolones and a modest selective advantage to that subpopulation. As members of this subpopulation survive, proliferate, and migrate from one host to another, further mutational events occur which, in the setting of repeated antibiotic exposures, lead eventually to the expression of high-level fluoroquinolone resistance. Likewise, a similar pathway describes the development of an enhanced spectrum of activity of the plasmid-borne beta-lactamase found in many gram-negative bacilli, as mutation and antimicrobial-mediated selection have led to the rise of potent extended-spectrum beta-lactamases [17,18].

Also situated within this spectrum are situations where expression of some or all of the resistance genes is not constitutive. The organisms may thus display in vitro susceptibility to a particular antibiotic, but in vivo exposure to that antibiotic for a period of time may induce the expression of the resistance gene, resulting in phenotypic resistance and, potentially, treatment failure during the course of therapy. Such is the case with inducible beta-lactamases in various gram-negative bacilli such as *Pseudomonas*, *Enterobacter*, and *Serratia* [19,20]. In some cases, exposure to one antibiotic can induce the expression of more than one resistance mechanism, resulting also in resistance to antibiotics other than the one used initially [21].

In each of the above examples, the action of antimicrobials is to function primarily as a selective force. There is evidence, however, that antibiotics may contribute to the emergence of bacterial resistance also by inducing a state of hypermutability [22•,23]. Two notable examples include the effects of streptomycin and quinolones on genetic mutation rates in *Escherichia coli*. There is appreciable evidence that exposure of *E. coli* to streptomycin, an antibiotic known to increase the number of translational errors, results in the induction of a mutator phenotype [24]. Put another way, streptomycin-mediated mistranslational stress essentially elevates the level of background mutagenesis, effectively allowing a greater opportunity for the microbes to evade the inducing stress (streptomycin). Another form of stress-induced mutagenesis, referred to as adaptive mutation [25], appears to occur when *E. coli* are exposed to quinolones. In this instance, evidence suggests that the mutation process—traditionally thought to occur only in actively dividing bacteria during the DNA replication process—can also occur in

nondividing cells while under selection for a particular phenotype (antibiotic resistance). In the case of *E. coli*, the presence of ciprofloxacin in the growth medium was shown to induce the emergence of ciprofloxacin-resistant colonies, despite the absence of detectable replication or death during the observation period [26].

Individual- and population-level antibiotic effects

Once resistant microorganisms emerge and their survival is enhanced through the action of antimicrobial agents, a subsequent hazard is that resistant species will escape from their host into the environment, with the potential for colonizing or infecting other individuals. Antimicrobials are capable of exerting some measure of influence on this process as well, albeit in a more indirect fashion (Table 1). By doing so, it could then be concluded that antibiotics have discernible effects not only on organisms and individuals, but also on the population as a whole [14,27].

The common denominator in the process by which antimicrobials exert their influence on the spread of resistant organisms appears to be the eradication of susceptible organisms within a host—both pathogens (intentionally) and colonizers (unintentionally). In those cases where antimicrobial use is clearly justified, this consequence is difficult, and often impossible, to avoid. Regardless, eradication of susceptible organisms leaves an ecologic void within the host, ultimately to be repopulated by other organisms from the local or external environment. Antimicrobials effectively shift the competitive balance for this repopulation toward resistant organisms. Also, by affecting the type and quantity of organisms shed into the environment by the treated host, antimicrobial treatment of one individual can potentially have consequences on other individuals in that environment [14,28]. Thus, through both direct and indirect mechanisms, antimicrobials are able to catalyze the spread of resistance through the environment and the population. The challenge to investigators at this point is to better define each mechanism and the role each plays in the spread of antimicrobial resistance.

Measures to Control Antimicrobial Resistance

Based upon the previous discussion, interventions aimed at controlling antimicrobial resistance can be classified as those that ultimately control the emergence of resistance and those that ultimately control the spread of resistance. These actions are not exclusive, however, as some measures can perform both functions simultaneously; in fact, any measure that modifies antimicrobial use could, theoretically, have an effect on both the emergence and the spread of resistance through an alteration in antibiotic selection pressure. A more useful approach, therefore, might be to classify strategies for controlling resistance as antimicrobial or nonantimicrobial measures (Table 2).

