T4 Bacteriophage Treatment for Escherichia Coli Infections

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INTRODUCTION

Acute infectious diarrhea is the second leading cause of death of children living in developing countries, accounting for 1.5 million deaths per year. One major agent causing this diarrhea is Escherichia Coli (E. coli). This bacterium is regularly transported to the developed world as well, particularly from those who develop traveler's diarrhea when visiting developing countries (11).

Escherichia Coli is gram-negative bacteria that live in the lower half of the intestinal tract of warm-blooded mammals. There are numerous kinds of E. coli in the human intestinal tract. Some of these strains are vital for healthy gut function, while others cause severe gastrointestinal distress (1). These infections can sometimes be resolved without treatment, but the most common form of medication administration is antibiotics; this is problematic, however, because the antibiotics are not selective in which bacteria they kill. Antibiotics kill both beneficial and harmful bacteria and possibly allow for harmful bacteria to flourish even after treatment ends. Further, antibiotic resistance rates are rapidly rising (2).

A possible alternative to antibiotic treatment is the administration of bacteriophages. Bacteriophages are viruses that bind protein receptors on the outside of bacteria. Bacteriophages inject a genome into the host bacteria, which forces the bacteria to produce bacteriophages identical to the one that infected the host; the bacteria then bursts (and dies), releasing the phages into the environment where they can infect adjacent bacteria. Phages are a useful alternative to antibiotics, as phages are specific to a strain of bacteria and thus only kill the targeted bacteria (3).

The strain of E. coli that frequently causes severe gastrointestinal distress in humans is referred to as enterotoxigenic E. coli. The bacteriophage that binds to enterotoxigenic E. coli is known as T4 Bacteriophage and is currently one of the most well-researched bacteriophages (4). Some research has already been instigated on clinical applications of phages as a cure for E. coli infections. This paper seeks to build upon existing literature and give guidance to future medical applications of T4 bacteriophage, particularly in determining the dosage necessary to cure enterotoxigenic E. coli infection.

METHODS

To determine the amount of phages required to effectively treat an *Escherichia Coli* infection, a box model was first constructed to get a general idea of how free phages interact with the *Escherichia Coli* over time. From this process, we were able to determine a set of differential equations that represent how phage therapy could be modeled as a treatment option. The equations entered into the Big Green Differential Equation Machine were as follows

