A Literature Review on the Role of Bacteria, Antibiotics, Antibiotic Resistance and Bacteriophages

By Ashna Kadambi

Ashna Kadambi

Table of Contents

Abstract

Bacteriophages are ubiquitous microorganisms found in various types of environments *(Clokie, et al)*. On Earth, there are an estimated 10^{31} phage particles, roughly equivalent to a trillion phages per grain of sand *(Keen)*. A bacteriophage is a virus which infects a bacterium *(Clokie, et al)*. Bacteriophages have been of interest recently as a result of its promising potential as a novel therapeutic agent, treating infectious bacteria without further perpetuating the spread of antibiotic resistant bacteria. Weighing the pros and cons of bacteriophages and analysing its role in reducing bacteria resistance can help in solving the worldwide crisis of antibiotic resistance. This literature review aims to discuss the role of bacteria, the immune system and antibiotics, along with the global issue of antibiotic resistance. Most importantly, this review will evaluate the environmental benefits and economic consequences of bacteriophages in order to determine whether bacteriophages are a viable option to reduce the spread of antibiotic resistance.

Introduction

The perplexing nature of biodiversity in our planet is driven by microbiology. Complex eukaryotic organisms flourish as a result of prokaryotic organisms such as bacteria. Bacteria encoded enzymes help assist in global key processes such as biogeochemical cycling, the nitrogen cycle, the carbon cycle and produce an estimated half of Earth's oxygen *(Clokie, et al)*. Additionally, in microorganisms, bacteria assists in important survival aspects such as nutrition and immune defence *(Clokie, et al)*. Amidst the countless benefits of bacteria, certain types of bacteria have the potential to be pathogenic and detrimental to organisms, specifically humans. For example, bacteria such as Salmonella have been attributed to over 1.35 million infections in

the United States alone *(Salmonella)*. However, natural immune system defence against infectious bacteria may not prove to be effective for every patient due to varying health conditions. Thus, the usage of antibiotics is crucial to save countless lives. Antibiotics have aided in doubling the lifespan of those in the 20th century, but the innovation of antibiotics are at risk as they become increasingly more ineffective against bacterial infections *(The Value)*. Antibiotic resistance prevails, as a result of natural selection and evolution, threatening humanity's dependence on antibiotics. The Centres for Disease Control (CDC) have estimated that 2.8 million antibiotic-resistant infections occur in the United States each year, and more than 35,000 people perish as a result *(Be Antibiotics)*. Antibiotic resistance is causing harmful worldwide environmental and economical impacts as the diversity of ecosystems are threatened and rising infections result in expensive hospitalisation costs and loss of workforce productivity. On the other hand, bacteriophages, a type of virus which targets bacteria, has shown potential in substituting or working alongside antibiotics. As bacteriophages are natural obligate parasites which only target a narrow spectrum or strain of bacteria, it results in effective targeting of resistant bacterial strains and prevents the overexposure of antibiotics to a wide range of bacteria, reducing the amount of bacterial resistance caused *(Davidson, et al)*. However, further discussion and debate is needed to whether bacteriophages have the potential to substitute or work alongside antibiotics. This review aims to discuss the environmental benefits and the economical consequences of bacteriophages, analysing whether it is beneficial to continue investing in the development of bacteriophages.

Part I: Overview of Bacteria, Antibiotics and the Immune System

Bacteria is a type of microorganism which are classified as prokaryotes. Characteristics of prokaryotes include; being unicellular, lacking a nucleus and having membrane bound organelles *(Bacteria (Updated))*. Bacteria have unique structures , the most common being the Coccus, Spirillum, Ballius structure. Bacteria can either be heterotrophs, or autotrophs *(Bacteria (Updated))*. Bacteria reproduce through binary fission and can also share its DNA through a process known as conjugation *(Bacteria (Updated))*. Heterotrophic bacteria consumes existing organic matter for energy whereas autotrophic bacteria produce its own food *(Bacteria (Updated))*. All pathogenic bacteria are heterotrophic, being either saccharolytic or proteolytic *(Goering) (Köpke, et al)*. Proteolytic bacteria create an enzyme known as protease *(Proteolytic Bacteria)*, whereas saccharolytic bacteria are able to metabolise sugars to create energy *(Perin, et al)*. Despite certain misconceptions, bacteria can be found on both contaminated and clean surfaces. Bacteria are not necessarily always pathogenic. For example, Bifidobacteria, a type of probiotic, are a healthy type of bacteria found in your intestines. Bifidobacteria aid in digesting fibre and help prevent infections *(O'Callaghan)*. On the contrary, harmful bacteria release toxins and cause disease. However, the immune system is naturally designed to protect the body from being infected by pathogenic bacteria. The immune system has three main lines of defence *(Crash Course)*:

- **First line of defence** - This includes your skin, mucus and tears. These are nonspecific to a particular pathogen and are a general defence system *(Crash Course)*.
- **Second Line of Defence** - This includes macrophages, a microorganism which can engulf pathogens. Macrophages are responsible for your inflammatory response as well. Additionally, non-specific white blood cells are included too *(Crash Course)*.

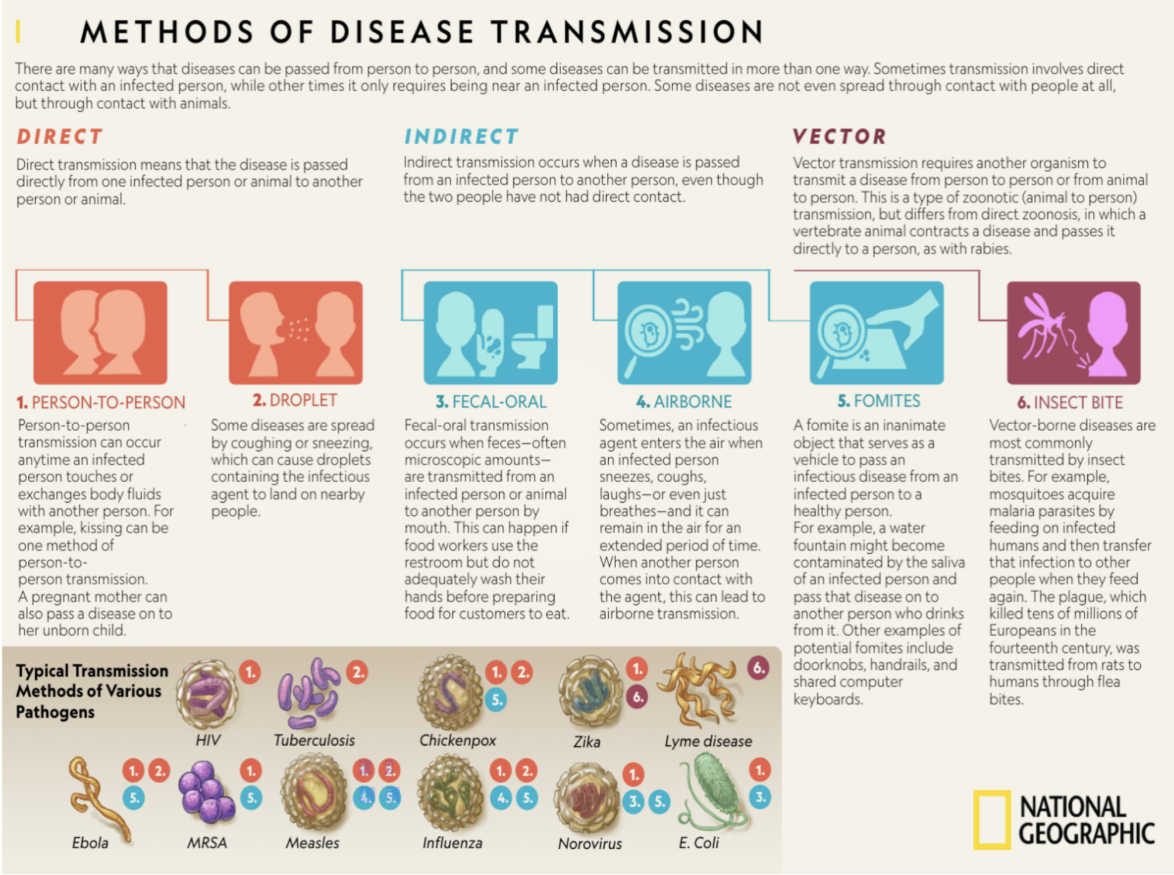
- **Third Line of Defence** - This includes B and T cells (lymphocytes which are specific white blood cells) and memory cells. Memory cells can target pathogens by recognising a specific pathogen to trigger a quicker immune response. B and T cells react to antigens found on the pathogen which triggers the release of antibodies *(Crash Course)*.

