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A Literature Review and Survey of Bacteriophage Therapy against Multidrug Resistant Organisms and the Perceived Barriers to Clinical Research

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Abstract

The emergence of antimicrobial resistance presents a global health concern. These infections can result in adverse or fatal health outcomes. Due to this phenomenon, the scientific community must consider effective alternative or supplemental treatments such as bacteriophage therapy. Bacteriophage therapy has been in existence for at least a century and has shown to alleviate multidrug resistant infections in multiple clinical trials in the former Soviet Union. However, at the present time, there are limited large-scale clinical evidence performed on patients *in vivo*, except for a few cases in the United States and Europe.

The research question for this capstone project is "What are the perceived barriers to clinical research for bacteriophage therapy?" In this capstone paper, we explore the perceived barriers to clinical research through the evaluation of laboratory articles, human compassionate case studies and phage expert surveys. Both recent non-human *in vitro* and *in vivo* animal laboratory articles (n=10, post-2010) and human clinical articles (n=10, post-2009) present perceived barriers as further lab testing needed and large-scale clinical trials needed, respectively. The online survey from 29 phage experts state that perceived barriers consist of lack of funding and willing laboratories to perform clinical research, the scientific unknowns of bacteriophage therapy (i.e. side effects, bacterial resistance development, long term effects post-treatment and genes with unknown functions), the pharmaceutical lobby and the lack of rigorous clinical trials data outlining pharmacology, safety and efficacy. These perceived barriers influence the current state of bacteriophage therapy and explain the slow and cautious progression of the field. Bacteriophage therapy potentially serves as a promising treatment to avert further global morbidity and mortality. The acknowledgement and eventual removal of these perceived barriers would help to make it more accessible in medicine.

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CHAPTER 1

INTRODUCTION

The existence of multidrug resistant organisms (MDROs) are an increasing concern in the field of medicine and global health. MDRO-infected individuals are unable to use standard antibiotics because they are rendered ineffective against such infections and lead to deteriorating health outcomes, including death. This is a relevant topic to global health because for certain people who contract MDRO worldwide, prognosis is dire. Bacteriophage therapy may serve as a potential treatment option. However, it is not a regular practice in most countries (Furfaro, Payne & Chang, 2018). Currently, the greatest urgency regarding multidrug resistance is among clinically significant ESKAPE organisms. This is an acronym that refers collectively to the genus *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter species.* This capstone project hopes to understand why the field has not gained faster momentum in the age of antimicrobial resistance (AMR) which are gradually becoming more common worldwide and requires useful interventions. The thesis statement is there are perceived barriers to clinical research for bacteriophage therapy. The goal is to prove or disprove this statement. The research question is "What are the perceived barriers to clinical research for bacteriophage therapy?" The goal is to answer the question and find a relationship between the barriers and the current progression of the field. It is important because the answers may explain why the therapy is not being administered urgently enough to cover the need for antibiotic supplements and alternatives. These goals will be achieved by performing a qualitative assessment of relevant sources. These sources will include laboratory studies, human case studies, and field expert responses derived from an online survey.

THE FRAMEWORK: PROBLEM, POLITICS AND POLICIES

Antimicrobial resistance (AMR) is one of the major threats to global health, animal health and environmental ecosystems. In this capstone paper, we discuss the possibility of multidrug resistant treatment with bacteriophage but first, it is important to understand how certain aspects of the globalized world contributes to AMR. In this chapter, we identify universal drivers of resistance using the multiple streams framework (MSF) or the Kingdon model (Kingdon, 1984) for how specific activities of globalization has contributed to the development of AMR. The Kingdon model states there are three categories of the policy making process: problem, politics and policy (Béland & Howlett, 2016). The three streams are interconnected and provide an opportunity for problem solving.

For AMR, the Kingdon problem has two parts: 1) the overarching theme of overuse or misuse of antibiotics in medicine, pets and livestock and 2) the ease of availability of antibiotics. The first part involves clinical misuse - when antibiotics are given, and they are not clinically justified. Antibiotics are designed for bacterial infections not viral ones. According to the article *Global Contributors of Antibiotic Resistance*, a study in Egypt showed that 81% of pharmacists prescribe antibiotics for colds (Chokshi, Sifri, Cennimo & Horng, 2019). The second part of the problem is the ease of accessibility. This creates an instant gratification culture and complacency. At any sign of discomfort or presumed bacterial infection, consumers think it is the correct course of action. The article goes on to say that in Asia, Africa and Latin America, antibiotics are readily available from local pharmacies, hospitals, drugstores, roadside stalls and hawkers without a prescription (Chokshi et al., 2019). These problem streams propagate the global emergence of MDR organisms. The Kingdon politics involve the efforts by the people to campaign for change, the national political "mood" and the different elements that affect policy, regulation, and legislation (Béland & Howlett, 2016). The political players of AMR are a whole network of people: patients

(consumers), healthcare professionals, pharmaceutical industry, academic researchers, lawmakers, healthcare authorities, and politicians. They all influence politics which can lead to policy making regarding antibiotics and bacteriophage therapy. From the demand side, there needs to be a culture shift and education about the proper use of antibiotics. From the supply side, pharmacies and health systems are businesses that also care about making profits, their stakeholders and drug company kickbacks but must control administration and distribution (Chokshi et al., 2019). Most of the responsibility falls on those who distribute antibiotics, which desperately need proper policies and regular auditing. AMR has become a geopolitical issue where international efforts are being made by international health agencies such as the World Health Organization (WHO) and many non-governmental organizations (NGOs) to form alliances and coalitions to address it. According to the article *Antibiotic Resistance: a geopolitical issue*, the author says that global interventions such as minimizing the use of antibiotics, increasing vaccination and good infection control will reduce the circulation of antibiotic-resistant bacteria (Carlet, Pulcini & Piddock, 2015). However, it goes on to say that currently there is a lack of political vision and will to implement these ideas.

