Brandon Hale

Class: Junior
Major: Mathematics
Minor: Biology

I am working in the relatively young field of mathematical biology. I first became interested in this area when I was taking a microbiology class taught by Dr. James Stuart from Murray State University's Department of Biological Sciences. We discussed the problem of antibiotic resistance. By coincidence, I was also taking a mathematical modeling class taught by Dr. Renee Fister in the math department. Using what I learned in both classes, I was able, with the guidance of these two faculty members, to develop a mathematical model for antibiotic resistance. I have refined and presented the model twice at Murray State University. Today, under the direction of Dr. Maeve McCarthy, I am developing a mathematical model and computer program to describe a number of ecological phenomena.

ABSTRACT

Mathematical Model for Antibiotic Effectiveness

It is known that in any given population of bacteria, a certain percentage can be resistant to one antibiotic or another. Bacterial resistance carried on a plasmid can be transferred from parent to offspring (vertical transfer), as well as from a resistant cell to a non-resistant cell (horizontal transfer). As a result, an antibiotic becomes less effective over time when it is used more often because the percentage of antibiotic resistance increases in the bacterial population being fought. This is often seen in hospitals, where patients' immune systems are weakened and the usage of antibiotics is higher than normal. A mathematical model is presented that can be used to minimize the rate of horizontal transfer while also minimizing the amount of time that the infected person is sick. Different "patient types" are examined. Shown in the study is how the horizontal transfer rate increases as the patient's immune system and/or drug efficiency decrease.

FACULTY MENTOR

K. Renee Fister is an associate professor in the Department of Mathematics and Statistics. She has been at Murray State for eight years where she teaches a variety of university studies and upper level mathematics courses. Her research interests focus on optimal control techniques applied to differential equations. Recently, she has been studying optimal treatment strategies for breast and ovarian cancers. Through her involvement in Murray State's Howard Hughes Medical Institute award and through other grants, she has been able to involve and support both undergraduate and graduate students in these research endeavors.

Mathematical Model for Antibiotic Effectiveness

A rather common practice in today's agricultural industry is the addition of antibiotics into food for livestock. This practice is primarily used to protect the animals from bacterial infections, which could otherwise kill the livestock and thus lower the profits of the farmer or company. One such antibiotic, shown in Figure 1, is tetracycline (European Commission, 1999).

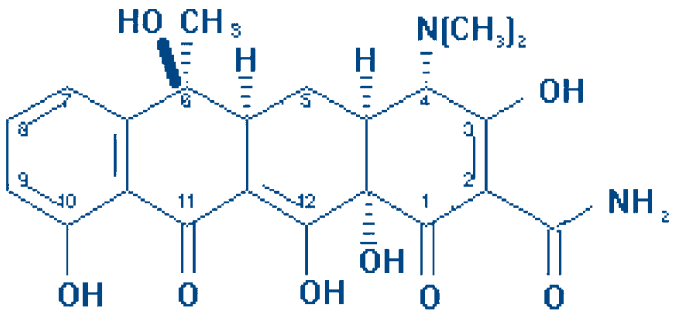

Figure 1. Chemical Structure of Tetracycline.

The addition of tetracycline does not directly pose a threat to human health. However, it poses a type of natural selection on bacteria that may inhabit the livestock food, areas around the cattle, or perhaps even the meat or dairy products that we buy. A good example is the bacterium *Bacillus cereus*, which can be found in all of these places as well as airborne (Iowa State University [ISU], n.d., p.1).

According to the United States Food and Drug Administration (USFDA), *Bacillus cereus* is a gram-positive rod-shaped bacterium that is rather commonly found in food. The structure of Gram-positive cell walls typically contain extra layers and sometimes contain toxins or other chemicals that aid in infecting another organism. Most infectious bacteria are Gram-positive. *B. cereus* bacteria are easily killed at temperatures above 50 ˚C (122 ˚F). Usually, cooking prevents the bacterium from causing disease in humans. In some rare cases, ingestion of a large number of *B. cereus* bacteria in undercooked food has been known to result in food poisoning. *B. cereus* food poisoning can be accompanied by either diarrhea or vomiting, depending on the type of toxin in the cell walls of the ingested *B. cereus* strain (USFDA, 2003, p.1-2).