Nonantimicrobial measures consist primarily of infection control measures for preventing the horizontal cross-transmission of resistant organisms (hand disinfection, contact precautions, patient isolation, and cohorting), and specialty consultation to aid in the diagnosis, treatment, and handling of infected or colonized patients. A considerable amount of clinical research has been dedicated to the study of these practices, in many cases demonstrating the efficacy and cost effectiveness of these measures for the reduction of the spread of resistant organisms [29–31]. Typically, the greatest impact of these measures is seen when several approaches are used concurrently, but in those situations it can be difficult to ascertain the impact of each individual control measure on the overall effect on antimicrobial resistance. More comprehensive reviews of these interventions have been published recently elsewhere [32,33].

Antimicrobial measures, conversely, consist of measures aimed primarily at the minimization of antimicrobial selection pressure through the reduction of unnecessary antibiotic use and/or the optimization of antibiotic effectiveness. As mentioned, the selective pressure exerted by an antimicrobial agent appears to be the primary catalyst for the emergence and spread of resistant organisms. Since any changes in the pattern of antimicrobial use in a clinical setting should have a discernible effect on the selection pressures experienced in that setting, carefully designed changes in antimicrobial use could promote changes in the emergence and/or spread of resistance at both the individual and population levels. A variety of mechanisms have been employed to promote changes in antimicrobial use in the hospital setting, with varying results [32,34] (Table 2).

One common approach for preventing emergence of resistance in individual hosts is to use drugs or combinations of drugs that diminish the effective rate with which nonsusceptible subpopulations are able to grow. The common thread to these strategies is that for resistance to the antimicrobial regimen to become manifest, multiple independent mutations are required. Thus, as in the case of tuberculosis, a regimen containing two to four antibiotics is given to prevent the emergence of a subpopulation of resistant organisms.

In addition to drug selection, however, a number of other factors related to drug delivery and exposure have been shown to be major determinants of the in vivo efficacy of antimicrobial agents. The success of a particular antibiotic in the treatment of an infection correlates with one or more pharmacokinetic or pharmacodynamic (PK/PD) parameters, including peak serum level, the area under the concentration time curve (AUC), and the duration of time serum levels exceed the minimum inhibitory concentration (MIC), depending on the antimicrobial agent and the offending organism [35,36•]. Thus, for agents such as aminoglycosides and fluoroquinolones that exhibit concentration-dependent killing, maximizing their antimicrobial activity may best be achieved through the maximization of the ratio of peak serum level to the MIC. Conversely, for agents that exhibit time-dependent killing such as beta-lactams, macrolides, and clindamycin, maxi-

Table 2. Strategies to control antimicrobial resistance

Antimicrobial measures
Academic detailing
Antibiotic cycling
Antibiotic management teams
Audit/feedback
Area-specific empiric antibiotic regimens
Combination antimicrobial therapy
Computerized decision support and order entry
Formulary restriction/antibiotic approval
Implementation of clinical evidence-based guidelines
Individual application of pharmacokinetic/ pharmacodynamic parameters
Nonantimicrobial measures
Active surveillance cultures
Contact precautions
Hand disinfection
Specialty consultation (infectious diseases, pharmacy, infection control)
Isolation of patients colonized or infected with resistant organisms
Patient/staff cohorting

mizing the duration of time above the MIC should best exploit their antimicrobial effects. Some agents, such as azithromycin, tetracyclines, and vancomycin, exhibit time-dependent killing but with prolonged persistent effects; in these cases, the 24-hour AUC to MIC ratio should be maximized to achieve the greatest antimicrobial effect [36•].

The magnitude of the PK/PD parameters required for the most efficient killing of bacteria also appears to depend on the target organism. Specifically, there appear to be differences based on whether the organism is gram-positive or gram-negative, such as the differences seen in the 24-hour AUC/MIC ratios required for the clinical efficacy of fluoroquinolones against gram-negative bacilli and *S. pneumoniae* [36•,37,38]. There is also the suggestion that the use of these parameters can contribute not only to improved clinical efficacy, but also to a reduced risk of the emergence of antimicrobial resistance during therapy, particularly with gram-negative bacillary infections [36•,39,40].