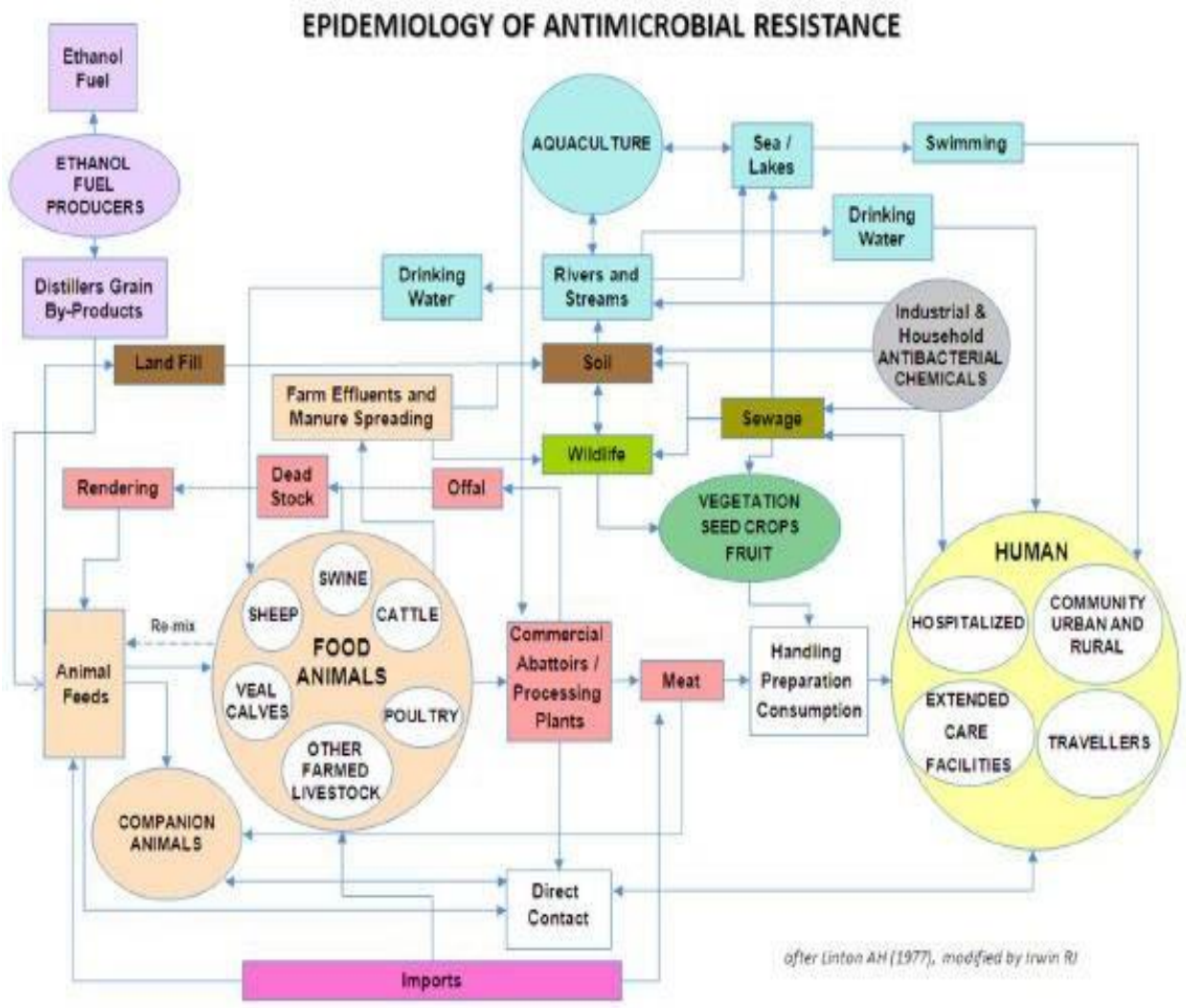
Political intervention is essential to address this issue and to develop an adequate regulatory framework for bacteriophage therapy. Thus, moral reasoning may be necessary to justify the need for a feasible regulatory framework and force politicians and regulators to take action for their responsibility to guarantee the accessibility of phage therapy to patients who need it. Politically speaking, the safety and efficacy of bacteriophage therapy has yet to be fully presented by large-scale controlled clinical trials (Furfaro et al., 2018). Moreover, the therapy is still generally considered as a last resort, not as an effectual and feasible means. Thus, we have not arrived at an appropriate political climate for policy making (Furfaro et al., 2018). However, this can be associated with normative ethical considerations of bacteriophage therapy, which is the fact that

the therapy has the potential to save thousands of people with MDR infections. The application of the therapy can also be flexible and sustainable, such as patient-specific-made (tailor-made) and local development of bacteriophages (Verbeken et al., 2014). The state of politics in a given location dictates the quality of the policies that follow. Bacteriophage therapy can temper the complex global problem of AMR, but it requires collaboration between countries, political leadership and tremendous will to overcome.

The Kingdon policies involve the lack of regulations behind antibiotic prescription and distribution. It is common for people to self-diagnose and obtain antibiotics without a prescription (Merrett et al, 2016). People believe in the effectiveness of antibiotics - much like a placebo effect- but unfortunately have little to no knowledge about drugs and infections. The ease and availability of antibiotics without a prescription in many countries is a significant enabling factor which stems from the lack of prescription-only regulation, lack of law enforcement and other aspects such as poverty, culture and norms (Merrett, Bloom, Wilkinson & MacGregor, 2016). When the arbitrary use of antibiotics occurs frequently and routinely, then it becomes a cultural norm. This pattern must be broken through education and practicing good antibiotic stewardship where the goal is appropriate usage and the re-establishment of social and institutional norms (Merrett et al, 2016). Policies should be stricter and better structured. Policies such as by prescription-only or, even better, prescribing antibiotics when an appropriate diagnostic lab test confirms a bacterial infection. The former is a good start, but the latter is more stringent and evidentiary. Antibiotic medications are still an indispensable and effective form of chemotherapy today, but these events have had the unintended consequences of antimicrobial resistance. The AMR crisis and its corresponding drivers, many of which are deep and systemic, truly go beyond the scope of this capstone paper. (Please refer to Figure 2.1 which shows the complexity of antimicrobial

resistance.)

Regarding regulations and policies of bacteriophage therapy, considering the potential of the therapy, rigorous regulations and policies are required to vitalize the application of phage therapy (Fauconnier, 2019). Initial policy framework may refer institutes in Eastern European countries such as Georgia where phage therapy has been used in the past century. At a global level, the involvement of the WHO on the development of phage therapy is essential to foster official public standards (Fauconnier, 2019). Credible normative standards and frameworks will guide the development of suitable regulatory approaches in each country, especially for lower income developing countries where phage therapy is urgently needed (Fauconnier, 2019). These collective mechanisms of problem, politics and policy have contributed to the current phenomenon of antimicrobial resistance and is equally applicable to bacteriophage therapy. These streams need to be recognized together as an open window of policy making which leads to a formalized political framework, regulations, and policies.