Antibiotics, a type of medicine, can assist the immune system against a bacterial infection. Antibiotics work by blocking vital processes in the bacteria's cell, killing the bacteria or preventing it from multiplying *(Antibiotics Explained)*.

Part II: Ramifications of Antibiotics

Bacteria can evolve, becoming resistant to antibiotics through the process of natural selection. Natural selection was a theory discovered by naturalist Charles Darwin in 1859 *(National Geographic Society)*. Natural selection is a well-known phenomenon that illustrates all organisms' process of 'survival of the fittest'. Charles Darwin obtained this theory by observing the Finches on the Galápagos Islands, a species of birds who all had uniquely structured beaks on various parts of the island *(National Geographic Society)*. Darwin concluded that differences in beak shape was an adaptation of each individual bird based on the different food each bird consumed. For example, Finches which consumed insects had longer and sharper beaks as opposed to Finches who consumed fruits. This adaptation to the environment was caused by natural selection as each type of Finch with favourable beak traits increased its own chances of survival and thus passed on its genetics to its offspring *(National Geographic Society)*. Bacteria too, can undergo the process of natural selection. Bacteria which are antibiotic resistant, are not eradicated by antibiotics and thus, through binary fission- reproduce *(What causes antibiotic)*.

Additionally, bacteria can naturally undergo mutations due to a slight change in their genetic material resulting in antibiotic resistant bacteria *(Antibiotic Resistance)*. Certain strains of bacteria share its advantageous genetic mutation with other non-resistant bacteria through a process known as conjugation *(Antibiotic Resistance)*. Case studies of antibiotic resistant strains include E. Coli and Salmonella, which result in food poisoning and kidney failure. In severe cases, antibiotic resistant E. Coli can expel any antibiotics which penetrate the cell membrane *(Antibiotic Resistance)*. Certain emerging strains of Salmonella have shown potential to create powerful enzymes, known as the integrase enzymes, which break down antibiotics before even creating any damage to the bacteria *(Nair, et al)* . Strains of antibiotic resistant bacteria can be spread through numerous forms of disease transmission, depending on the nature of the pathogen. The three main types of disease transmissions include, direct, indirect and vector transmissions *(Methods of Disease)*. Further detail on the forms of transmissions are shown in the National Geographic infographic below *(Methods of Disease)*.



Methods of Disease Transmission (Methods of Disease)

"The rise in antibiotic-resistant bacteria is a global health emergency that could kill 10 million people by 2050."

- United Nations, 2017

The environment globally is contaminated with antibiotics. Antibiotic resistant bacteria are thriving in ecosystems contaminated with antibiotics, creating a major global health threat. Antibiotics are prevalent in the environment as a result of human sewage waste as 40% - 90% of the antibiotic a patient consumes is excreted *(Polianciuc, et al)*. Additionally, antibiotic use is also rising in livestock farming, due to global intensive farming *(Polianciuc, et al)*. Globally, ⅔

of antibiotics produced are used in agriculture for livestock. Antibiotic consumption in livestock reached 63,151 tons in 2010 and is predicted to increase by another 67% by 2030 *(Polianciuc, et al)*. For this reason, animal excretions are found to contaminate soil and consequently cause soil runoff to contaminate bodies of water. Livestock can become vectors for disease, as human populations may consume contaminated meats while agricultural communities could potentially contribute to the spread of 'superbugs' to urban areas *(Gallagher)*. Lastly, pharmaceutical companies and industrialised factories further produce biohazardous antibiotic/ chemical waste which can further contaminate waterways. For example, in 2020, Singapore had to dispose of 5,700 tonnes of hazardous waste *(Amount of Medical*). Antibiotics are naturally part of many microorganisms' immune systems, thus as antibiotic resistant bacteria spreads by contaminated wastewater, certain microorganisms perish from disease *(World's rivers')*. This creates an imbalance in the food chain, affecting an entire ecosystem *(World's rivers')*. For example, The Yale Environmental Review reported on the findings of Croatian researchers who investigated the effects of antibiotic contaminated water on algae and fishes in 2017 *(Bielen, et al) (Lehman)*. The investigation involved the analysis of river water nearby major pharmaceutical plants. Researchers found high concentrations of macrolides, a resilient type of antibiotic *(Bielen)*. High levels of antibiotics halted all algae growth, decreased overall bacterial diversity, and caused fish embryos to have development issues *(Bielen)*. Antibiotic pollution has the potential to destroy our marine ecosystem.

