To date, the majority of pharmacokinetic/pharmacodynamic (PK/PD) discussions have focused on PK/PD relationships evaluated at steady-state drug concentrations. However, a concern with reliance upon steady-state drug concentrations is that it ignores events occurring while the pathogen is exposed to intermittent suboptimal systemic drug concentrations prior to the attainment of a steady state. Suboptimal (inadequate) exposure can produce amplification of resistant bacteria. This minireview provides an overview of published evidence supporting the positions that, in most situations, it is the exposure achieved during the first dose that is relevant for determining the therapeutic outcome of an infection, therapeutic intervention should be initiated as soon as possible to minimize the size of the bacterial burden at the infection site, and the duration of drug administration should be kept as brief as clinically appropriate to reduce the risk of selecting for resistant (or phenotypically nonresponsive) microbial strains. To support these recommendations, we briefly discuss data on inoculum effects, persister cells, and the concept of time within some defined mutation selection window.

When considering the human or the veterinary patient, the clinical importance of selecting antimicrobial dosing regimens based upon exposure-response relationships is supported by published in vitro and in vivo evidence. As described by Am brose et al. (2), this pharmacokinetic/pharmacodynamic (PK/PD) paradigm has had a critical role in human medicine for dose determination and drug development from the vantage point of predicting the clinical outcome for the individual patient.

Most antimicrobial compounds produce their effects by acting on specific microbial targets such as enzymes that regulate cell wall synthesis (e.g., the blockage of cross-linking enzymes in the peptidoglycan layer of cell walls or sequestration of substrate required for peptidoglycan cross-linking), protein synthesis (aimed at the 23S rRNA and associated proteins in the peptidyl transferase center of the ribosome to inhibit protein chain elongation), and targets responsible for DNA repair or replication (inhibition of DNA gyrase and the related enzyme topoisomerase IV in mid-catalytic cycle) (58). A summary of drug classes versus generalizations regarding the corresponding PK/PD parameter(s), the type of antimicrobial activity (i.e., bactericidal and/or bacteriostatic), and the mechanism(s) of action is provided in Table 1 (42).

It is believed that aminoglycosides, beta-lactams, and fluoroquinolones act by using the bacterium itself to produce self-destructive chemicals (Table 2). In the presence of inadequate drug exposure, these targets can be corrupted. Within this context, “exposure” refers to the magnitude of the active drug concentration(s) available to interact with the bacterial site of action and is usually considered from the perspective of the duration and extent of exposure. “Inadequate exposure” refers to conditions under which the drug exposure is not sufficient to kill (or inhibit) first-step mutants or bacteria with borderline susceptibility. Inadequate exposure leads to the amplification of drug-resistant subpopulations. Target modification can result in a loss of bacterial killing effect. Examples of mechanisms that disrupt the drug-effect relationship are reviewed elsewhere (42).

Antimicrobial resistance can be described as a continuum, ranging from those situations where effectiveness can be recovered by an increase in exposure (what we will term “relative resistance”) to those situations where a response to the antimicrobial agent cannot be achieved by an increase in exposure (which we will refer to as high-level resistance). Organisms exhibiting relative resistance can be controlled at “elevated” drug concentrations that are achievable only by increasing the dose. Alternatively, for beta-lactams, bactericidal effects against less susceptible organisms may be achieved by extending the infusion time. In contrast, organisms exhibiting high-level resistance will not be affected by the drug even upon exposure to very high concentrations (61). The latter can lead to concerns about the safety of a human or animal patient or to human food safety issues when the drug is administered to a food-producing animal.

Typically, PK/PD assessments are based upon the MIC for the pathogen and the unbound drug concentration in the host plasma (44). By convention, these relationships are classified as being primarily time or concentration dependent (42). Dosages of time-dependent drugs that exhibit a negligible postantibiotic effect (PAE; e.g., β-lactams) are generally evaluated on the basis of the portion of a 24-h dosing interval when the drug concentration exceeds the MIC for the targeted pathogen (T>MIC). Time-dependent drugs that exert a prolonged PAE (e.g., macrolides) are generally evaluated on the basis of the 24-h area under the concentration-versus-time curve (AUC) divided by the MIC for the pathogen (AUC/MIC). Drugs associated with concentration-dependent killing may be described by either the AUC/MIC (e.g.,
fluoroquinolones) or the peak drug concentrations divided by the MIC ($C_{\text{max}}$/MIC; e.g., aminoglycosides).