Complexity of Antimicrobial Resistance (AMR) (Retrieved November 15, 2019 from http:// tdvglobal.com/en/about-us/news/ development-of-canadian- roadmap- for-amu-surveillance-in-food-animal- production)

Figure 1.1 The complexity of AMR

BACKGROUND

Before the development of antibiotics, people suffered from and succumbed to infectious diseases. The invention of antimicrobials in the 20th century was considered a medical revolution that grossly reduced fatal cases. This time frame was labelled the "antibiotic era" and it was one of the most successful forms of chemotherapy in the history of medicine (Aminov, 2010). Unfortunately, because of the overuse of antibiotic drugs, the limited amount of new drug development, the lack of prescription regulations in certain countries and other evolutionary mechanisms, drug resistance has emerged onto the medical and public health landscapes. According to one article, more than 25,000 people die from an MDR bacterial infection in the EU, and more than 63,000 deaths from nosocomial infections in the United States (Aminov, 2010). It is for this reason that new therapeutics must be considered and tried to avoid future mass affliction and losses. Antibiotics are still beneficial today and play a vital role in clinical medicine and therefore, contributes overall to the positive welfare of the general public. However, there is no denying that AMR is growing as a result of both human antibiotic exploitation and natural biological evolution. Every time antibiotics are used, it inadvertently creates a selective pressure for bacteria to mutate or exchange pieces of DNA resulting in drug resistance (Ventola, 2015). It is in the concept that bacteriophage therapy becomes a new testing platform for use in the cases of MDR infections and should be put into the treatment pipeline.

Bacteriophages are the most naturally abundant and ubiquitous organisms in the world. For every bacterium, there exists many more bacteriophages (Clokie, Millard, Letarov & Heaphy, 2011). They reside in water, soil, sewage and other natural ecosystems (Clokie et al., 2011). In order to discuss bacteriophage and its effects on multi-drug resistant bacteria, it is important to understand the traits and nature of bacteriophages. Bacteriophages are viruses, often known as

phages, which prey on bacteria for their own survival. The word is derived from the meaning "bacteria-eater" since they parasitize host cells. Bacteriophages are composed of a nucleic acid molecule housed by a protein structure. A bacteriophage attaches to its target bacterium and infects it. The bacteriophage uses the bacterial cellular material for reproduction and proliferation (Clokie et al., 2011). The discovery of bacteriophage and its potential uses arose roughly at the same time as antibiotics (D. Lin, Koskella & C. Lin, 2017). However, bacteriophage treatment was a foreign and controversial topic, so antibiotics became the favored treatment of choice because it was easier to manufacture as opposed to testing and finding the bacteriophages of interest (Wittebole, De Roock & Opal, 2014). According to *Bacteriophages: A Therapy Concept against Multi-Drug Resistant Bacteria*, there are several advantages to using phage as an alternative or antibiotic-supplement therapy because they are 1) bacteria specific, 2) non-toxic and 3) self-limiting (Rhode, Wittmann & Kutter, 2018). Furthermore, the use of antibiotics has given rise to several problems. First, while some antibiotics are blatantly bactericidal (kills), others can be described as bacteriostatic which means they inhibit bacterial growth but leave room for potential persistence. Second, depending on the antibiotic drug, they can have toxic side effects such as ototoxicity and nephrotoxicity. Lastly, antibiotic administration interferes or alters the human microbiome, which is largely responsible for immunological functions, disrupting natural homeostasis and exacerbating the risk of internal opportunistic infections such as *Clostridium difficile* (Saha & Mukherjee, 2019). Other considerations include bacteriophage phenotype, mode of infection and administration, host specificity and the degree of host range also play a role in the selection of therapeutic mediation (Saha & Mukherjee, 2019). Combine the unique advantages of bacteriophage with the disadvantages of antibiotics and there is a valid argument for why bacteriophage should enter clinical trials to test for efficacy.

JUSTIFICATION

Multidrug resistant organisms are bacteria and other microorganisms that have developed resistance to antimicrobial medication through antibiotic-mediated pressure and other biological mechanisms (Ventola, 2015). Internationally, there is a growing concern over antimicrobial resistance (AMR), a term used often in the literature, which is currently estimated to account for more than 700,000 deaths per year worldwide (Tadesse et al., 2017). AMR concerns every continent and every country regardless of wealth and development status as resistant pathogens do not respect borders (Tadesse et al., 2017). Globalization from travel, trade and migration have allowed resistant bacteria to travel faster than ever before. In the 2013 *Global Risks Report of the World Economic Forum*, it warned about the growing risks associated with antibiotic complacency towards AMR. It discussed two main reasons for the phenomena. The first reason is the overuse and misuse of antibiotics in both medicine and livestock management and the second reason is the fact that new classes of antibiotics are not being invented fast enough. According to the Centers for Disease Control, in the United States 2 million people get an antibiotic resistant infection annually, and at least 23,000 people die (CDC, 2018). The Alliance for the Prudent Use of Antibiotics (APUA) estimated that the costs for the U.S. range from $24 billion to $38 billion per year (Mammina, 2013). In Europe, it is estimated that 33,000 people die of MDRO infections and that it costs 900 million euros each year (European Centre for Disease Prevention and Control). On the continent of Africa, AMR data was not available for 42.6% of the countries making a gap in surveillance problematic (Tadesse et al., 2017). According to a recent review of AMR data in Africa which fixated on sub-Saharan Africa, the authors found a high level of resistance to the commonly used antibiotics in the sub-Saharan African regions such as 90% of gram negatives were resistant to chloramphenicol, a commonly used antibiotic. In contrast, resistance to third-generation cephalosporins, such as ceftriaxone, was less common, recommending this group for