B. cereus food poisoning is fairly rare. The USFDA states that fewer than 50 cases have been reported to the Centers for Disease Control during the past 25 years. This is likely due to the fact that *B. cereus* food poisoning typically goes unreported or misdiagnosed as food poisoning caused by a different species of bacteria. As is the case with any type of food poisoning, all people are equally susceptible to *B. cereus* poisoning (USFDA, 2003, 1). However, the concern over *B. cereus* is not due to the disease it causes, but rather the DNA which some *B. cereus* carry.

Some bacteria contain plasmids, self-replicating circular strands of DNA that can carry certain genes that are not necessary for a bacterium's survival but can sometimes be useful to it. A good example of a plasmid-carried gene would be one which encodes for resistance to an antibiotic. Plasmids copy themselves during cell replication, so any offspring from a plasmid-carrying cell will also get a copy of the plasmid. This is called vertical transfer. In addition, plasmids can also be transferred horizontally (that is, from a plasmid-carrying cell to another cell that does not carry it) through a process called conjugation (Nester, Anderson, Roberts, Pearsall and Nester, 2004, p. 209-210). In some cases, conjugation has been observed between different species of bacteria and even between bacteria and certain eukaryotic species such as yeast.

An antibiotic-resistance plasmid can allow a bacterium to survive in the presence of an antibiotic that would otherwise kill it, but this resistance comes at a price. Extra genes create extra proteins, and the creation of these proteins costs the bacterium some energy that could otherwise be used for vital functions of its metabolism or replication. This metabolic cost is thought to be what generally keeps the prevalence of a plasmid in a population of bacteria at a relatively low percentage. However, the use of antibiotics creates a selective pressure on the bacteria, allowing the resistant bacteria to survive and multiply while the susceptible bacteria die off. This metabolic cost is not trivial and should be included in the model,

but currently little data regarding this cost is available. For this reason, the metabolic cost will be disregarded and left for a future version of the model.

Some *Bacillus cereus* strains contain a plasmid designated pBC16, which codes for tetracycline resistance. The protein that this plasmid codes for acts like a pump, forcing the tetracycline out of the cell before it can stop the bacterial protein synthesis (Collard, 2003, 1; Nester et al., 2004, p. 512, 522). A study by Schlegelova, Brychta, Klimova, Napravnikova, and Babak (2003) found the ratio of Tet-resistant to Tet-susceptible *B. cereus* bacteria to be about 2 to 129 (p. 336).

General Model for Antibacterial Resistance

A model for a disease with an antibiotic-resistant strain (R_t) and an antibiotic-susceptible strain (S_t) must treat each strain as separate population equations, with each equation being dependent upon the other (due to conjugation). It must also take into account the birth rate of the bacteria as well as a death rate due to the immune system for each equation and a death rate due to the drug for the susceptible population. The drug's effectiveness should also be included as a percentage term, in order to accommodate the fact that some of the drug can be eliminated by the body before it is able to destroy any invading bacteria. The natural death rate of the bacteria can be included in the death rate due to the immune system's actions, since the immune system probably would destroy an invading bacterium regardless of whether it is alive or not. Therefore, a reasonable model is shown in Equations 1 and 2, where the equations are written in difference form (excluding time) for simplicity. The corresponding differential equations include a time variable for any terms in which time is not included for as part of another variable.

$$S_{t+1} = P_S * i_0 + S_t * \beta - \delta * S_t - \gamma * R_t * S_t - E * C_t * S_t \qquad (1)$$

$$R_{t+1} = P_R * i_0 + R_t * \beta - \delta * R_t + \gamma * R_t * S_t \qquad (2)$$

