CASE STUDY 1 Kill Curves and Antibiotic Effectiveness

Antibiotics are often prescribed to patients who have bacterial infections. When a single dose of antibiotic is taken, its concentration at the site of infection initially increases very rapidly before slowly decaying back to zero as the antibiotic is metabolized.[1] The curve shown in Figure 1 illustrates this pattern and is referred to as the *antibiotic concentration profile*.

The clinical effectiveness of an antibiotic is determined not only by its concentration profile but also by the effect that any given concentration has on the growth rate of the bacteria population. This effect is characterized by a *dose response relationship*, which is a graph of the growth rate of the bacteria population as a function of antibiotic concentration. Bacteria typically grow well under low antibiotic concentrations, but their growth rate becomes negative (that is, their population declines) if the antibiotic concentration is high enough. Figure 2 shows an example of a dose response relationship.[2]

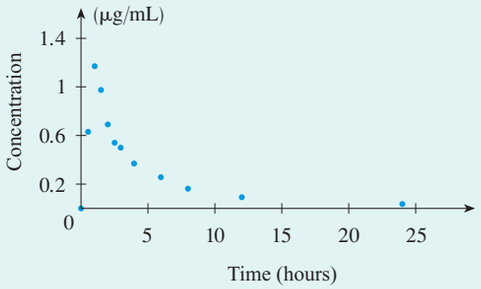

FIGURE 1
Antibiotic concentration profile in plasma of a healthy human volunteer after receiving 500 mg of ciprofloxacin

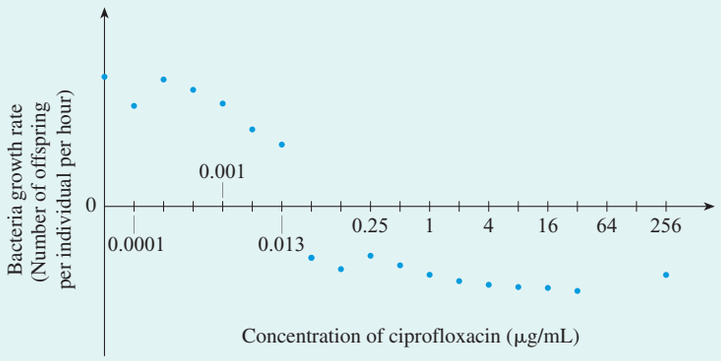

FIGURE 2
Dose response relationship for ciprofloxacin with the bacteria *E. coli*

Together, the antibiotic concentration profile and the dose response relationship determine how the bacteria population size changes over time. When the antibiotic is first administered, the concentration at the site of infection will be high and therefore the growth rate of the bacteria population will be negative (the population will decline). As the antibiotic concentration decays, the growth rate of the bacteria population eventually changes from negative to positive and the bacteria population size then rebounds. The plot of the bacteria population size as a function of time after the antibiotic is given is called the *kill curve*. An example is shown in Figure 3.

To determine how much antibiotic should be used to treat an infection, clinical researchers measure kill curves for different antibiotic doses. Figure 4 presents a family of such curves: Notice that as the dose of antibiotic increases, the bacteria population tends to decline to lower levels and to take longer to rebound.

When developing new antibiotics, clinical researchers summarize kill curves like those in Figure 4 into a simpler form to see more clearly the relationship between the

1. Adapted from S. Imre et al., "Validation of an HPLC Method for the Determination of Ciprofloxacin in Human Plasma," *Journal of Pharmaceutical and Biomedical Analysis* 33 (2003): 125–30.

2. Adapted from A. Firsov et al., "Parameters of Bacterial Killing and Regrowth Kinetics and Antimicrobial Effect Examined in Terms of Area under the Concentration-Time Curve Relationships: Action of Ciprofloxacin against *Escherichia coli* in an In Vitro Dynamic Model," *Antimicrobial Agents and Chemotherapy* 41 (1997): 1281–87.