To date, the predominance of PK/PD discussions have focused on PK/PD relationships evaluated at steady-state drug concentrations. In addition, in vitro studies generally base evaluations on simulated steady-state concentrations (56). However, as will be discussed within this minireview, when considering the impact of inherent mutation rates or the time needed for upregulation by

**TABLE 1 Relationships among drugs, their effects, and the PD surrogate(s) most closely aligned with their clinical effects**

<table>
<thead>
<tr>
<th>Mechanism of action and drug</th>
<th>Activity</th>
<th>Bacterial effect</th>
<th>Duration of in vitro PAE</th>
<th>PD parameter(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agents affecting the function of 30S and 50S ribosomal units, resulting in reversible inhibition of protein synthesis (and therefore generally bacteriostatic activity)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Macrolides</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Erythromycin</td>
<td>Bacteriostatic</td>
<td>Time dependent</td>
<td>Brief$^a$</td>
<td>$T$ &gt; MIC</td>
</tr>
<tr>
<td>Azalide</td>
<td>Bacteriostatic</td>
<td>Time dependent</td>
<td>Brief</td>
<td>AUC/MIC</td>
</tr>
<tr>
<td>Lincosamides (clindamycin)</td>
<td>Bacteriostatic</td>
<td>Time dependent</td>
<td>Brief</td>
<td>AUC/MIC</td>
</tr>
<tr>
<td>Ketolides (telithromycin)</td>
<td>Bacteriostatic or bactericidal (e.g., bactericidal for <em>S. pneumoniae, S. pyogenes</em>).</td>
<td>Time dependent</td>
<td>Prolonged</td>
<td>AUC/MIC</td>
</tr>
<tr>
<td>Chloramphenicol, florfenicol, thiamphenicol</td>
<td>Primarily bacteriostatic but bactericidal against some pathogens; exhibit both Gram-positive and Gram-negative activities</td>
<td>Time dependent</td>
<td>?</td>
<td>$T$ &gt; MIC</td>
</tr>
<tr>
<td>Tetracyclines</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Traditional (e.g., chlortetracycline)</td>
<td>Bacteriostatic</td>
<td>Time dependent</td>
<td>Prolonged</td>
<td>AUC/MIC</td>
</tr>
<tr>
<td>Atypical (e.g., chelocardin, anhydrochlortetracycline)</td>
<td>Bactericidal</td>
<td>Time dependent</td>
<td>Prolonged</td>
<td>AUC/MIC</td>
</tr>
<tr>
<td>Agents that inhibit cell wall synthesis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beta-lactams (e.g., penicillins, carbapenems, cephalosporins, monobactams)</td>
<td>Bactericidal</td>
<td>Time dependent</td>
<td>Gram-negative bacteria, none or brief; Gram-positive bacteria, may be prolonged</td>
<td>$T$ &gt; MIC</td>
</tr>
<tr>
<td>Glycopeptides (e.g., vancomycin, teicoplanin)</td>
<td>Bactericidal (slower than beta-lactams)</td>
<td>Time dependent</td>
<td>Prolonged</td>
<td>AUC/MIC</td>
</tr>
<tr>
<td>Agents that bind to the 30S ribosomal subunit, inhibiting bacterial protein synthesis, leading to aberrant proteins and eventually cell death, i.e., aminoglycosides (e.g., gentamicin, tobramycin)</td>
<td>Primarily bactericidal</td>
<td>Concentration dependent</td>
<td>Prolonged</td>
<td>AUC/MIC, $C_{\text{max}}$/MIC</td>
</tr>
<tr>
<td>Agents that alter nucleic acid metabolism, inhibit DNA gyrase and, in some cases, topoisomerase IV, thus preventing transcription and replication, i.e., fluoroquinolones</td>
<td>Bactericidal</td>
<td>Concentration dependent</td>
<td>Prolonged</td>
<td>AUC/MIC, $C_{\text{max}}$/MIC</td>
</tr>
<tr>
<td>Agents that act as antimetabolites (e.g., block folate metabolism by inhibiting dihydrofolate reductase)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>Bacteriostatic alone, bactericidal with sulfonamides</td>
<td>Time dependent</td>
<td>Brief$^a$</td>
<td>$T$ &gt; MIC</td>
</tr>
<tr>
<td>Sulfonamides</td>
<td>Bacteriostatic</td>
<td>Time dependent</td>
<td>Brief$^a$</td>
<td>$T$ &gt; MIC</td>
</tr>
<tr>
<td>Agents that inhibit initiation of protein synthesis (at 50S ribosomal subunit), i.e., oxazolidinones (e.g., linezolid)</td>
<td>Bacteriostatic (staphylococci and enterococci) or bactericidal (most streptococci)</td>
<td>Time dependent</td>
<td>Brief$^a$</td>
<td>$T$ &gt; MIC</td>
</tr>
<tr>
<td>Agents that depolarize bacterial cell membrane,$^b$ i.e., cyclic lipopeptides (e.g., daptomycin)</td>
<td>Bactericidal (Gram-positive bacteria)</td>
<td>Concentration dependent</td>
<td>Prolonged (&gt; 6.8 h)</td>
<td>AUC/MIC, $C_{\text{max}}$/MIC</td>
</tr>
</tbody>
</table>