Where:

P_S and P_R = Percent of general bacterial population that is susceptible and resistant, respectively

i = Number of bacteria that infects the person (i_0 being the initial value)

β = Birth rate of the bacteria (should be the same for either strain)

δ = Percent death rate due to actions of the immune system

γ = Percent rate of conjugation between resistant and susceptible strains

E = Percent effectiveness of the drug

C_t = Concentration of a drug at a given time

Note: $0 \le P_S, P_R, I, \gamma, E \le 1$ since they are all percentages.

For simplicity, it will be assumed that $\beta = i * 2^{(t/G)}$, where t = time and G = the amount of time required for the bacterial population to double. This corresponds to the simple binary fission method through which bacteria reproduce. The susceptible bacteria population will be affected by drug dosages that can be calculated using the drug dosage model in Equation 3, created by Horelick and Koont (as cited by Giordano, Weir, and Fox, 2003, p. 382-389).

$$C_t = C_0 + \frac{C_0 \mathbf{e}^{(-kT)}(1 - \mathbf{e}^{(-nkT)})}{1 - \mathbf{e}^{(-kT)}} \qquad (3)$$

Where:

C_t = Concentration of the drug at time t

C_0 = Initial concentration of the drug

k = Elimination constant of the drug

T = Time between doses (differs depending on the drug)

n = Number of doses

Using these two models together, the goal of the problem will be to minimize the rate of conversion from the susceptible strain into resistant strains (i.e. $-\gamma * R_t * S_t$). This can be achieved by killing the susceptible bacteria as quickly as possible with the drug while maintaining safe dosage levels. However, before the problem can be solved for a particular disease and bacterium, values for the parameters γ, δ, and E are needed.

Determination of Generalized/Specific Parameters

Andrup, Smidt, Hojgaard, and Jensen (2003) of the National Institute of Occupational Health in Denmark have developed a formula for calculating the conjugative rate (γ) of *Bacillus thuringiensis*, a species of bacteria closely related to *Bacillus cereus*. His team created a model analogous to the Michael-Menten enzyme kinetics model, which relates the rate of an enzyme-catalyzed reaction to the enzyme's substrate concentration. Similarly, the Andrup team's model for conjugation kinetics relates the rate of conjugation with the titer (cells/ml) of the susceptible

$$\gamma_{[S]} = \frac{\gamma_{max}[S]}{[S] + K_M} \quad (4)$$

Where:

[S] = Concentration (titer) of susceptible cells

$\gamma_{[S]}$ = Rate of conjugation at concentration [S]

γ_{max} = Maximum rate of conjugation possible

K_M = Concentration of S that yields the rate at half of the maximum

cells. Changing variable designations to fit the bacterial population model mentioned before (Equations 1 and 2), the Andrup et al. model is shown in Equation 4 (Andrup et al., p.1-5).

Figure 2 shows the probable rate curves which fit the data which Andrup et al. (2003) used to determine the conjugative rate for the 200 kb plasmid pXO16 (note that one kb = 1000 base pairs of DNA nucleotides). The plasmid pBC16 in *B. cereus* is considerably smaller, being only about 0.31 kb (Collard, 1998, 1). Andrup and his team found that the rate of plasmid transfer is approximately 1 kb/sec for the *B. thuringiensis* plasmid. This same rate can likely be applied to *B. cereus* since the species are so closely related, and this creates a γ_{max} for the plasmid pBC16 of about 0.31 kb/sec. The constant K_M cannot be determined without experimentation, but because of the small size of the plasmid, it turns out that the K_M has little effect on the graph, as shown in Figure 3. However, when applying this model to other species with larger plasmids, γ would have a larger impact on the overall model. In the case of *B. cereus*, since the maximum conjugative rate occurs at less than one third of a second, a constant $\gamma = 0.3$ titer/min x time will be used.