use (Leopold, Leth, Tarekegn & Schultsz, 2014). Furthermore, because many countries do not possess effective surveillance technology, there is a good chance that the international numbers for MDR are actually higher (Please refer to the AMR world maps courtesy of World Economic Forum in Figure 1.2). South America is facing similar circumstances as North America and Europe (Casellas, 2011). However, the risk in Asia and Africa is much higher than other continents (Piotrowski, 2015). Multidrug resistance is also ever-present in the region of southeast Asia, most especially, India. India is one of the world's largest consumers of antibiotics and has the highest levels of AMR. In a 2016 study it found that resistant infections kill 60,000 babies (sepsis) in India every year (Davies, 2018). Greater than 80% of some types of bacteria causing sepsis in babies were multidrug resistant, immune to nearly all antibiotics (Davies, 2018). There are many reasons for this such as a high burden of disease, poor public health infrastructure, rising incomes, and unregulated sales of cheap antibiotics, have intensified AMR (Das, Chaudhuri, Srivastava, Nair & Ramamurthy, 2017). Clearly, multidrug resistance is a rampant and complex global health dilemma. AMR is currently on the rise and if there are no new effective interventions then it could lead to 10 million deaths globally and $100 trillion (USD) by the year 2050 (de Kraker, Stewardson & Harbarth, 2016). We live in a time where microscopic pathogens are evolving to elude previously effective treatments. Research personnel of diverse disciplines must work together to develop new and innovative intervention strategies. It is an interesting time to be amidst this kind of obstacle, but it also requires ingenuity and urgency. Multidrug resistance poses a critical threat to our world and bacteriophage therapy may be a contender in the fight against AMR.

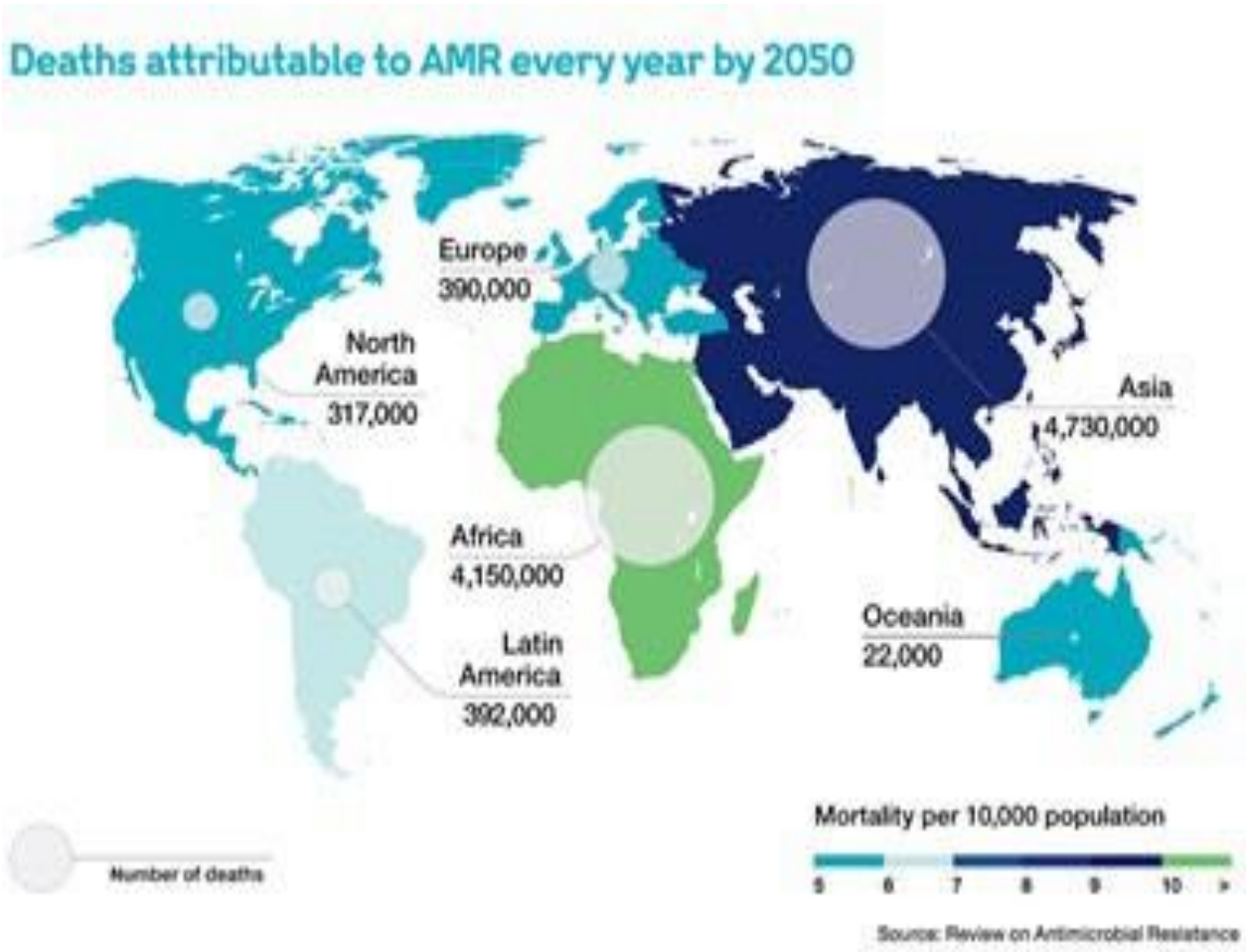


Figure 1.2 Deaths Attributable by AMR map credit: Public Health Post website

CHAPTER 2

LITERATURE REVIEW ON LABORATORY AND CLINICAL ARTICLES

Background

The emergence of antibiotic-resistant bacteria due to indiscriminate use of antibiotics has led to the need for solutions. The persistency of antimicrobial resistance has compelled the scientific community to take action by conducting bacteriophage testing as it pertains to resolving multidrug resistant organisms. Due to the unfamiliar nature of bacteriophage as a means of healing MDR infections, it is only natural for research scientists to perform non-human *in vitro* and *in vivo* animal laboratory testing prior to the consideration and application of bacteriophage medicine on human patients.

There are advantages and disadvantages to laboratory testing. An advantage is that laboratory experiments take a theoretical concept, such as bacteriophage therapy, and attempts to prove or disprove it under controlled conditions. It also provides a practical simulation setting and reveals unanticipated problems which prompt researchers to develop solutions before clinical trials are conducted. A potential disadvantage is that lab results may not necessarily translate as working the same way in more complex animal models, let alone human subjects. Laboratory studies are designed to understand the many biological mechanisms involved, the behaviors between a target MDRO and their respective bacteriophage counterpart and the nature of bacteriophages as microscopic mercenaries.

Laboratory study is essential because it serves as the foundational step to initiating human clinical trials. Since the threat of these bacteria is clinically certain, raising severity is predicted in the foreseeable future. An effective and practical treatment strategy to this inevitable threat was explored because failure of current standard antibiotic treatment against MDR infections.