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$^a$ Brief is less than 1 h; prolonged may be up to 6 h.

$^b$ Unique mechanism of action may help minimize the risk of microbial resistance.

$^c$ Based upon a table created by Martinez and Silley (modified with permission from reference 42).

To date, the predominance of PK/PD discussions have focused on PK/PD relationships evaluated at steady-state drug concentrations. In addition, in vitro studies generally base evaluations on simulated steady-state concentrations (56). However, as will be discussed within this minireview, when considering the impact of inherent mutation rates or the time needed for upregulation by
bacterial resistance mechanisms, such as efflux pumps or porin expression (38, 40, 47), to minimize the amplification of resistant strains, the PK/PD target should ideally be achieved by the first dose and not several doses later. In fact, efflux pump upregulation can occur within only a few hours, as shown by the change in Streptococcus pneumoniae pump expression at a rate of three times in 28 h (33) and the rapid induction (within about 6 h) of patA and patB (encoding the corresponding ABC transporter proteins) when S. pneumoniae is exposed to suboptimal concentrations of ciprofloxacin and norfloxacin (22).

A concern about the conventional approach of relying upon steady-state drug concentrations is that it ignores events occurring while the pathogen is exposed to intermittent suboptimal systemic drug concentrations prior to the attainment of a steady state. In other words, PK/PD paradigms that neglect the time delays associated with the achievement of the targeted steady-state drug concentrations, by their very nature, neglect the changes in pathogen susceptibility that can occur during the period of suboptimal drug exposure that occurs before a steady state is reached. Therefore, rather than basing the dosage estimation upon population models of steady-state drug concentrations, it may be preferable to consider the use of models that describe likely day 1 exposure characteristics or to explore the potential benefits that may be achieved through the use of a loading dose.

Suboptimal (inadequate) exposure can produce amplification of resistant bacteria so that a resistant strain dominates at an infection site (56). This point was demonstrated by Jumbe et al. (32), who showed that in a thigh infection model, suboptimal levofloxacin exposure selected for resistant strains within only 12 h after drug administration. The resistant strains proliferated after subsequent exposures to suboptimal doses. The suboptimal dosing regimen resulted in an infection site bacterial population with MICs higher than that of the original inoculum. As shown in these studies, a consequence of the shift in the MICs is that larger doses (drug exposure) are needed to overcome the inadequacy of the initial kill. Clinically, this point was further demonstrated by the rapid emergence of ciprofloxacin resistance in Canada subsequent to the use of suboptimal ciprofloxacin for the treatment of pneumococcal infections (1).

With these points and concerns in mind, this minireview examines published evidence to support the position that in most disease situations, it is the exposure achieved during the first dose that is relevant (and often undervalued) for predicting the microbial response to a therapeutic intervention. (Note that although it is relevant to the issue of minimizing the risk of resistance selection and improving drug efficacy, the utility of combination drug therapy is outside the scope of this minireview.) In addition, time to treatment and duration of treatment are also important variables to consider as part of our efforts to minimize the selection of resistant strains. The fundamental variables in this argument include (i) data on the amplification of resistant subpopulations when drug exposure is inadequate to suppress amplification of the less susceptible bacterial population, (ii) the relationship between the size of the inoculum and the likelihood of mutational events, (iii) the potential risk of encouraging the conversion of actively growing bacteria into persister cells when the dosing regimen fails to kill the pathogen but is sufficient to evoke the expression of phenotypic changes, and (iv) information supporting shortening of the total drug exposure when clinically appropriate.

Although some of the in vitro and animal model studies cited may include drug-pathogen combinations no longer used or not considered acceptable for use in clinical practice, all of these studies nevertheless describe important overarching principles that influence microbial responses to therapeutic interventions. The corresponding clinical relevance of these findings is supported by the various points included in our discussion.