$$P_F' = akE_I - pE_SP_F - CP_F$$

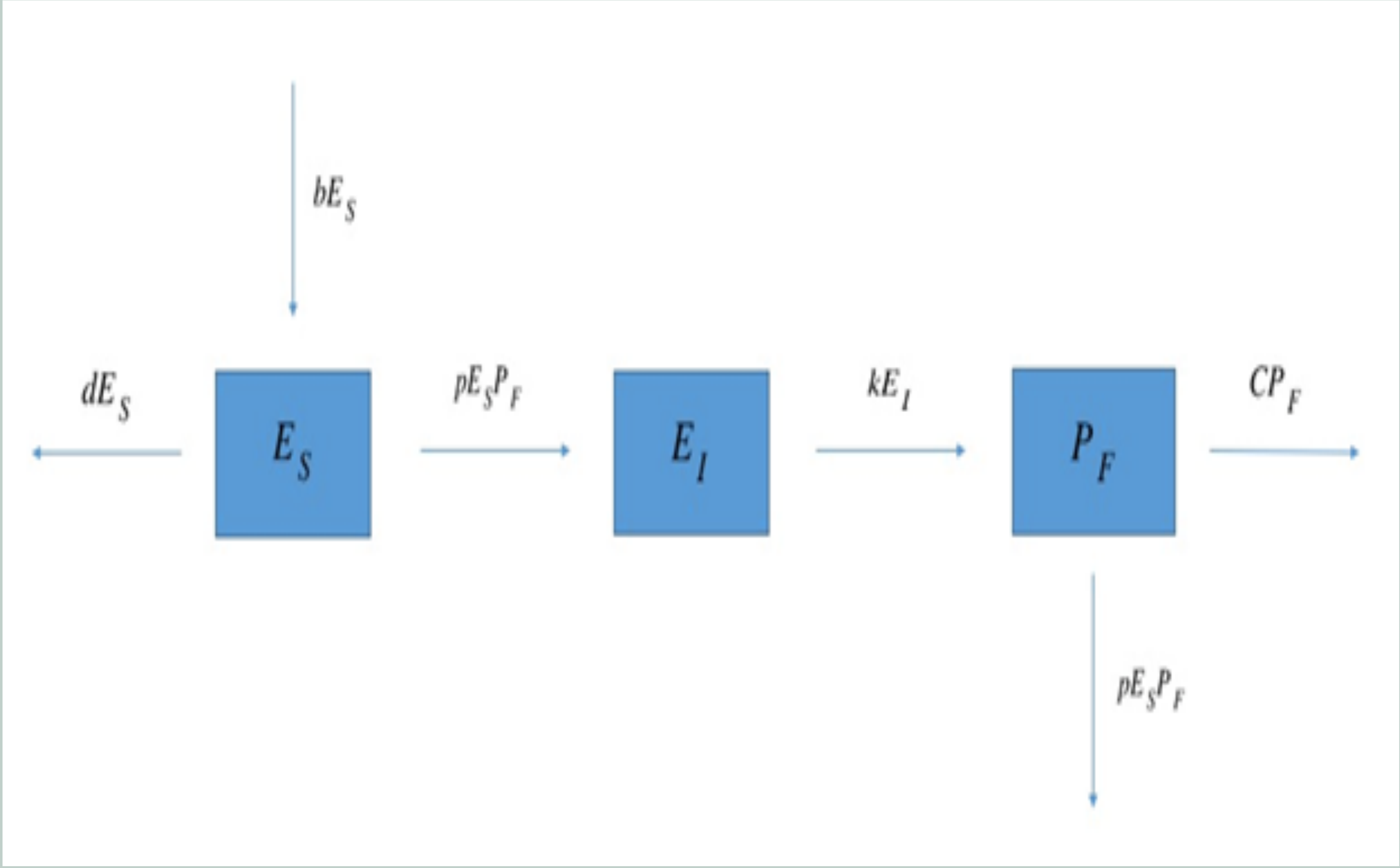
$$E_S' = bE_S - dE_S - pE_SP_F$$

$$E_I' = pE_SP_F - kE_I$$

Where P_F represents the free phages, E_S represents the E. coli that susceptible to infection and E_I represents the E.coli that are infected with phages. It was necessary to distinguish the E. coli s E_S from E_I to demonstrate two states that the bacterial cells could be in upon introduction of the T4 phages.

The following values represent the constants that were either based on literary research or estimation. The number of free phages produced from the burst of an infected E. coli cell was determined to be 200 represented by (a). This was calculated due to the literature value that a single E. coli cell, 100 phages burst every 30 minutes, so this was converted to 200 per hour to keep units consistent throughout the model. The natural death rate of the free phages was estimated to be 0.10 represented by (c). The natural reproduction rate of E. coli doubles every 2 hours based on literature, thus the was calculated to be ln2/12 which equals 0.025 represented by letter (b). To determine the death rate of bacterial cells due to the immune response, we found that it takes between 5-10 days for the immune system to respond to an E. coli infection. Since the time frame for phage therapy occurs in a number of hours, the death of E. coli cells due to the immune system was assumed to be negligible, represented by letter (c) as 0. The bursting rate of an E. coli cell due to phage infection calculated to be 2 E. coli per hour based on the literature rate of 100 phages out of one E. coli every 30 minutes represented by letter (k). The probability that a free phage will infect an E_s was estimated to be a 0.01 contact rate represented by letter (p).