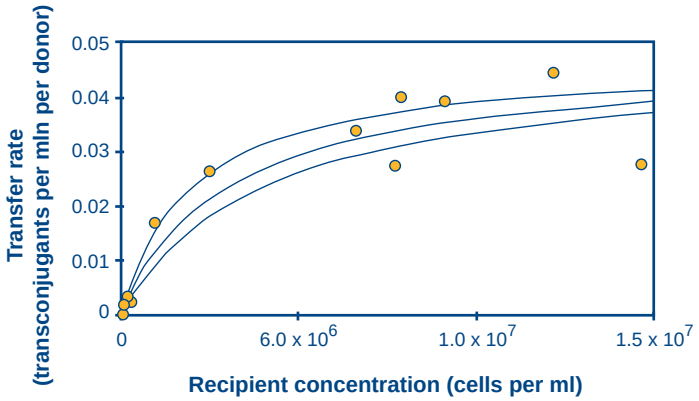

Figure 2. Conjugation rate developed for Andrup et al. (2003) research

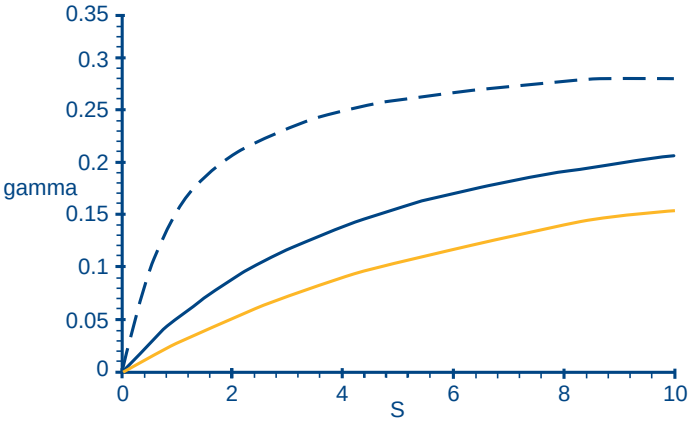

Figure 3. Predicted rate of conjugation for *B. cereus* when K_M = {1, 5, 10}

Next, the model requires a parameter δ to describe the efficiency of the patient's immune system (or simply, the patient's immunoefficiency). Determining an equation to describe the immune system is a research project in itself. However, it is known that the immune system of some people works better than the immune system of other people. Some people seem to never get sick, while others seem to be sick quite often. The worst-case scenario is, of course, a full-blown AIDS patient. Imagine a hypothetical group of patients with varied levels of immunity (as in Figure 4). This graph allows for δ values relative to the most immunoefficient patient (the peak of the graph) to be used. These δ values will be percents of the titer of bacteria per hour that the most immunoefficient patient's immune system can destroy. For the tetracycline resistance model, the most immunoefficient patient will be able to kill 500 titer/hr.

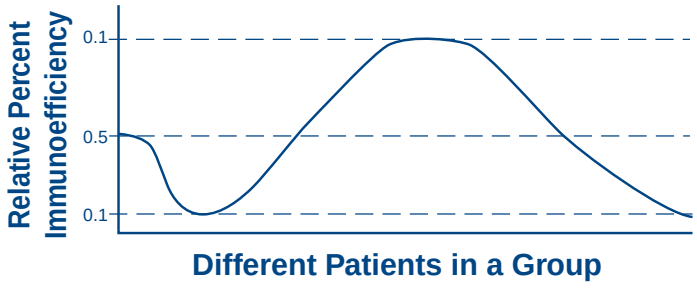

Figure 4. Immunoefficiency graph of a hypothetical group of patients

A more accurate equation for δ could be developed with additional work. It should be variable, depending on the patient's age, the disease in question, and possibly the geographic location of the

patient as well. It is likely that this may have to be examined at the cellular level, by monitoring the actions of the patient's T-cells and B-cells. In addition, this equation should also be able to incorporate various factors which could also affect the immunoefficiency, including secondary infections of another disease, immunosuppressant drug therapy or chemotherapy, and patient vaccinations to the bacteria species' toxin.