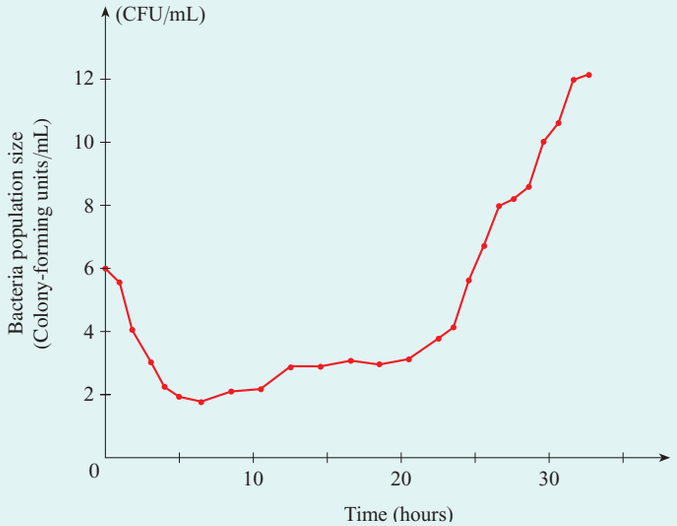

FIGURE 3
The kill curve of ciprofloxacin for *E. coli* when measured in a growth chamber. A dose corresponding to a concentration of 0.6 μg/mL was given at $t = 0$.

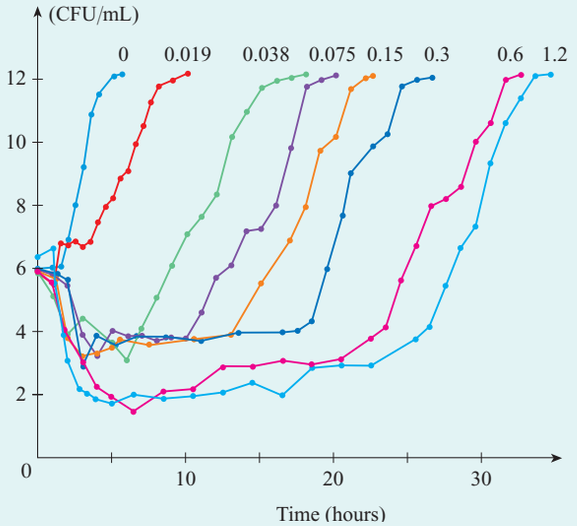

FIGURE 4
The kill curves of ciprofloxacin for *E. coli* when measured in a growth chamber. The concentration of ciprofloxin at $t = 0$ is indicated above each curve (in μg/mL).

magnitude of antibiotic treatment and its effectiveness. This is done by obtaining both a measure of the magnitude of antibiotic treatment, from the antibiotic concentration profile underlying each kill curve, and a measure of the killing effectiveness, from the kill curve itself. These measures are then plotted on a graph of killing effectiveness against the magnitude of antibiotic treatment.

As an example, Figure 5 plots the magnitude of the drop in population size before the rebound occurs (a measure of killing effectiveness) against the peak antibiotic concentration (a measure of the magnitude of antibiotic treatment). Each of the eight colored points corresponds to the associated kill curve in Figure 4. (Peak concentration is measured in dimensionless units, as will be explained in Case Study 1a.) The points indicate that, overall, as the peak concentration increases, the magnitude of the drop in population size increases as well. This relationship can then be used by the researchers to choose an antibiotic dose that gives the peak concentration required to kill the bacterial infection.