Emphasizing the relationship between antimicrobial concentration and selection of resistant mutants even further, the concept of the mutation prevention concentration (MPC) has been proposed. The MPC is operationally defined as the lowest antibiotic concentration that completely prevents the emergence of mutant resistant strains from a large starting inoculum [16]. Another explanation of the MPC is that it represents a concentration of an antimicrobial agent beyond which double mutations should be required for resistance to emerge. It has been suggested that antimicrobial drugs that do not achieve tissue concentrations above the MPC should only be used as part of combination therapy [16]. Much more data from clinical and animal studies are needed before this principle can be adopted as the core strategy for preventing emergence of resistant organisms.

At the population level, one method of optimizing the effectiveness of antimicrobials worth mentioning that has been under increased investigation in recent years is the use of antibiotic cycling. A combination of other measures including antibiotic restriction and area-specific antimicrobial regimens, antibiotic cycling is a strategy to potentially reduce resistance through the temporary withdrawal of one antibiotic or antibiotic class and substitution with another, to allow resistance rates to the withdrawn agent(s) to stabilize or decrease. The cycle continues as the withdrawn agent is then reintroduced at a later date in place of the substitute, a key difference between this method and a simple policy of antibiotic class restriction. Although antibiotic restriction policies have been shown to lead to a reduction in the prevalence of resistance to the restricted antibiotic [41], the restriction of one class of antibiotics frequently results in the equivalent use of a different class, with concomitant increases in resistance to the alternate [41]. Antibiotic cycling works in a similar fashion, but each cycle is theoretically scheduled to occur before resistance is allowed to rise significantly to the substitute agent, potentially reducing the overall prevalence of resistance. A number of studies [9,42,43] have shown encouraging results in this regard, including one intriguing study reporting concurrent reductions in the incidence of antibiotic-resistant infections and infection-related mortality [44], but limitations in study design and analysis prevent broad applicability of the results. As well, at least one analysis suggests that alternatives to cycling, such as the simultaneous use of alternative drugs at the population level, may be more efficacious overall [45]. Thus, there remains a lack of clinical experience with this method and uncertainty regarding its overall efficacy, particularly since it fails to address some of the larger issues concerning excessive or inappropriate antibiotic use [46]. Clearly, additional controlled studies will be needed before this modality receives widespread acceptance.

Improving Control of Antimicrobial Resistance: Role of Mathematical Models

Although most of the practices currently employed to control antimicrobial resistance have been shown in controlled studies to have some measure of effect on the emergence and/or spread of resistance, from a practical standpoint they are used almost always in combinations, particularly in the setting of an outbreak of resistant organisms. As such, it can be very difficult to ascertain the impact each individual mechanism might have, if any, in the overall effort to eliminate or reduce the spread of resistant species. Because each of these practices are interdependent to a degree and the relationships between them quite complex, designing experimental or observational studies to assess the efficiency of these measures is extremely challenging.

Models are theoretical frameworks of interactions based on existing knowledge that can be useful in situations like this to help us understand the complexity of the real world. When these models are simulated in mathe-

matical formulas, we are able to quantify different components of the overall process, and to express in quantitative terms the overall effect of manipulating each component. Quantification in this way allows for more direct and reasonable comparisons of the values of the different components relative to each other. Although the model cannot nearly approach the complexity of the system it is describing, it does allow the investigator to simulate complex interactions among those components that are believed to be important, and to evaluate the quantitative effects of each component process.

Mathematical models have been in use for many years in the study of infectious diseases epidemiology, even as far back as the early 20th century, when they were used to understand malaria transmission for the purpose of disease control [47]. They have been applied over the years to the epidemiologic study of numerous viral, bacterial, and parasitic diseases, but in many cases the perception that mathematical theory was too detached from clinical reality resulted in a general lack of enthusiasm and acceptance for this approach to the solution of clinical problems [47,48•]. The past few years, however, have seen a resurgence in the use of this approach to address the epidemiology of antimicrobial resistance, particularly as it relates to nosocomial transmission and infection control practices in hospital settings such as intensive care units (ICUs). These studies have helped us gain a better understanding of transmission dynamics in a hospital setting, as well as the potential efficacies of different infection control techniques.