"The release of antibiotics in the environment is driving bacterial evolution and the emergence of more resistant strains."

- United Nations, 2017

A socio- economic burden is caused by antibiotic resistance as extensive hospitalisation and testing is required for patients affected with superbugs, leading to large hospital expenses. Annually, 47 million unnecessary antibiotic prescriptions are issued globally to patients *(CDCa)*. These prescriptions were intended for bacterial infections, but instead are provided to cure a viral infection or an antibiotic resistance bacteria strain, in which both do not respond to antibiotics. In addition, with COVID, an average hospital visit in the United States cost $12,530 *(NHE Fact*). However, healthcare spending for patients with resistant bacterial infections is 165% higher than for patients with non-resistant infections *(CDCb)*. Healthcare becomes more complex as families suffer economic losses as a result of high medical bills and increased insurance rates. Unaffordable costs for treatment lead to an increase in mortality rates and long-term illnesses, mentally affecting the family of ill individuals who can suffer from feelings of depression or grief. Annually 48,700 families lose a loved one as a result of antibiotic resistant bacteria infections *(CDCb)*. Depression and grief causes US employers to lose $24 billion USD annually due to lost productive work time *(Okun)*. The Centres for Disease Control (CDC) estimates that antibiotic resistance causes an average loss of $2 billion USD every year. Lastly, Healthcare Associated Infections (HAIs), an antibiotic resistant bacteria obtained from a hospital, can further cause economic losses *(CDCb)*. The majority of HAI cases are a result of antibiotic bacteria causing sepsis or death. According to the CDC, 1 in 31 hospital patients has an HAI. In U.S. hospitals, HAIs have direct medical costs of at least $28.4 billion each year, further perpetuating the socio-economic burden of antibiotic resistant bacteria.

Part III: Bacteriophage - A Solution to Antibiotic Resistance

The bacteriophage or in short, a phage, is a virus which target bacteria *(Clokie, et al)*. Phages have shown potential to aid in solving the worldwide antibiotic resistance crisis *(Viruses (Updated))*. This is due to each phage's ability to evolve, targeting its host cells, bacteria, far more effectively despite bacteria evolving *(Viruses (Updated)*. As phages are obligate parasites towards bacteria, both bacteria and phages are binded in an evolutionary arms race, competing endlessly to gain a survival advantage *(Davidson, et al)*. Phages are ubiquitous microorganisms found in various types of environments *(Clokie, et al)*. Phages are known to be diverse in its size, morphology, and genomic organisation *(Porter)*. Phages consist of having an icosahedral capsid which contains its DNA or RNA genome *(Bacteriophages)*. The capsid of a bacteriophage can be icosahedral, filamentous, or head-tail in structure *(Bacteriophages)*. Each phage's head-tail structure is unique, as a similar structure is not found in other eukaryotic viruses *(Bacteriophages)*. Similarly to other viruses, phages infect a host in order to replicate itself *(Viruses (Updated)*. Phages are species specific towards its hosts and infect a single bacterial species or specific bacteria strains within a species *(Viruses (Updated)*. Phages reproduce either using a lytic lifecycle or lysogenic lifecycle *(Bacteriophages)*. In a lytic reproduction cycle, upon attaching to a susceptible host, a phage introduces its genome into the host's cytoplasm and utilises the host's ribosome to produce copies of the phage genome *(Clokie, et al)*. The host cell then consumes its resources to produce phages, creating viral genomes and capsid proteins which are then assembled into identical copies of the bacteriophage *(Clokie, et al)*. Once enough phages are produced, the phage and its host undergo a process of lysis *(Clokie, et al)*. The bacteriophage damages the host's plasma membrane and cell wall, causing the bacteria to burst *(Clokie, et al)*. On the contrary, a lysogenic reproduction cycle involves each phage's genome being integrated

into the bacterial chromosome *(Clokie, et al)*. Thus, creating identical daughter cells containing the phage's genome after the insertion and duplication of a phage's genome. Phages which use a lysogenic reproduction cycle have the potential to convert to a lytic reproduction cycle as a response to changing environmental conditions *(Clokie, et al)*.