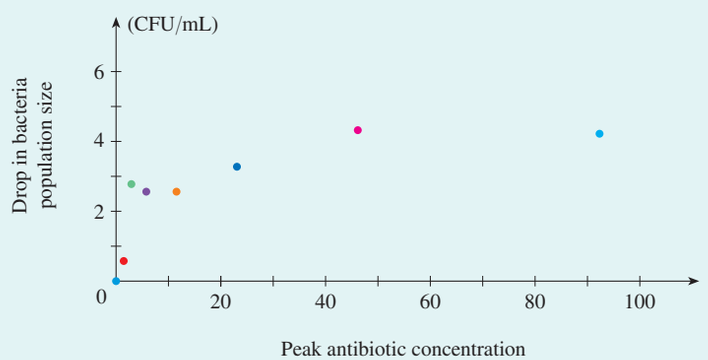

FIGURE 5

This approach for choosing a suitable antibiotic dose may seem sensible, but there are many different measures for the killing effectiveness of an antibiotic, as well as many different measures for the magnitude of antibiotic treatment. Different measures capture different properties of the bacteria–antibiotic interaction. For example, Figure 4 shows that many different antibiotic doses produce approximately the same magnitude of drop in bacteria population despite the fact that the doses result in large differences in the time necessary for population rebound to occur. Thus the magnitude of the drop in population size before rebound occurs does not completely capture the killing effectiveness of the different antibiotic doses.

For this reason, researchers typically quantify antibiotic killing effectiveness in several ways. The three most common are (1) the time taken to reduce the bacteria population to 90% of its initial value, (2) the drop in population size before rebound occurs, as was used in Figure 5, and (3) a measure that combines the drop in population size and the duration of time that the population size remains small (because effective treatment not only produces a large drop in bacteria population but maintains the population at a low level for a long period of time).

Similarly, there are many measures for the magnitude of antibiotic treatment. The most commonly used measures include (1) peak antibiotic concentration, as was used in Figure 5, (2) duration of time for which the antibiotic concentration is high enough to cause negative bacteria growth, and (3) a measure that combines both peak concentration and duration of time that the concentration remains high.

The conclusions clinical researchers obtain about suitable antibiotic doses can differ depending on which measures are used. For example, Figure 6 shows the relationship between the time taken to reduce the bacteria population to 90% of its initial value plotted against the same measure of peak antibiotic concentration as was used in Figure 5 for the kill curves shown in Figure 4. Unlike Figure 5, Figure 6 shows no consistent relationship between effectiveness (as measured by the speed of the population decline) and strength of treatment.

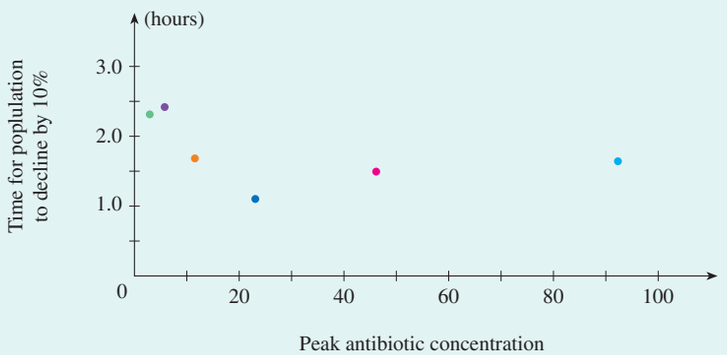

FIGURE 6

To use appropriate measures to formulate effective antibiotic doses, we therefore need to understand what determines the shape of the relationships between measures, and when and why these relationships will differ depending on the measures used. This is where mathematical modeling can play an important role: By modeling the biological processes involved, we can better understand what drives the different patterns, and we can then use models to make predictions about what we expect to observe in other situations. Making such predictions is the goal of this case study.

The order in which mathematical tools are used by researchers is not always the same as the order in which they are best learned. For example, when analyzing the problem in

this case study, researchers would first use techniques from Chapter 3 and then Chapter 6 to model the dynamics of the drug and bacteria and to quantity the strength of treatment and effectiveness of killing. They would then analyze these models using the techniques of Chapters 1 and 2.

For our learning objectives, however, this case study will be developed in the opposite order: In Case Study 1a we will use a given model for the effect of antibiotics on bacteria growth to draw conclusions about the differences in the relationships shown in Figures 5 and 6. In Case Study 1b we will begin to fill in the gaps by deriving the model used in Case Study 1a. In Case Study 1c we will continue to fill in gaps from Case Study 1a by deriving different measures for the magnitude of antibiotic treatment. We will also show how a process called dose fractionation can be used to alter various aspects of these measures. Finally, in Case Study 1d we will use the model derived in Case Study 1b to make new predictions about the effectiveness of antibiotics and compare these predictions to data.