### TABLE 2 Examples of bacterial actions resulting from bactericidal antimicrobials

<table>
<thead>
<tr>
<th>Drug class</th>
<th>Target</th>
<th>Self-destructive chemical action</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aminoglycosides</td>
<td>Disruption of translation</td>
<td>Production of misfolded proteins with antimicrobial activity</td>
<td>15</td>
</tr>
<tr>
<td>Beta-lactams</td>
<td>Inhibition of peptidoglycan synthesis</td>
<td>Inhibition of peptidoglycan synthesis results in activation of autolysins present in bacterial cell wall</td>
<td>5</td>
</tr>
<tr>
<td>Fluoroquinolones</td>
<td>Inhibition of ligase step of the DNA gyrase and topoisomerase</td>
<td>Trapping of enzymes on DNA during topoisomerization reaction, forming a physical barrier to replication fork movement, RNA polymerase, and DNA helicase</td>
<td>30</td>
</tr>
</tbody>
</table>

**SUBOPTIMAL EXPOSURE AND SELECTION OF RESISTANT MICROBIAL STRAINS**

Within any bacterial population, there is the possibility of bacterial subpopulations that are less susceptible (i.e., higher MICs) to the antimicrobial agent and this probability is proportional to the bacterial burden and the frequency of mutation to resistance. As demonstrated by Blaser et al. (7) and Drusano (16), unless these less susceptible pathogens are killed, the more resistant bacteria will be amplified and will populate the infection site. Therefore, drug concentrations need to be adequate to either eradicate the bacteria at the site of the infection or (at least) to reduce the number of infecting bacteria to the point where the host’s defense mechanisms can successfully control and eliminate the remaining pathogens. Assuming that absolute resistance has not occurred, subsequent doses would need to be increased by a considerable amount to achieve the desired clinical outcome (24). It should be noted that the dose increase necessary to treat infections associated with bacterial strains exhibiting relative resistance (i.e., an increase in the MIC) is due not to an increase in the PK/PD target (e.g., a higher AUC/MIC ratio) but rather to the MIC increase necessitating a larger dose to achieve the same PK/PD target (54).

The mathematical models of Lipsitch and Levin (41) suggest that the selection of resistant mutants is most efficient at drug concentrations where there is some, but not maximal, killing. Similarly, the mathematical model of Handel et al. (29) suggested that reducing the size of the total population through antimicrobial killing allows for an increase in the net growth of the resistant population. These model-based conclusions have been experimentally confirmed with the “inverted-U” plot by Tam et al. (56) and the inverted-U mountain by Drusano et al. (18) (Fig. 1 and 2, respectively). The inverted U represents the relationship between
drug exposure (which can be expressed as the AUC or as the AUC/MIC ratio) versus the log CFU count.

The relationship between an index of drug exposure (e.g., AUC/MIC ratio or T/H11022MIC) and the ability of the index to result in bacterial cell killing is a monotonic function. That is, as the index increases, the amount of antibacterial effect increases, up to a maximum value. With resistance suppression, this is not true. The relationship between the intensity of the index and resistance amplification suppression is nonmonotonic. At relatively low-intensity exposures, there is little amplification of any pre-existent less susceptible population because little pressure is exerted upon the system. As intensity increases, the susceptible population is killed but there is maximal amplification of the less susceptible portion of the population. Consequently, the most harmful regimen from the perspective of resistance is one that is robust for the susceptible portion of the population but maximally amplifies the less susceptible portion of the population (peak of the inverted U). Finally, with very large exposures, a sufficient intensity of drug exposure is achieved that even the less susceptible portion of the population is suppressed. This forces down the size of the less susceptible population and is seen as the descending limb of the inverted U.

These studies confirmed that selection pressure tends to increase to some maximum (increase in the CFU count), after which further increases in drug exposure result in a reduction in the CFU count. When the initial drug doses are not adequate to achieve the desired antimicrobial effect, the clinical outcome becomes more highly dependent on the host immune response and is consistent with the findings of Firsov et al. (26), where two strains of methicillin-resistant Staphylococcus aureus were exposed to various in vitro concentrations of ciprofloxacin, simulating twice-daily dosing that oscillated within different regions of what has traditionally been termed the mutation selection window (MSW).