The parameter to describe the effectiveness of the drug (E) is probably a function somewhat similar to that described for the immunoefficiency rating. It should represent the removal rate of the antibiotic by the body before the antibiotic has a chance to destroy an invading bacterium. It should also include other factors that affect the effectiveness of the antibiotic, such as spontaneous mutations in the bacterial genome that prevent the antibiotic from working, without necessarily being carried on a plasmid. Again, since this drug efficiency equation is likely a complicated paper in itself, the value of E will instead be parameterized with various percentage values, and the possibility of spontaneous mutations will be ignored. Therefore, for example, an E value of 0.75 will represent 75 percent of the drug actually killing bacteria, while 25 percent is absorbed by the body.

The Specific Model for Tetracycline Resistance in *Bacillus cereus*

The objective of this model is to minimize the spread of the tetracycline resistance. That is, the objective function of this model is the conjugation between resistant and susceptible bacteria (the term $\gamma * R_t * S_t$ in Equations 1 and 2) should be minimized while also keeping the tetracycline dosage within safe levels. Since the conjugation term is nonlinear, this problem cannot be solved with linear optimization methods such as the Simplex method. Instead, a complicated technique called the Primal Dual Log Barrier method is needed. The Primal Dual Log Barrier method finds a minimum optimal solution value in multiple variable models such as this. Jason Schattman of Maplesoft, Inc. has created a package for Waterloo, Inc.'s Maple versions 6.0-7.0 that utilizes this method to optimize nonlinear models (2001). To download a copy of and/or learn more about this package, please visit Schattman's website at http://www.mapleapps.com/powertools/optimization/optimization.shtml.

For the tetracycline-resistance model, the initial number of infecting bacteria (i_0) will be 10,000 bacteria for each patient. As previously stated, the ratio of resistant to susceptible bacteria will begin as 2/129 (Schlegelova et al., 2003, p. 336). According to Malahyde Information Systems, the minimum effective level of tetracycline is 8×10^{-6} g, and the maximum safe level is 3 g for adults (Malahyde Information Systems, 1993). The half-life of tetracycline is about 14 hours (Doxycycline.com, 2000, 3), and the doubling time (G) of *B. cereus* is about 30 minutes, so the birth rate (ß) can be calculated as $ß = 2^{t/30}$ (ISU, n.d, 1). Based on the 14 hours half-life using the Horelick and Koont model, $k_{Tet} = 0.0495$. Therefore, the problem is as follows:

$$\text{Min } (\gamma * R_t * S_t)$$

subject to Equations 1, 2, and 3 as well as the following logical bounds:

$$8 \times 10^{-6} \leq C_n \leq 3$$

$$0 \leq n \leq 10$$

Values of all variables are nonnegative.

Table 1 shows the results of the Schattman (2001) package based on these conditions and some of the different patient types tried. The final iterations were considered to be those in which S_f and R_f were closest to zero but still nonnegative or when C_f reached the maximum safe level, as reported in the table. Also, Table 1 is calculated assuming that the drug's efficiency level (E) is 1 (that is, 100 percent efficient). Changes in the drug efficiency level will be examined in the next table.