Phages offer a multitude of benefits, from preventing antibiotic resistance bacteria, treating antibiotic resistant bacteria, and having a low environmental impact. Phages differ greatly from antibiotics, as different mechanisms are used to eradicate bacteria *(Loc-Carrillo)*. This allows for phages to treat antibiotic resistant bacteria, as antibiotic resistance does not translate to phage resistance *(Loc-Carrillo)*. Phages lower the risk of antibiotic resistance bacteria as phages target a narrower spectrum of bacteria, either a specific type of bacteria or a strain *(Loc-Carrillo)*. On the contrary, chemical antibiotics which are administered have the potential to eradicate a broad spectrum of bacteria, allowing a wider range of bacteria to be exposed to the antibiotic which in turn develops resistance *(Loc-Carrillo)*. By lowering the risk of developing resistant bacteria, phages can be used in the agricultural sector, specifically livestock. Sewage waste of both animals and humans will be far less likely to perpetuate the issue of antibiotic resistance in the environment, protecting ecosystems *(Polianciuc, et al)*. Lastly, phages are composed of nucleic acids and proteins, a natural component of many environments *(Keen*). There are an estimated 10^{31} phage particles, roughly equivalent to a trillion phages per grain of sand globally *(Keen)*. Phages naturally possess narrow host ranges, refraining from damaging a broad spectrum of vital bacteria in the environment *(Keen*. Phages are not adapted to degradative environmental factors *(Loc-Carrillo)*. This includes factors such as sunlight, desiccation, or temperature extremes *(Loc-Carrillo)*. As phages are sensitive to

degradation environmental factors, inactivation of phages can further prevent environmental harm.

Phages are a promising new avenue to combat bacteria, however the expense to develop this medical innovation will hinder its progress. Worldwide antibiotic innovation funding is shown to be woefully inadequate *(How can)*. To produce an antibiotic it is estimated to cost $1.5 billion USD *(Plackett)*. Meanwhile, industry analysts estimate that the average revenue generated from an antibiotic's sale is roughly $46 million per year *(Plackett)*. Large pharmaceutical companies have halted its production of antibiotic innovations as a result of low profits *(How can)*. As antibiotic resistance prevails, newly developed antibiotics gradually become effective for a narrower spectrum of infections *(How can)*. Thus, fewer doses of certain antibiotics are sold, making the market less profitable *(How can)*. Similarly, phages are shown to have single or double dose potential and targets only a narrow scope of bacteria *(Loc-Carrillo)*. Along with its self-replicating nature in targeting a specific scope of bacteria, it further lowers a pharmaceutical's overall volume of medication sold. Additionally, due to the nature of phages and the diverse types of interactions between phages and bacteria, challenges will arise in creating a large industrial scale production for phage medications *(Rodrigo, et al)*. As phages are a protein based live biological agent, ensuring the safe-usage of phages will be costly as a result of the numerous clinical trials needed. For example, during the manufacturing or usage process of phages, phages can evolve by replicating and interacting with the body's immune system *(Loc-Carrillo)*. Phages which lyse bacteria can cause a release of bacterial toxins, or during the process of replication and evolution, phages can negatively infect body tissues *(Loc-Carrillo)*. Thus, prior to patient use, phages need to have thorough clinical trials which can be costly. With

limited funding for antibiotic innovations, low profits gained from selling antibiotics, the narrow spectrum of bacteria phages target and the complexity of developing phages, pharmaceutical companies instead gain a larger profit over life-long medications such as cholesterol medications, antidepressants and anti-anxiety medications *(How can)*. As a result of profits, development of new antibiotic innovations have slowed.

Conclusion

With the prevailing issue of antibiotic resistance threatening the effectiveness of antibiotics globally, action must be taken immediately. Annually, over 2.8 million antibiotic resistant infections occur in the United States solely, resulting in over 35,000 deaths *(Be Antibiotics)*. An estimated 47 million unnecessary prescriptions of antibiotics are issued annually, further perpetuating the overexposure of bacteria to antibiotics which results in the greater spread of bacterial resistance *(CDCa)*. According to the United Nations, the rise in antibiotic resistant bacteria is a global health emergency which will result in 10 million deaths by 2050 *(CDCa)*.