The MSW has been defined as the range of concentrations that are bounded by the MIC (lower limit) and the mutation prevention concentration (MPC), where the MPC is the drug concentration necessary to kill bacteria expressing resistance mutations (4, 59, 60). Firsov et al. (26) confirmed that even if the times within the MSW are identical between dosing regimens, it is the location of the oscillation that determines whether or not there will be resistance amplification. In other words, what was important was that drug concentrations exceeded the concentrations needed to prevent resistance amplification. Like Tam and Drusano, Firsov observed that when the drug exposure was in the left portion of the inverted-U plot, there was an increase in bacterial growth (an increase in the number of CFU/ml) within 24 h of exposure. Conversely, amplification was prevented for at least 48 h when AUC/MIC values are located in the right part of the inverted-U plot.

Based upon the results of these studies, considering only the duration of time during which drug concentrations are within the MSW (9, 27, 59, 60) without simultaneously factoring in the proximity of the drug concentrations to the MPC can bias conclusions about the likelihood of resistance selection. In other words, the

FIG 1 Resistant populations at 48 h under various levels of drug exposure (56). The data at an AUC/MIC ratio of 0 represent the densities of the resistant subpopulation at baseline (time zero). All other data points denote the densities of the resistant subpopulation present at 48 h. KP, Klebsiella pneumoniae ATCC 13883; MRSA, methicillin-resistant S. aureus; MRSA-CS, ciprofloxacin-susceptible MRSA; MRSA-CR, ciprofloxacin-resistant MRSA.
MSW is not a range of concentrations associated with a uniform probability of mutation selection but rather should be considered a range of concentrations within which multiple factors influence the likelihood of selecting for resistant microbial strains. In this regard, the critical variables include the prevailing drug concentration relative to the pathogen’s MIC and MPC (where the likelihood of mutation selection ranges from highest near the MIC to negligible as concentrations approach the MPC) and the duration of time during which this exposure is maintained within some discrete concentration range. This conclusion is consistent with the observation that use of adequately high doses within the first 3 days of therapy has a clinically and statistically significant positive impact on the probability of pathogen eradication in nosocomial pneumonia patients (50).

For this reason, both dose and duration of therapy can influence the likelihood of resistance selection and amplification. This is shown in Fig. 3, where a hollow-fiber infection model was used to examine the influence of the duration of drug treatment (garenoxacin) on the size of the bacterial population (expressed as log_{10} CFU/ml) and on the relative proportions of susceptible versus resistant bacterial strains. The test pathogen in this study was methicillin-susceptible S. aureus. The investigation included a no-treatment control and three other regimens, each producing a daily AUC/MIC ratio of 100 but with a treatment duration of 4, 5, or 6 daily doses, respectively. The response of the bacterial population to a cessation of drug exposure was evaluated by following the bacterial populations through study day 13 (19). Strains were tested for garenoxacin susceptibility using the broth microdilution method described by the Clinical and Laboratory Standards Institute (CLSI).

To explore the differences in the effects of drug exposure on the resistant and susceptible subpopulations, the size of the resistant subpopulation was subtracted from the size of the total population. Drusano et al. (19) estimated the relative proportions of susceptible versus resistant strains using modeled data (Fig. 4). From this set of plots, it is evident that the resistant pathogens made up an increasingly greater proportion of the total population as the duration of drug exposure increased. Most importantly, although the majority of the bacterial population consisted of susceptible strains for up to 4 days of drug exposure, the bacterial population consisted almost entirely of the resistant strain when garenoxacin exposure continued for a duration of 6 daily doses. Therefore, even when garenoxacin concentrations were inadequate to completely eradicate the resistant subpopulation, the susceptible population dominated during a short course of treatment. However, as the duration of suboptimal exposure continued, the resistant population began to increase. Thus, if drug exposure continued, only the resistant strains would remain, with
the corresponding risk of an infection populated nearly entirely by the resistant pathogen. When dosing was terminated, the proportion of susceptible and resistant strains returned to the pretreatment values.

These results lead to the conclusion that with longer courses of therapy, the drug exposure needs to be greater than predicted based upon the MIC for the susceptible pathogen to suppress the amplification of the resistant population. Furthermore, it follows that the risk of transmitting resistant microbes to other hosts is substantially greater with a prolonged exposure at concentrations inadequate to kill the resistant population than with a short duration of therapy (19).

Because strategies that target only susceptible organisms can lead to the amplification of resistant strains, the impact of dose and duration on successful therapeutic outcomes has been extensively investigated (e.g., 16, 19, 56). This recognition has promoted proposals of unconventional dosing schedules in human medicine, such as front-loaded dosing regimens, a shortened dose duration, and once-daily high-dose therapies (e.g., see references 8, 17, 19, 21, 46, and 52).