Re-attention in revitalizing bacteriophage therapy has brought interest to be clinically tested. Currently, clinical application of bacteriophage therapy is often provided as a compassionate treatment, also known as compassionate phage therapy (cPT), which may be conducted as the last treatment option for patients who have failure to antibiotic treatments and no suitable clinical trials or conventional treatments. In response to the increasing demands of cPT, 25 clinical trials have been conducted since 2000 and more than half of the trials were made in the past two years, 2017 to 2019 (McCallin, Sacher, Zheng & Chan, 2019). Especially, ESKAPE pathogens, that are a typical group of multidrug resistant organisms, mainly reported in human clinical trials. In such a situation, there is no reason not to study and invigorate clinical use of bacteriophages as an alternative or adjuvant treatment against MDROs. In this section, we evaluate scientific laboratory articles and human clinical articles of bacteriophage therapy against various MDR infections caused by ESKAPE organisms to demonstrate current state of bacteriophage therapy.

Methods

Literature searches for laboratory and human clinical studies were conducted on the PubMed website. For laboratory studies, inclusion criteria comprised of key words such as multidrug resistant ESKAPE organisms, bacteriophage therapy and *in vitro* studies. It was also decided that all articles selected must be published after the year 2010 to ensure updated material. Exclusion criteria consisted of specific kinds of multidrug resistant mechanisms (i.e. extended beta lactamases or carbapenemase producers), non-ESKAPE organisms, fungal and parasitic infections, bacteriophage combined with antibiotics as a synergistic therapy and articles published before 2010.

For human articles, same inclusion and exclusion criteria were shared, except the search period that targeted articles published after the year 2009. The literature search was both traditional

and non-traditional. For starters, we were given 2 human case study articles via email from Dr. Stephanie Strathdee of UCSD. A literature search for human clinical trials was conducted through PubMed search engine for the rest of 8 human case study articles, and several were retrieved and selected from the reference section of the scientific PubMed articles *Systematic and Critical Review of Bacteriophage Therapy Against Multidrug-resistant ESKAPE Organisms in Humans* (El Haddad, Harb, Gebara, Stibich, & Chemly, 2018), *Bacteriophage Therapy: Clinical Trials and Regulatory Hurdles* (Furfaro et al., 2018), and *Current State of Compassionate Phage Therapy* (McCallin et al., 2019). All articles were selected in the range of 10 years, since 2009, to review the most updated reports. The articles are hand-selected and used to perform a thematic analysis using case study characteristics, methods, phage effectiveness, and perceived barriers to research.

Results

Laboratory Articles

A total of 10 laboratory appropriate articles on the PubMed website using our inclusion criteria. From ten articles, there were a variety of multidrug resistant pathogens represented along with their correlating bacteriophage or "cocktail" (mixture of bacteriophages). Altogether, there were 8 articles that featured a single bacterium of interest. One article tested against *Staphylococcus aureus (Methicillin resistant Staphylococcus aureus and Vancomycin Intermediate Staphylococcus aureus)*, two articles tested against *Klebsiella pneumoniae*, two articles discussed *Pseudomonas aeruginosa,* two articles were about *Acinetobacter baumannii* and one article for *Enterococcus faecalis* and *Enterococcus faecium*. The other two articles researched on multiple MDROs. One of them featured *Escherichia coli*, *Klebsiella pneumoniae* and *Enterobacter cloacae* and the other article presented information on *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Escherichia coli*. Each bacterial organism is

paired against their respective bacteriophage counterparts. Please refer to *Figure 2.1* for the breakdown of bacteriophage match-ups. For the methods, 5 articles were *in vitro* tests only, 2 articles were performed on mice only, 1 article did their study on *Galleria mellonella* (wax moth) larvae only and 2 articles were done on both *in vitro* and mouse models. In terms of bacteriophage effectiveness, each article demonstrated bacteriophage effectiveness. However, the article *Activity of Bacteriophages in Removing Biofilms of Pseudomonas aeruginosa Isolates from Chronic Rhinosinusitis Patients* said that the phage cocktail for *Pseudomonas aeruginosa* was 76% reduced after 48 hours (Fong et al., 2017). For this scenario, it is difficult to assume whether it would have reached 100% after a longer time frame. Furthermore, 2 articles address bacteriophages' ability to break down biofilms, mucus-like substances formed by bacteria used as a protective refuge, found in some persistent infections (Fong et.al, 2017). Regarding perceived barriers to research, 4 articles did not mention perceived barriers to clinical research, 1 said that phage resistance must be better understood and studied and 5 said more laboratory tests must be conducted before moving on to clinical experiments. Of the five articles that said further evaluation should take place, two articles said that more testing should be done on more complex mammalian models before proceeding onto human research. Also, the article *A Potential Treatment for Klebsiella pneumoniae Isolated from Diabetic Foot Patients*, mentioned the importance of ensuring bacteriophage cannot transfer resistance mechanisms to resident microbiota (Taha, L. Connerton, F. Connerton & El Shibiny, 2018). These laboratory studies show that testing is being performed for the purpose of multidrug resistant treatment and understanding.

Human Clinical Articles

A total of 10 articles were selected from 28 publicly available human clinical trial articles for this literature review. The selected articles represented clinical reports of various phage therapy

applications on ESKAPE organisms, reported from 2009 to 2019.

Enterococcus faecium, three patients with chronic bacterial prostatitis caused by *E. faecium* received phage therapy due to failure of long-term targeted antibiotic treatments (Letkiewicz, Międzybrodzki, Fortuna, Weber-Dąbrowska & Górski, 2009). Enterococcal lytic bacteriophages were prepared by isolation from 134 samples of sewage or drinking water samples. Prior to application of phage on patient, phage-lytic activity was examined by testing prepared bacteriophage on the bacterial strains isolated from each patient. The bacteriophages were administered rectally. The treatments were successful and *E. faecium* was eradicated for all three patients after phage therapy, and lack of disease recurrence showed (Letkiewicz et al., 2009).