Table 1

Results of Optimization when Altering δ and Holding Efficiency at 100 percent

Patient δ Value(E as a	Drug Efficiency percent)	Final Tet-Susceptible Bacteria (S_f in titer)	Final Tet-Resistant bacteria R_f in titer)	Final Drug Concentration on (C_f in grams)	Treatment Time Elapsed (hours)	# of Doses (n)	Value of Objective Function ($0.3*R_t*S_t$) in titer/hr
1.00	1	4.31	0.00016	1.73	30.8	5	0.0002
0.90	1	6.47	0.0003	1.41	46.2	3.108	0.0006
0.75	1	7.71	5.308	2.99	32.48	1.4	12.27
0.67	1	19.21	15.13	2.17	49.84	4.3	87.19
0.50	1	19.79	22.36	2.99	29.96	1.83	132.75
0.33	1	21.38	21.6	2.29	37.52	2.88	138.54
0.25	1	7.68	67.62	2.99	35	1.33	155.79
0.10	1	2.58	264.08	2.38	37.1	2.67	204.40
0.01	1	1.80	436.77	2.57	36.32	2.95	235.86

Notice the bacterial conjugation rate (the objective function) increases as the immunoefficiency rate decreases. In the lower immunoefficiency cases, a combination of drugs may be necessary to destroy all of the bacteria. The specific model being examined only considers tetracycline by itself, but a second drug could be considered by copying Equation 3 and adding a new term or terms into Equation 1 to accommodate the new drug(s). This would, of course, create a considerably more complex system.

The data in Table 1 provides some mathematical evidence to explain why bacterial antibiotic resistance is highly prevalent in hospital environments. Some bacterial infections that reside in the highly selective environment of a hospital can have plasmids that code for resistance to several different antibiotics (Nester et al., 2004, p. 211-212). The data on Table 1 show that the combination of the low immunoefficiency state of the patients in the hospital, along with the high prevalence of antibiotics being used by them, creates a natural selection-like pressure for the resistant strain. The increase and subsequent decrease in the susceptible strain as the patient immunoefficiency decreases reflects the increased reliance of the patient on the drug. At the 0.5 immunoefficiency mark, the conjugation rate has increased so significantly that fewer susceptible bacteria remain since they are so quickly converted into the resistant strain (by conjugation). This results in an increase in the rate of conjugation between the bacterial strains.

Next, alterations to the tetracycline efficiency (E) will be examined to mimic overuse of the antibiotic. Table 2 shows the effects of this in three particular patient types ($\delta = \{1.00, 0.5, 0.01\}$).

Table 2

Results of the Algorithm with Drug Efficiency of 75 percent and 50 percent.

Patient δ Value	Drug Efficiency (E as a %)	Final Tet-Susceptible Bacteria (S_f in titer)	Final Tet-Resistant bacteria (R_f in titer)	Final Drug Concentration (C_f in grams)	Treatment Time Elapsed (hours)	# of Doses (n)	Value of Objective Function ($0.3*R_t*S_t$) in titer/hr
1.00	0.75	7.53	29.72	2.56	27.26	5.85	67.15
1.00	0.50	8.66	94.56	2.50	25.29	4.16	245.70
0.50	0.75	44.31	11.08	2.53	22.69	4.88	490.99
0.50	0.50	41.84	48.21	2.99	21.21	3.07	605.15
0.01	0.75	1.36	674.6	2.60	22.82	3.59	275.24
0.01	0.50	0.42	1590.32	2.54	31.92	2.19	200.38

Table 2 shows a clear indication of the dangers of overusing antibiotics. It clearly shows that the drug works too slowly to prevent the antibiotic resistant bacteria from conjugating with (and thereby converting) the antibiotic susceptible bacteria. One can easily see how quickly the population of the antibiotic resistant strain explodes in each case.

It should be reiterated that *Bacillus cereus* is not a dangerous species. However, a strain of this species could easily mutate into a more virulent form. In addition, *B. cereus* is closely related to another *Bacillus* species that causes one of the deadliest diseases known to man—*Bacillus anthracis*, known by most as anthrax (ISU, n.d., p. 1). Treatment of anthrax involves ciproflaxin, tetracycline or pencillin, in that order of preference (United States Center for Disease Control, 2003, p. 3). Interspecies conjugation is known to occur, so continued addition of tetracycline into animal food poses a serious threat of allowing *B. anthracis* to obtain the tetracycline resistance plasmid from *B. cereus*.