Dose and duration can also lead to a phenotypic switch from growing to nongrowing “persister” cells. Persistence of wild-type strains is ubiquitous and is responsible for a variety of chronic infections (6, 31). These cells can exist in the planktonic state (and therefore be accessible to the host immune system) or in a biofilm, where they are protected from the host defenses (37, 38, 39). Bacteria grown from these persister cells are sensitive to antimicrobial treatment, indicating that persister cells are not genetically different from wild-type growing cells (45). However, while in the persister phase, the cells remain unaffected by antimicrobials. Never-

FIG 3 Impact of differing durations of garenoxacin therapy on susceptible and resistant bacterial subpopulations subjected to a regimen with an AUC/MIC ratio of 100 (reproduced from reference 19 with permission).
theless, there is a brief period during which dormant bacteria can respond to and be killed by antimicrobials (28).

The ability to transform into a persister cell appears to be pre-determined and not simply a response to antimicrobial exposure. Constant and prolonged drug exposure is believed to encourage persister cell formation. For this reason, alternative exposure regimens (pulse dosing) have been suggested to encourage persister cell to convert back to a sensitive (growth) condition, where it can be effectively eradicated by the antimicrobial (39).

**DURATION OF TREATMENT**

Much of the experimental data cited above point to the benefits of high doses for fairly short durations in order to minimize the risk of resistant strains. Likewise, there is an increasing body of evidence suggesting that for the use of bactericidal compounds in the treatment of acute infections, the administration of larger doses for short durations of about 3 to 5 days is often as good as, or possibly better than, the administration of lower doses for 10 to 14 days.

Published examples of uncompromised clinical responses to shorter durations of therapy include studies on the use of high-dose, short-duration (750 mg for 5 days versus 500 mg for 10 days) treatment of community-acquired pneumonia associated with a variety of pathogens (20, 53). Similarly, shortening the duration of amoxicillin administration from 8 days (3 days of 1,000 mg given intravenously every 6 h [q6h], followed by 5 days of oral amoxicillin at 750 mg q6h) to 3 days (3 days of 1,000 mg given intravenously q6h, followed by 5 days of oral placebo) did not compromise the treatment of moderate-to severe pneumococcal pneumonia (23). Similar study results are reviewed elsewhere (51).

In their review of antimicrobial stewardship protocols used in intensive care units (ICUs), Kaki et al. (34) observed that a reassessment of the need for continued therapy in patients with ventilator-associated pneumonia (VAP) generally resulted in a decision to reduce the duration of therapy. Looking across the various studies, maintaining interim assessments of the need to continue therapy tended to reduce the duration of therapy and decrease the prevalence of resistant microbial strains. In one hospital, a reevaluation at day 3 for patients
with pulmonary infiltrates led to a much shorter duration of antimicrobial treatment (from 9.8 days to 3 days). In the hospital where resistance was monitored following an ICU protocol of including a day 3 assessment for a duration of 2 years led to a 24% reduction in extended-spectrum beta-lactamase-producing *Klebsiella* and a 20% reduction in carbapenem-resistant *Pseudomonas*. In another example, a 10-day reassessment protocol, maintained over a 3-year period, resulted in a reduction in methicillin-resistant *S. aureus* (from 61% to 13%) and ceftriaxone-resistant *Enterobacteriaceae* (from 37% to 13%).

Using information contained in the Cochrane Central Register of Controlled Trials (Central), Pugh et al. (49) examined the impact of duration of therapy (7 to 8 days versus 10 to 15 days) on clinical success versus the likelihood of recurrence of infection due to multidrug-resistant organisms. Their meta-analysis included eight studies (1,703 patients) with hospital-acquired pneumonia (HAP), the majority of whom were also being mechanically ventilated, and VAP. The selection of antibiotics was aminoglycosides or a fluoroquinolone plus a beta-lactam. In all cases, the bacteria were classified as susceptible to the selected antibiotic regimen. Pugh et al. observed that, with the exception of VAP due to non-fermenting Gram-negative bacteria (which were very difficult to eradicate), a 7- to 8-day course of antibiotics resulted in a recurrence of VAP due to multidrug-resistant pathogens lower than that achieved with the 10- to 15-day course of therapy.