Environmentally, antibiotic resistance further paints a bleak picture. Ecosystems contaminated with antibiotics can result in detrimental biodiversity consequences, causing certain microorganisms to perish from stronger, antibiotic resistant bacterial disease *(World's rivers')*. The environment, such as contaminated waterways, can become vectors for disease, passing on antibiotic resistant strains to human populations *(Gallagher)*. Additionally, livestock can become vectors for disease as two-thirds of global antibiotic production is for livestock *(Polianciuc, et al)*. Thus, antibiotic resistant bacteria can thrive in macroorganisms, passing from

commercially farmed livestock to urban areas, or infecting the environment through livestock excretions *(Polianciuc, et al)*.

From an economic standpoint, hospitalisation treatments will become more expensive for those patients infected with antibiotic resistant bacteria. Healthcare spending for patients with resistant bacterial infections is 165% higher than for patients with non-resistant infections *(CDCb)*. With COVID, the average US hospital expense is $12,530 USD and will increase dramatically as the antibiotic resistant crisis worsens *(NHE Fact)*. Lastly, the CDC estimates that antibiotic resistance causes an average loss of $2 billion USD annually *(CDCa)*.

Bacteriophages are a potential solution to the antibiotic resistance crisis as it is a virus which targets bacterium *(Clokie, et al)*. As bacteria evolves, phages also have the ability to evolve, targeting resistant bacteria effectively due to the theory of natural selection and evolution *(Viruses (Updated)*. Phages are composed of nucleic acids and proteins, a natural component of many environments *(Keen)*. Additionally, phages target a narrow spectrum or strain of bacteria, thus phage exposure to the environment will not cause detrimental ecosystem harm, as a limited strain of bacteria will be affected and widespread bacterial resistance will not be developed *(Loc-Carrillo)*. In turn, the environment will not become a vector for infectious resistant bacteria. From an economics standpoint, phage testing and development will require large amounts of funding. Economically, with limited funding for antibiotic innovations, low profits gained from selling antibiotics and the nature of new antibiotics targeting only a narrow spectrum of bacteria, it is unprofitable for pharmaceutical companies to invest in new antibiotic innovations such as phage testing. Phage testing will be intensive and costly as a result of the narrow spectrum of

bacteria each phage targets and the complexity of developing phages, a live biological agent *(How can)*.

Overall, phages have the potential to solve antibiotic resistance, a world health crisis predicted to kill millions. Additionally, phages have shown to have limited impacts on the environment as opposed to antibiotics. To solve the economic burden of funding phage innovations, governments can shift pharmaceutical profit schemes away from the volume of antibiotic or phages sold and use various nationwide programs to incentivise ways for antibiotic innovation development *(How can)*. However, such political programmes are still in the early stages and need further research. Thus, as a result of all the evidence collected in this literature review, innovation in phage technology has proven to be a plausible solution for antibiotic resistance.

Bibliography ***Primary Sources***

"Amount of Medical Waste From Hospitals and Clinics in Past Year Compared With Five Years Ago and Measures to Reduce and Manage Such Waste." *Ministry of Health*, Government of Singapore, 10 May 2021, www.moh.gov.sg/news-highlights/details/amount-of-medical-waste-from-hospitals-and-clinics-in-past-year-compared-with-five-years-ago-and-measures-to-reduce-and-manage-such-waste. Accessed 14 Apr. 2022.

Antibiotic Resistance." *BrainPOP*, www.brainpop.com/health/diseasesinjuriesandconditions/antibioticresistance/. Accessed 14 Apr. 2022.

"Antibiotics, Explained." *NPS MedicineWise*, www.nps.org.au/consumers/antibiotics-explained#:~:text=1%2F7-,How%20do%20antibiotics%20work%3F,against%20different%20types%20of%20bacteria. Accessed 13 Apr. 2022.

Arnaud, Celia Henry. "Penicillin." Chemical and Engineering News, 20 June 2005,cen.acs.org/articles/83/i25/Penicillin.html. Accessed 9 Apr. 2022.

"Bacteria (Updated)." *Amoeba Sisters*,YouTube, 31 Jan. 2019, youtu.be/ORB866QSGv8. Accessed 13 Apr. 2022.

"Bacteriophages." *Khan Academy*, www.khanacademy.org/science/biology/biology-of-viruses/virus-biology/a/bacteriophages . Accessed 18 Apr. 2022.