BOX MODEL



EQUATIONS

The administration of T4 bacteriophages for Escherichia Coli infections is modeled with the following equations:

Variables:

Table 1: Variables	
Symbol	Description
E^s	E. coli that are susceptible to phage infection
E^I	E. coli that have been infected with phages
P^F	Free phages (that have not yet infected an E. coli)

Parameters:

Table 2: Parameters				
Symbol	Description	Value	Units	Reference
b	Reproduction rate of susceptible E. coli	0.025	E. coli per hour	(5)
d	Death rate of susceptible E. coli due to immune response	0	E. coli per hour	(6)
p	Probability that a free phage infects a susceptible E. coli	0.01		Educated guess
k	The number of infected E. coli that burst per hour due to phage infection	2	E. coli per hour	(7)
a	The number of free phages produced from the bursting of an infected E. coli	200	Free phages per hour	(7)
c	Death rate of free phages	0.1	Free phages per hour	(8)

Changes in susceptible E. coli:

$$E_S' = bE_S - dE_S - pE_SP_F$$

Changes in Infected E. coli:

$$E_I' = pE_SP_F - kE_I$$

Changes in Free Phages:

$$P_F' = akE_I - pE_SP_F - CP_F$$

CONCLUSIONS

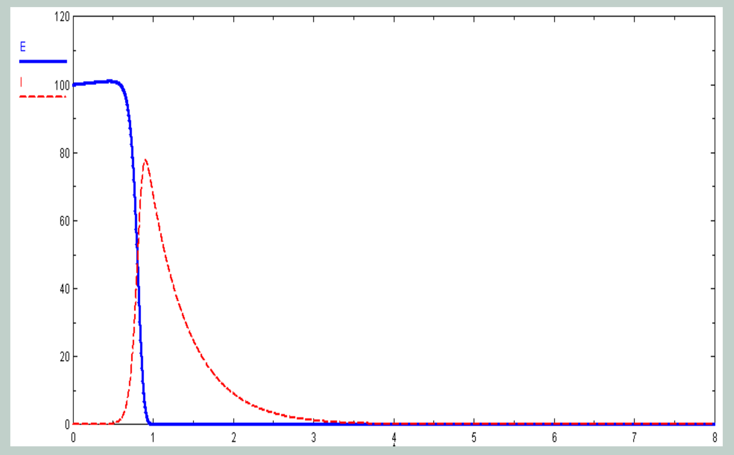


Figure 1: The amount of susceptible and infected Escherichia coli following T4 bacteriophage treatment