Staphylococcus aureus, the series of compassionate phage therapy were provided for nine patients who have diabetic foot ulcer caused, and the patients' bone and soft tissue were infected by *S. aureus* (Fish, Kutter, Wheat, Blasdel, Kutateladze, & Kuhl, 2016). All antibiotic treatments were poorly responded or failed, and the only other acceptable treatment option was toe amputation. Thereby, commercially available staphylococcal phage Sb-1 was administered topically to the ulcerations once weekly. All patients responded to phage therapy and were able to heal in seven weeks, except the one who had an extreme infection and required 18 weeks of treatment. This application of phage therapy was effective and successful without complication (Fish et al., 2016). A 65-year-old female with nosocomial left-eye corneal abscess and interstitial keratitis infected by *S. aureus* received phage therapy at Phage Therapy Center in Georgia (Fadlallah, Chelala & Legeais. 2015). The commercially available bacteriophages SATA- 8505 was administered topically and intravenously for 4 weeks. The treatment was successful, and the eradication of S. *aureus* was confirmed with examination of her ocular signs and laboratory cultures after three and six months of treatment (Fadlallah et al., 2015).

Klebsiella pneumoniae, clinical application of phage therapy was reported in Russia that targeted *K. pneumoniae*. The outbreak of nosocomial infections of pneumonia caused by *K. pneumoniae* in the neonatal intensive unit resulted in 15 infected newborns and antibiotic measures were not successful to control the infections (Aslanov, Lubimova, Dolgiy & Pshenichnaya, 2018). The commercially available phage cocktail targeting *K. pneumoniae* was administered via oral route for 5 days. The application of phage therapy successfully eradicated *K. pneumoniae* among all patients (Aslanov et al., 2018). A successful phage treatment was done on a patient who had a left tibial infection complicated by *K. pneumoniae* and *A. baumannii* infections (Nir-Paz et al., 2019). The bacteriophages were administered intravenously, and it showed a rapid clinical improvement. The phage therapy effectively eradicated the infections and the amputation of the patient's leg was no longer needed (Nir-Paz et al., 2019).

Acinetobacter baumannii, a personalized phage treatment was conducted on a 68-year-old diabetic male (Schooley et al., 2017). The patient developed necrotizing pancreatitis which complicated by MDR *A. baumannii* infection on pancreatic pseudocyst. FDA approved clinical administration of cocktails of bacteriophages (ΦPC, ΦIV, and ΦIVB) as an emergency investigational new drug (eIND) (Schooley et al., 2017). A cocktail of phages, ΦPC, was administered through intracavitary for 18 weeks, and ΦIV and ΦIVB were intravenous administration for 16 weeks and 8 weeks, respectively. The patient showed signs of decline indicating the organism was slowly building resistance to the first round of phage therapy. However, upon additional administration of new phage cocktails, ΦIV and ΦIVB, it showed significant clinical improvement, eventually eradicated *A. baumannii* (Schooley et al., 2017). Another phage therapy against *A. baumannii* was reported in the U.S. The patient was a 77-year-old male who had subdural hematoma and traumatic brain injury (LaVergne et al., 2018).

Craniectomy was performed and MDR *A. baumannii* was grown intraoperatively. Five bacteriophages were selected from 104 *A. baumannii* bacteriophages which was provided by the Naval Medical Research Center (NMRC) phage-Biolog system. FDA also approved the bacteriophage cocktail as an eIND on this case. The cocktail was administered intravenously every 2 hours for 8 days. Any significant clinical improvement and efficacy of the phage therapy were not able to be monitored and evaluated due to a withdrawal request of phage therapy and extubation from the patient's family (LaVergne et al., 2018).

Pseudomonas aeruginosa, in the UK, 24 patients were recruited who are with chronic otitis caused by an antibiotic resistant *P. aeruginosa* (Wright, Hawkins, Anggard & Harper, 2009). A half of randomly selected patients received phage therapy with a single dose of Biophage-PA cocktail (six bacteriophages contained; BC-BP- 01 to BC-BP-06) administered into the ear, whereas the rest received placebo. The treatment showed significant clinical improvements in the phage group compared to the placebo group. Even though single dose of phage cocktail was a challenge to completely cure patients with long-term infection history, the results demonstrated this clinical trial of phage therapy against *P. aeruginosa* was effective and safe (Wright et al., 2009). Total of 27 patients from France and Belgium who have burn wound infections with *P. aeruginosa* were recruited and randomly received standard medical care or PP1131 bacteriophages (a cocktail of 12 natural lytic anti- *P. aeruginosa*) (Jault et al., 2019). Phage therapy was administered topically to 13 patients for 7 days. This application of phage therapy did not demonstrate significant efficacy and safety than standard medical care. The report revealed the failure of PP1131 phage treatment due to low doses (Jault et al., 2019). Another clinical trial was conducted on a 26-year-old female who had cystic fibrosis with confirmed colonization of MDR *P. aeruginosa* (Law et al., 2019). FDA approved AB-PA01 which is a cocktail of 4 lytic phages for application of phage therapy

on MDR *P. aeruginosa* infection. The phage was administered intravenously for 8 weeks. The treatment was effective and successful with exacerbation of cystic fibrosis and no recurrence (Law et al., 2019).

Enterobacter spp., although clinical application of phage therapy against MDR *Enterobacter spp*. infection was not found in the search period, from 2009 to 2019, the risk of this organism is recognized, as it is included in ESKAPE.

Discussion

Scientific literature for bacteriophage therapy is available on both laboratory and human compassionate case studies. The laboratory articles present information that substantiates the need for preclinical research on multidrug resistant organisms and serve as a precursor to human clinical trials. In terms of study characteristics, it was observed that testing against several genuses of bacteria indicates the severity and complexity of the AMR problem. There is not one type of bacterium that is clinically problematic but rather a myriad of microorganisms. Furthermore, there is a matching bacteriophage for every bacterial target demonstrating how the natural environment provides supply. These lab and human studies also represent a variety of infection types (i.e. keratitis, diabetic foot ulcers, burn wounds, septicemia and pulmonary infections) that MDROs can cause commonly.

In terms of methods, the utilization of different procedures (*in vitro* and *in vivo* animal models) suggests that researchers are making every effort to employ all possible methods to ensure validation. Some of the articles emphasized the prudent use of bacteriophage cocktails because if a target bacterium develops resistance to one bacteriophage then the remaining bacteriophages within the cocktail can still battle it. However, even the singular use of bacteriophage demonstrates efficacy against its specific MDRO. For human cases, conducting trials are limited to

compassionate use. The use of bacteriophage is often performed with commercially available bacteriophages on relatively well-known infections, or it is rarely approved by a government agency such as FDA, as an eIND for clinical trial. Regarding bacteriophage effectiveness, the majority of the laboratory studies and human clinical trials have shown effectiveness which means this could be a viable option for multidrug resistant infections, especially ones that are life-threatening.