"Be Antibiotics Aware: Smart Use, Best Care." *CDC*, U.S. Department of Health and Human Services,

www.cdc.gov/patientsafety/features/be-antibiotics-aware.html#:~:text=We%20rely%20on%20antibiotics%20to,high%20risk%20for%20developing%20infections. Accessed 18 Apr. 2022.

Bielen, Ana, et al . "Negative environmental impacts of antibiotic-contaminated effluents from pharmaceutical industries," *Water Research*, Volume 126, 2017, Pages 79-87, ISSN 0043-1354. DOI: 10.1016/j.watres.2017.09.019. Accessed 14 Apr 2022.

CDCa. Antibiotic Use in the United States, 2017: Progress and Opportunities. Atlanta, GA:US Department of Health and Human Services, CDC; 2017. Accessed 15 Apr 2022.

CDCb. Antibiotic Resistance Threats in the United States, 2019. Atlanta, GA: U.S. Department of Health and Human Services, CDC; 2019

Clokie, Martha Rj et al. "Phages in nature." *Bacteriophage* vol. 1,1 (2011): 31-45. doi:10.4161/bact.1.1.14942

Crash Course. "Immune System, Part 1: Crash Course A&P #45." *YouTube*, 8 Dec.2015, youtu.be/GIJK3dwCWCw Accessed 12 Apr. 2022.

Crash Course "Immune System, Part 2: Crash Course A&P #46." *YouTube*, 14 Dec. 2015, youtu.be/2DFN4IBZ3rI. Accessed 12 Apr. 2022.

"The dangers of mixing drugs - Céline Valéry." TedEd. YouTube, 12 Nov. 2019. Accessed 9 Apr. 2022.

Davidson, P.M., et al. "The Use of Natural Antimicrobials in Food: An Overview." *Elsevier*, 2015, pp. 1-27. *Elsevier*, https://doi.org/10.1016/B978-1-78242-034-7.00001-3. Accessed 18 Apr. 2022.

Gallagher, James. "Infections resist 'last antibiotic' in US." BBC, 27 Apr. 2018, www.bbc.com/news/health-36396663. Accessed 14 Apr. 2022

Goering, Richard V. "Heterotroph." *Elsevier*, new series. *Elsevier*, www.sciencedirect.com/topics/immunology-and-microbiology/heterotroph. Accessed 13 Apr. 2022.

"How can we solve the antibiotic resistance crisis? - Gerry Wright" TedED, *YouTube* , 6 Mar. 2020, https://youtu.be/ZvhFeGEDFC8. Accessed Apr 18 2022.

Jacobs, Andrew. "Crisis Looms in Antibiotics as Drug Makers Go Bankrupt." *The New York Times*, 25 Dec. 2019, www.nytimes.com/2019/12/25/health/antibiotics-new-resistance.html. Accessed 18 Apr. 2022.

Keen, Eric C. "A century of phage research: bacteriophages and the shaping of modern biology." *BioEssays : news and reviews in molecular, cellular and developmental biology* vol. 37,1 (2015): 6-9. doi:10.1002/bies.201400152

Köpke, Michael, et al. "Clostridium Difficile Is an Autotrophic Bacterial Pathogen." *National Library of Medicine*. *National Library of Medicine*, https://doi.org/10.1371/journal.pone.0062157. Accessed 13 Apr. 2022.

Lehman, Rebecca. "Antibiotics: Helping our bodies, hurting our environment." Yale Environment Review, 11 Apr. 2018, https://environment-review.yale.edu/antibiotics-helping-our-bodies-hurting-our-environment#:~:text=However%2C%20once%20they%20are%20excreted,potential%20to%20upend%20fragile%20ecosystems. Accessed Apr 14 2022.

Loc-Carrillo, Catherine, and Stephen T Abedon. "Pros and cons of phage therapy." *Bacteriophage* vol. 1,2 (2011): 111-114. doi:10.4161/bact.1.2.14590

Nair, Divek V. T., et al. "Antibiotic-Resistant Salmonella in the Food Supply and the Potential Role of Antibiotic Alternatives for Control." *National Library of Medicine*, https://doi.org/10.3390/foods7100167. Accessed 14 Apr. 2022.