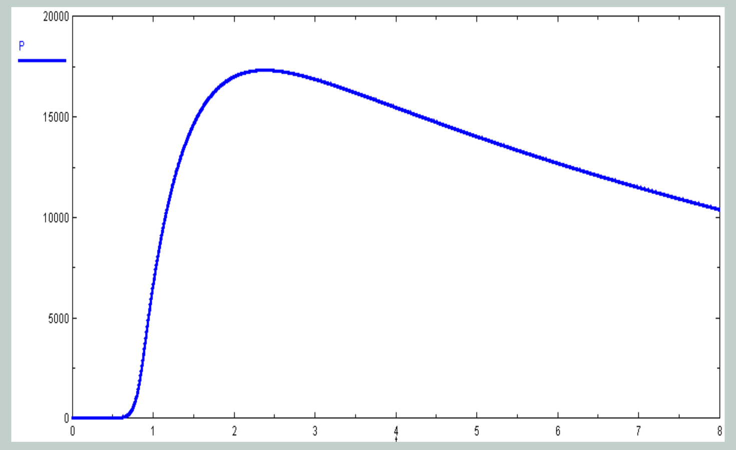


Figure 2: The amount of free phages following T4 bacteriophage treatment

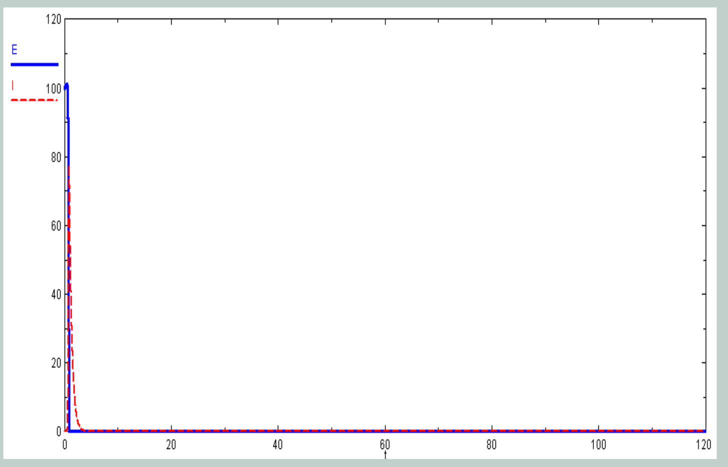


Figure 3: The amount of susceptible and infected Escherichia coli following T4 bacteriophage treatment

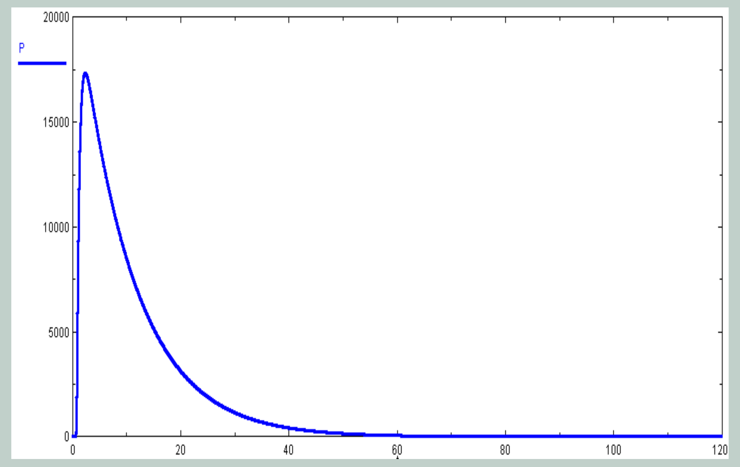


Figure 4: The amount of free phages following T4 bacteriophage treatment

DISCUSSION

Our model is based on a few assumptions. The first few assumptions are related to the context of our setup in the intestine. We denoted the time of administration of T4 bacteriophages as the time in which the phages enter the intestine; however, realistically the phages would be administered orally and take some time to digest and reach the intestine. We assumed that the bacteriophages are specific enough to the E. coli bacteria that they can only kill off and reproduce via E. coli -- they do not interact with anything else. Second, we assumed that all susceptible E. coli are situated in the intestine such that the phage is able to interact with each bacterium with equal probability. Further, we assumed that there is no movement of E. coli from the intestine to elsewhere--the intestine is largely a closed system. We also assumed that the phages were not killed or denatured during ingestion.

Additionally, we made a few assumptions more specifically for our model. We assumed the death rate of E. coli due to immune response was negligible, considering that the rate is small enough to necessitate 5-10 days to clear up the infection, and our model operates on a per hour time scale.

While each term and each equation that comprise our model seem reasonable, the fact that all bacteria die out after 3 hours of phage administration seems a bit unexpectedly fast. It is likely that phage treatment would take longer than this. We suspect the major source of error in our results is due to a software limitation; the Big Green Differential Equation Machine cannot handle an initial E. coli concentration as large as 400,000. As a result, we had to modify our model to represent the necessary phage dosage as a percentage of the initial E. coli amount. However, further research could use more sophisticated software to work with the true initial amounts and compare results.

Nevertheless, our results are useful for further clinical application of bacteriophages for the treatment of E. coli infections. According to the literature, at least 100,000 E. Coli must be present in the intestine for a patient to start experiencing symptoms. However, we assume that a patient would not seek treatment for at least 24 hours, since medical treatment cannot be obtained immediately. Thus because the E. coli amounts double every 12 hours, the initial E. coli amount upon phage administration would be 400,000. Our model indicates that 0.00001% phages of the starting E. coli are needed to terminate the infection, or 4 phages.

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