Regarding perceived barriers to clinical research, the majority of articles stated more lab tests and large-scale controlled clinical trials must be performed. A few laboratory articles did not address barriers to research. This theme suggests that more empirical evidence must be collected to convince the medical community that bacteriophage could challenge multidrug resistant organisms. A limitation of human clinical cases is also that possible side- and long term- effects which are not yet well demonstrated. These cases will require follow up to see if there are unforeseen consequences. Recently, more clinical trials of phage therapy are being conducted and successful results are reported. However, we may also need to investigate those clinical phage therapy which failed because that is still a valuable data and it will help to advance the methodology and technology of phage therapy on human applications. Furthermore, increasing public and professional perception of phage therapy is important. For a clinical trial, patient's and family's consent is an essential factor to advance the therapy, for example, a clinical trial conducted by LaVergne had to stop during application of phage therapy on the patient due to request from the patient's family. This may require acknowledge and belief on bacteriophage therapy. Demand of phage therapy from patients will bring better frequency of application of phage therapy. And, these factors are closely associated with each other and synergize to solve the perceived barriers. The laboratory articles and human clinical articles presented in this paper

exhibit the initial stages of bacteriophage therapy as a way of confirming its usefulness in treating antimicrobial infections.

Significance of Articles

The significance of laboratory and human studies in the fight against AMR is that they must be more vitalized and conducted in larger scale. Researches have been conducted in the past and will continue to be as there is no such thing as performing too many experiments. When researchers declare that more studies must be performed, there is no finite number of lab tests to perform before starting clinical trials. Laboratory studies will forever be an ongoing process. The more tests performed, preferably with successful results, the greater the validation. In lab science, the validation of methods is incredibly important because it examines the precision (reproducibility) and accuracy (how close the result is to the real value or measurement) of a test and proves effectiveness. Bacteriophage therapy is not yet a widely-accepted concept as a treatment as it is in its infancy stages. Research scientists currently performing bacteriophage lab testing are diligently providing results. The significance of these studies helps determine whether bacteriophage is worth using in practical applications. Thus, it is important to continue laboratory testing even if concurrent with human compassionate cases as we make the transition from a theoretical framework to clinical implementation. As long as AMR exists and there is interest in alternative therapies such as bacteriophage, lab tests will always have a significant role and value.

CHAPTER 3

FIELD EXPERT SURVEY

Background

Bacteriophage use is a relatively new concept in therapeutic interventions. Laboratory experiments have been performed and their articles published to demonstrate effectiveness against MDROs. They are the theoretical groundwork for severe multidrug resistant infections in animals and people. Currently, there are limited clinical human evaluations using this type of therapy. From the information dispensed in Chapter 2, it is only applied in life-or-death compassionate cases. There is useful information in these literature pieces but sometimes it is equally informative to directly question field sources about their observations and sentiments. To obtain further information, an online survey was made available to phage experts in academia, research or clinical experience - people who have a finger on the pulse of this specialty. The purpose of the survey was to determine the status of bacteriophage therapy from their perspective. This was a decent method for determining the opinions, beliefs and attitudes surrounding the themes of interest: the demand, the frequency of use, availability, eligibility criteria, perceived barriers to human research and recommendations. The survey was used to assess the current climate of bacteriophage and what the future may hold for the field based on respondent expertise and experience.

Methods

We conducted a bacteriophage expert survey using a short questionnaire of 10 questions regarding the current science of bacteriophage therapy (Please refer to appendix for questionnaire). For the field expert survey of our capstone project, we had to find, identify and contact phage experts in the field of bacteriophage. We sent out emails to the Georgia Eliava Institute (GEI), the phage directory website, and scientific article authors. The online survey was created and

conducted through the survey monkey website (Link →Phage Field Expert Survey "https://www.surveymonkey.com/r/B6K8BK2"). The online survey was administered in three ways: 1) via email (Please refer to appendix section for email template), 2) a link to the survey was posted on the Phage Directory community message board Community Board "https://phage.directory/community" and 3) Twitter post. Through these methods, we were able to secure a sufficient quantity of survey results. These materials will serve as an additional layer of field intelligence.

Results

The survey was made available on 8/4/2019 to 10/3/2019. We received 31 responses. Two of the participant answers were incomplete and disregarded from analysis. Question 1 asked respondents about country location. From 31 responses, 14 were from the United States, 3 from the republic of Georgia, 2 from Belgium, 2 from Canada, 2 from Spain, one from Poland, one from France, one from Chile, one from Germany, one from Iraq, one from Israel, one from Japan and one from Mexico. (Please refer to Figure 3.1 to visualize the distribution of international participation) Question 2 inquired about years of professional experience. The lowest was one year and the highest was 20 years with an average of 8.8 years. Question 9 and 10 involved demographic information regarding age and highest level of education, respectively. The answers from question 9 revealed the youngest participant was 25 (PhD candidate) and the eldest 69 and the average age was 41 years old. The majority of the participants' education level were medical doctor or PhD, with the exception of one who was a PhD candidate and one who has a Master of Science degree. Questions 3, 4 and 5 were scaled using Likert scales, and the frequency of response distribution analyzed using SPSS and EXCEL software. Question 3 states "In your country, how high is the demand (by patients/physicians) for bacteriophage therapy for the purpose of multidrug resistant

infections?" The response options were very high, high, neutral, low, and very low. 10.3% answered very high, 31.0% answered high, 20.7% answered neutral, 20.7% answered low and 17.2% answered very low. Question 4 states "In your country, how frequent is bacteriophage therapy clinically administered to human patients?" The response options were very frequently, frequently, occasionally, rarely and never. 10.3% answered very frequently, 0% answered frequently, 13.8% answered occasionally, 51.7% answered rarely and 24.1% answered never. Question 5 states "In your country, how readily available is bacteriophage therapy? The response options were readily available, somewhat available, neutral, available with limitations and not available. 10.3% answered readily available, 24.1% answered somewhat available, 0% answered neutral, 27.6% answered available with limitations and 37.9% answered not available. (Figures 3.2, 3.3 and 3.4 for bar graphs.)