Aside from anthrax, many other diseases are treated with tetracycline as well. These include bacterial pneumonia, gonorrhea, syphilis, surgical infections, acne, cholera, Rickettsial disease, malaria and Lyme's disease, among others (Malahyde Information Systems,1993, p. 2-3). Continued abuse of this drug will ultimately result in an increase in tetracycline resistance in the microorganisms that cause some or even all of these diseases, as a result of the natural selection-like pressure demonstrated in Tables 1 and 2. In order to prevent these and other species of bacteria from becoming predominantly tetracycline-resistant, the selective pressure being imposed on them must be eased. In short, the practice of adding tetracycline into animal food must be halted, and much more care must be taken to help limit the usage of antibiotics.

Acknowledgements

I thank Dr. K. Renee Fister for her valuable assistance on this paper. I also thank Dr. James Stuart, whose microbiology lectures provided much of the inspiration for this model.

References

Andrup, Lars, Lasse Smidt, Maj-Britt Højgaard, and Jensen, Gert. (2003). *Conjugation in Bacillus thuringiensis.* Retrieved December 8, 2003, from Denmark National Institute of Occupational Health website: *http://www.ami.dk/research/andrup/research.html.*

Collard, James. (1998). *Tetracycline Resistance Gene Classification: Class L.* Retrieved December 7, 2003, from the Belgian biosafety and biotechnology server: *http://biosafety.ihe.be/AR/Tetracycline/Tetdescription/bodyL.html.*

Doxycycline.com. (2000). *Doxycycline Prescribing Information.* Retrieved December 8, 2003, from *http://www.doxycycline.com/prescribing.htm.*

European Commission. (1999*). Assessment by the Scientific Committee on Animal Nutrition of a Microorganism Product: Esporafeed Plus.* Retrieved October 7, 2003, from *http://europa.eu.int/comm/food/fs/sc/scan/out39_en.pdf.*

Giordano, Frank, Weir, Maurice, and Fox, William. (2003). *A First Course in Mathematical Modeling* (3rd ed.). Pacific Grove, CA: Brooks and Cole.

Iowa State University (ISU), Department of Food Science and Human Nutrition. (n.d.). *Bacillus cereus.* Retrieved December 8, 2003, from ISU website: *http://www.ag.iastate.edu/departments/foodsci/classes/fshn420/bcereus.htm.*

Malahyde Information Systems. (1993). *Acu-Oxytet Capsules. Online drug information.* Retrieved December 7, 2003, from *http://home.intekom.com/pharm/apotex/acuoxyt.html.*

Nester, Eugene, Anderson, Denise, Roberts, C. Evans, Pearsall, Nancy, and Nester, Martha. (2004). *Microbiology: A Human Perspective* (4th ed.). New York: McGraw-Hill.

Schattman, Jason. (2001). Maple Powertools – Nonlinear Programming. Retrieved from Maplesoft website: *http://www.mapleapps.com/powertools/optimization/optimization.shtml.*

Schlegelova, J., Brychta, J., Brychta, Klimova, E., Napravnikova, E., and Babak, V. (2003). The prevalence of and resistance to antimicrobial agents of *Bacillus cereus* isolates from foodstuffs. *Czech J Vet Med 48(11),* 331-338.

United States Centers for Disease Control. (2003). *Anthrax Questions and Answers.* Retrieved March 03, 2004, from CDC Bioterrorism website: *http://www.bt.cdc.gov/agent/anthrax/faq/index.asp.*

United States Food and Drug Administration (USFDA), Center for Food Safety and Applied Nutrition. (2003). *Bacillus cereus and other Bacillus spp. Foodborne Pathogenic Microorganisms and Natural Toxins Handbook* [online database]. Retrieved December 8, 2003, from USFDA, CFSA website: *http://vm.cfsan.fda.gov/~mow/chap12.html.*