General recommendations pertaining to an appropriate duration of therapy need to be weighed against the host’s physiological status, the virulence of the pathogen, and the clinical consequences of the infection. For example, a short course (5 days) of therapy (piperacillin-tazobactam at 4.5 g q8h plus gentamicin at 5 mg/kg once daily) for many patients with Gram-negative HAP had a clinical outcome (time to resolution and relapse rate) similar to that reported in other studies. However, there were a few patients whose duration of therapy needed to be adjusted based upon respiratory culture results (e.g., see reference 48). Similarly, in the case of childhood bacterial meningitis, while a shortened course (5 days) of intravenous ceftriaxone often produced a survival rate and neurological outcomes comparable to those observed following a 10-day course of therapy (35, 43), there were some patients who required a longer treatment duration. Clearly, careful clinical judgment needs to be exercised before discontinuing therapy (55).

### THE INOCULUM EFFECT AND IMPORTANCE OF A SHORT TIME TO TREATMENT

Using the mouse thigh infection model of Craig (13), Jumbe et al. (32) demonstrated that the decrease in CFU counts in response to various levofloxacin doses is also a function of the size of the initial inoculum. With a larger inoculum, the size of the microbial population exceeded the mutation frequency of the pathogen, resulting in the amplification of resistant strains. As a result, higher drug concentrations were needed to achieve the desired endpoint. Most importantly, for rapidly mutating pathogens such as *Pseudomonas aeruginosa*, this resistance amplification occurred within only 24 h of exposure to suboptimal drug concentrations. This outcome indicates that not only dose and duration but also time to onset of treatment is an important consideration in minimizing the total amount of drug needed to effect a clinical cure and avoid the selection of resistant strains.

By convention, the drug concentration needed to treat an infection has been linked to the MIC for the pathogen. However, the MIC estimate is based upon carefully controlled conditions, including medium, temperature, pH, and inoculum size. Indeed, the standard methods of susceptibility testing provided in CLSI documents (11, 12) are quite specific with respect to these parameters. For example, the inoculum size is approximately $1 \times 10^{5.5-6}$ CFU/ml, as determined by a McFarland standard. However, clinical infections may reflect a wide range of bacterial densities, raising the question of how inoculum size may influence the drug concentration needed to achieve a positive therapeutic outcome.

The probability of a random mutation conferring resistance is proportional to the total number of rounds of replication, which is, in turn, proportional to the size of the bacterial burden and the turnover rate. In infectious disease processes where there is a large bacterial burden (inoculum effect), the risk of a mutational event is increased simply because of the laws of probability (14, 17). Therefore, it is important to ensure the adequacy of drug exposure at the onset of treatment, which is when the bacterial numbers are at their highest. Craig and Dalhoff (14) concluded that for drugs that exhibit concentration-dependent killing properties, such as fluoroquinolones, the $C_{\text{max}}/\text{MIC}$ ratio may be particularly important for a pathogen with a high MIC or one that is proliferating rapidly.

When the bacterial population burden exceeds the inverse of the mutational frequency, the probability of bacterial subpopulations is high. It is hypothesized that the bacterial mutation frequency can range from $10^{-6}$ to $10^{-10}$. Purulent fluids have been shown to contain bacterial cell counts averaging $2 \times 10^{6}$ CFU/ml (soft-tissue and intra-abdominal infections), with some human patients having counts as high as $10^{9}$ CFU/ml (36). Therefore, there is a high likelihood that random mutation events will occur. The site of infection also appears to influence the bacterial turnover rate, which can, in turn, influence the relationship between dose and effect. For example, a pneumonic lung lobe or fluid-filled abdomen is expected to contain greater numbers of bacteria than an isolated infection of the skin, oral cavity, or other tissues that are limited by organ size (e.g., eye, prostate) (25).

Using a range of drugs and drug concentrations, Udekwu et al. (57) demonstrated that there is a decrease in antimicrobial effectiveness in the presence of a large ($1 \times 10^{6}$) versus a small ($5 \times 10^{5}$) inoculum of *S. aureus*. Using *in vitro* models, significant decreases in the killing of *S. aureus* by ciprofloxacin, daptomycin, gentamicin, and vancomycin occurred as the size of the inoculum increased. Although the MIC estimated using the broth microdilution and Etest methods (i.e., testing filtrates of cultures bearing $5 \times 10^{5}$ CFU/ml *S. aureus* based upon independent colonies from the high-density samples) did not change over an 18-h duration of *in vitro* drug exposure, the MIC estimates based upon the CFU count and optical density clearly showed that the MICs of these compounds substantially increased if evaluated within the presence of the large inoculum. Although in some cases (e.g., daptomycin and vancomycin), the decline in drug effectiveness was attributable to drug degradation (as measured by bioassay), in other cases (e.g., ciprofloxacin, gentamicin, linezolid, and oxacillin), no quantifiable drug degradation was observed. It should be noted that the loss of daptomycin and vancomycin activity appeared to be bacterium dependent because it was observed only in medium containing a large inoculum. No loss of activity was seen in bacterium-free medium.