National Geographic Society. "Natural Selection." *Society*, National Geographic, www.nationalgeographic.org/encyclopedia/natural-selection/. Accessed 14 Apr. 2022.

"NHE Fact Sheet." *U.S. Centers for Medicare and Medicaid (CMDS)*, US Federal Government, www.cms.gov/Research-Statistics-Data-and-Systems/Statistics-Trends-and-Reports/NationalHealthExpendData/NHE-Fact-Sheet. Accessed 15 Apr. 2022.

O'Callaghan, Amy, and Douwe Van Sinderen. "Bifidobacteria and Their Role as Members of the Human Gut Microbiota." *National Library of Medicine*, https://doi.org/10.3389/fmicb.2016.00925. Accessed 13 Apr. 2022.

Okun, Barbara. "Can Grief Morph into Depression?" Edited by Joseph Nowinski. *Havard Health Publishing*, Havard Medical School, www.health.harvard.edu/blog/can-grief-morph-into-depression-201203214511. Accessed 15 Apr. 2022.

Perin, Luana, et al. "The Microbiology of Raw Milk." *Chapter 3*, pp. 45-64. *Elsevier*, https://doi.org/10.1016/B978-0-12-810530-6.00003-1. Accessed 13 Apr. 2022. Excerpt originally published in *Elsevier*.

Plackett, Benjamin. "Why Big Pharma Has Abandoned Antibiotics." *Nature*, 21 Oct. 2020, www.nature.com/articles/d41586-020-02884-3#:~:text=A%202017%20estimate%20puts%20the,roughly%20%2446%20million%20per%20year. Accessed 18 Apr. 2022.

Polianciuc, Svetlana Iuliana, et al. "Antibiotics in the Environment: Causes and Consequences." *National Library of Medicine*. *National Library of Medicine*, https://doi.org/10.15386/mpr-1742. Accessed 14 Apr. 2022.

Porter, La Donna, and Laura M. Kasman. "Bacteriophages." *National Library of Medicine*. *StatPearls Publishing*, www.ncbi.nlm.nih.gov/books/NBK493185/. Accessed 18 Apr. 2022.

"Proteolytic Bacteria." *Volume 4*. *Elsevier*, www.sciencedirect.com/topics/agricultural-and-biological-sciences/proteolytic-bacteria. Excerpt originally published as "Encyclopedia of Dairy Sciences" in *Elsevier*, vol. 4, 2022.

Rodrigo, García, et al. "Bacteriophage Production Models: An Overview." *Frontiers in Microbiology*, vol. 10, 2019. *Frontiers in Microbiology*, https://doi.org/10.3389/fmicb.2019.01187. Accessed 18 Apr. 2022.

"Salmonella." *CDC*, U.S. Department of Health and Human Services, www.cdc.gov/salmonella/index.html#:~:text=CDC%20estimates%20Salmonella%20bacteria%20cause,%2C%20fever%2C%20and%20stomach%20cramps. Accessed 18 Apr. 2022.

"The Value of Antibiotics in Treating Infectious Diseases." *Pfizer*, www.pfizer.com/news/featured_stories/featured_stories_detail/the_value_of_antibiotics_in_treating_infectious_diseases.

"Viruses (Updated)." Amoeba Sisters,*YouTube*, 23 May, 2018. youtu.be/ORB866QSGv8. Accessed 18 Apr. 2022.

"What causes antibiotic resistance? - Kevin Wu." TedEd. *YouTube*, 7 Aug. 2014, youtu.be/znnp-Ivj2ek. Accessed 14 Apr. 2022.

Whelpton, Leigh. "Untreated wastewater kills coral." Yale Environment Review, 29 Apr. 2012, environment-review.yale.edu/untreated-wastewater-kills-coral-0. Accessed 10 Apr. 2022

World Health Organisation. "Lack of New Antibiotics Threatens Global Efforts to Contain Drug-Resistant Infections." *World Health Organisation*, 17 Jan. 2020, www.who.int/news/item/17-01-2020-lack-of-new-antibiotics-threatens-global-efforts-to-contain-drug-resistant-infections. Accessed 18 Apr. 2022.

"World's rivers' awash with dangerous levels of antibiotics.'" Guardian, www.theguardian.com/society/2019/may/27/worlds-rivers-awash-with-dangerous-levels-of-antibiotics. Accessed 10 Apr. 2022