One such study [49] simulated the spread of a resistant nosocomial pathogen (methicillin-resistant *S. aureus* [MRSA]) in a hypothetical ICU setting, and examined the impact of three infection control measures on the prevalence of colonization. The model was based on the direct and indirect interactions between patients and staff members, and set during the course of an MRSA outbreak. In addition to supporting the notion that staff-member colonization is critical for the spread of resistant organisms in an ICU setting, their results suggested that of the three infection control protocols studied—hand disinfection, antimicrobial policy, and curtailed admission of colonized patients—only the latter effectively and rapidly contained the outbreak. Interestingly, strong hand hygiene compliance (even up to 90%) had only a moderate effect on patient colonization rates, although the outbreak was attenuated and colonization among staff members was quickly eliminated. Despite the difficulty in implementing a strategy to restrict the admission of colonized patients to an ICU, their model did provide some insight into the role of different infection control techniques, and generated a series of testable hypotheses that could eventually lead to real-world experimental studies.

Similarly, a different set of investigators [50] developed a model to describe the transmission dynamics and persistence of vancomycin-resistant *Enterococcus* (VRE) in an ICU setting. Their model, based on the transmission dynamics of vector-borne diseases, viewed health care workers as vectors and patients as definitive hosts, and examined the impact of various infection control measures (handwashing, cohorting, and antibiotic restriction) on nosocomial cross-transmission. Combining predictions of the model with surveillance and monitoring data gathered directly from their ICU, they were able to demonstrate that the observed endemic prevalence of VRE was less than half of that predicted by the model, a decrease attributed to the effect of the infection control measures. Despite that observation, and despite the fact that handwashing compliance and cohorting were shown to be effective control measures, the model again demonstrated that restricting the admission of colonized patients was the only means of eradicating resistant organisms from the ICU. But like the previous study, this work provided a deeper understanding of the process by which resistant organisms maintain a stable endemicity in an ICU setting, and provided a strong theoretical basis for further testing of interventions to control infection and resistance in the hospital.

Despite their appearance of complexity, mathematical models are only simplified representations of much more complex real-world phenomena, and as such the interpretation of their results carries certain limitations. The predictive value of any model is directly related to the ability of the investigators to break down reality into important, easily quantifiable components, and to tie them together with sound mathematical formulas. To do this, however, modelers are required to make numerous assumptions upon which the framework is based, assumptions that are usually estimated from values derived from the literature. The applicability of any model, therefore, is contingent upon the validity of the assumptions that underlie it, some of which do not accurately reflect reality. In the first example, for instance, it was assumed that the numbers of staff members and patients remained fixed over time, and that patients discharged from the ICU were immediately replaced with others [49]. Although assumptions like these appear to detract from the validity of the model, often the alternative adds significant complexity to the model without providing much interpretive value. Even with these assumptions, models can prove to be very useful if their results are interpreted within the bounds of the stated assumptions and used to generate hypotheses testable in the real world through experimental studies. Through this work, we have gained a better understanding of the transmission dynamics of resistant organisms in the hospital setting and the impact of various infection control measures on this process.

Conclusions

Antimicrobial resistance is clearly one of the most challenging issues facing clinical medicine at the onset of the 21st century. Through studies of the pathways by which resistance emerges and spreads through the population, we have come to the understanding that antibiotics have a broad range of effects, not just on the microorganism and the individual,

but also on the population as a whole. This presents an important challenge to clinicians, since what may be good for the individual patient may not be as good from an ecologic standpoint for the population. Nevertheless, our understanding of these processes has allowed for the design of numerous interventions that can help slow or halt the emergence and spread of resistance. Still, the design of clinical studies to evaluate the impact of these interventions continues to be a challenge, particularly since the control of antibiotic resistance requires a multifaceted approach using many different control measures simultaneously. In cases such as this where the relationships between the measures are complex and the effects of each are interdependent, mathematical modeling can provide a means to generate testable hypotheses upon which to design useful clinical experiments. Because of the efficiency with which microorganisms adapt to or circumvent the various protective measures we currently employ, perhaps our most important goal should be to optimize our use of antimicrobial agents, and to this effect we must constantly strive to address the longstanding issues of inappropriate or excessive antibiotic use.

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