From the available study data, Udekwu et al. (57) could not...
ascertain whether the observed decrease in effectiveness was attributable to a smaller amount of available drug per bacterial cell, a phenotypic switch, or some other protective mechanism. Nevertheless, although the duration of the study may have been too brief to allow the quantifiable amplification of resistant strains, the conclusion remains that if a dosing regimen is based solely on conventional MIC estimates (e.g., MIC₉₀), the treatment may be more likely to fail if the inoculum is large, regardless of the MIC. To overcome large-inoculum effects, it is important to factor the bacterial load into the dosing regimens. This implies that, whenever possible, the load can be minimized by treating the infection as soon as possible. To this end, by the time bacterial infections produce clinical signs, the bacterial population is generally large enough to contain resistant mutants (16).

In consideration of these points, we conclude that antimicrobial administration should begin as soon as possible after a diagnosis to minimize the size of the bacterial population.

There may also be occasions where the “hit hard and hit fast for a short duration” paradigm may be appropriate, even for what is typically considered “self-healing” infections, such as sinusitis, bronchitis, uncomplicated skin infections, and uncomplicated urinary tract infections. For example, Ambrose et al. (3) showed that in patients with acute maxillary sinusitis attributable to Streptococcus pneumoniae, high-dose gatifloxacin resulted in sinus sterilization within 24 to 72 h after the initiation of therapy. The median sinus gatifloxacin AUC/MIC ratio in these patients was 122.8. Considering the rapid sterilization achieved with this high-dose intervention, such “short-duration, hard-strike” dosage regimens may be particularly helpful for the immunologically compromised patient. Optimally, this therapeutic strategy could also help minimize some of the resistance concerns that confound the positive therapeutic benefits associated with prophylactic antimicrobial treatments administered to patients with chemotherapy-induced neutropenia (10).
CONCLUSION

As illustrated in Fig. 5, the probability of therapeutic success is a multifactorial function. Limiting our considerations to the microbial response to therapies, this list summarizes the critical variables that can influence drug responses.

To maximize the likelihood of therapeutic success, for many infections where the inoculum is large enough to include resistant strains, it is important to consider aggressive early treatment with high drug concentrations (to minimize the increase in bioburdens [early intervention]) and to target a first exposure that is of a magnitude sufficient to kill both susceptible and resistant bacteria (i.e., well to the right side of the inverted-U plot). Optimally, the duration of dosing will also be considered from the perspective of minimizing a patient’s total drug exposure, both for the sake of the patient and to minimize the selection of drug resistance. PK/PD targets based upon steady-state drug exposure or dosing regimens do not factor the events occurring prior to the achievement of a steady state. Therefore, such estimates may not adequately predict the dose or support the determination of a clinical susceptibility breakpoint. In some cases, alternative dosing regimens (including front-loading dosage regimens and pulse dosages) may need to be considered.

Ultimately, four critical elements are important to consider. (i) In cases where upregulation or mutation events are likely, it is important to achieve levels of drug exposure that result in a kill rate that minimizes the total population and rounds of replication. (ii) To avoid opportunities to amplify resistant subpopulations, studies point to the importance of achieving the PK/PD target by the first dose at the primary infection site. (iii) To minimize the risk of selecting for resistant pathogens and the reduced drug effectiveness observed at high bacterial densities, treatment of the infection should be initiated as early as possible. (iv) Recent studies show that the duration of dosing should be tempered by the potential amplification of resistant strains that can occur with prolonged therapies and the risk of inducing the development of chronic infection. When possible, reduction of the number of days of treatment (e.g., 3 to 5 days, depending upon the drug, pathogen, and site of infection) should be considered. This is particularly relevant to cases where resolution of symptoms is apparent. However, in some cases (e.g., where the host immune system may be compromised or where there may other complications that may compromise the response to the antimicrobial intervention), clinical judgment should be exercised in deciding whether a longer duration of therapy is necessary. In some cases, alternative dosing regimens (such as pulse dosing) may be beneficial.

REFERENCES