Questions 6, 7 and 8 were open ended and assessed by qualitative thematic analysis. Question 6 was "In your country, what are the eligibility criteria for bacteriophage therapy?" Eighteen people said that eligibility only involved compassionate use or variations of this answer. Keywords would include multidrug resistant infections, last resort, life threatening, dying or close to, unresponsive to antibiotics and emergency situations. Four of the participants did not know and four said there are no eligibility criteria in their country. The remaining answers given were unclear for interpretation. There were common responses within certain country cohorts. For instance, participants from the United States are all aware of eligibility criteria for bacteriophage therapy being for compassionate cases and that the Food and Drug Administration must approve its use.

Another example is the three representatives from the Republic of Georgia said that bacteriophage is a registered medicine with eligibility criteria and that there is a screening process for individuals traveling abroad seeking to receive bacteriophage. Both participants from Canada

stated there are no eligibility criteria because they have not encountered the problem yet. The lone participant from Mexico replied that bacteriophage has only gotten clearance for the use in animal and fishing industry and thus, there are no human eligibility criteria. Question 7 was "In your professional opinion and experience, what are the practical and regulatory barriers that may prevent phage therapy clinical trials?" The answers to this question were many and complex (Table 3.1). However, the top most pressing practical barriers that were given by participants can be summed up as cost or lack of funding, lack of laboratories willing to perform clinical research, the scientific unknowns of bacteriophage therapy (i.e. side effects, bacterial resistance development, long term effects post-treatment and genes with unknown functions), the pharmaceutical lobby and the lack of rigorous clinical trials data outlining pharmacology, safety and efficacy.

Table 3.1 Question #7 Samples of Open-ended Responses

Participants	Responses
Participant #17 from the United States	"Societal resistance to change is a big part of the barrier. If medical professionals are not fully on board, most people will not be either."
Participant #21 from Spain	"The most important barrier is it's a virus! People have many preconceptions about viruses, and they cannot differentiate amongst them."

The second part of question 7 inquired about the regulatory barriers to clinical research where the majority of responses declare there is a lack of regulatory framework for this type of therapy.

Table 3.2 Question #8 Samples of Open-ended Responses

Participants	Responses
Participant #6 from the United States	"Purifying phage has created a bottleneck. There are only 3 labs in the U.S. that are capable of producing a high titer of pure, endotoxin-free batch of phage that is ready to administer to a patient. Also, the cost it would take for these labs to produce phage is very high. Regulatory barriers include FDA approval and finding labs willing to fund this research."

Participants	Responses
Participant #11 from the United States	"Approval of biologics come with difficulties as they may be less consistent than chemicals so there is going to need to be a bit of discussion about how they will be regulated."

Bacteriophage therapy is clearly in the initial stage of pre-regulation. The majority of respondents agree that decisions will have to be made in the near future by qualified individuals. Question 8 was "What are your professional recommendations for the field of bacteriophage therapy? Collectively, respondents believe that laboratory and human studies should continue, countries with AMR should apply for funding, conduct randomized control trials, develop public awareness and that a collaboration between governing health institutions, scientists and clinicians must agree on common protocols.

Figure 3.1 World map highlighting areas of survey participation.

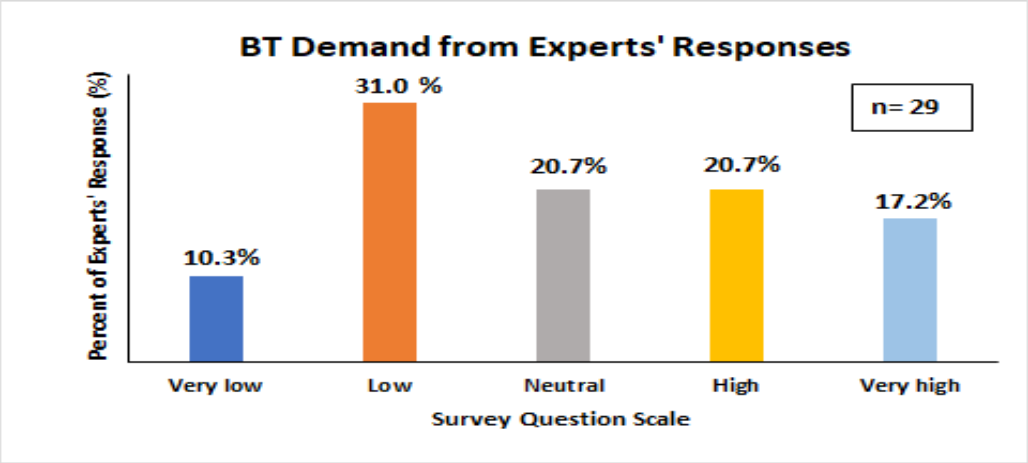


Figure 3.2 Graphs of demand for bacteriophage for multidrug resistant infections

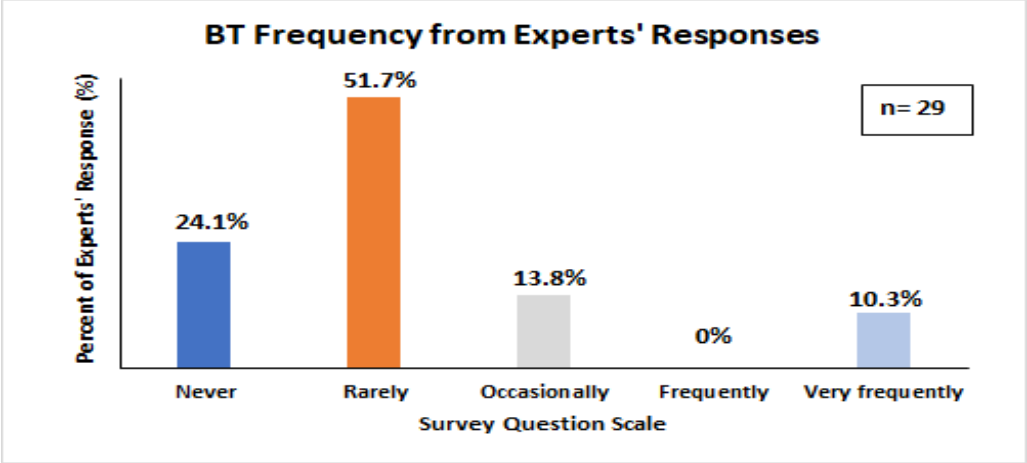


Figure 3.3 Graphs of the frequency of bacteriophage use on human patients

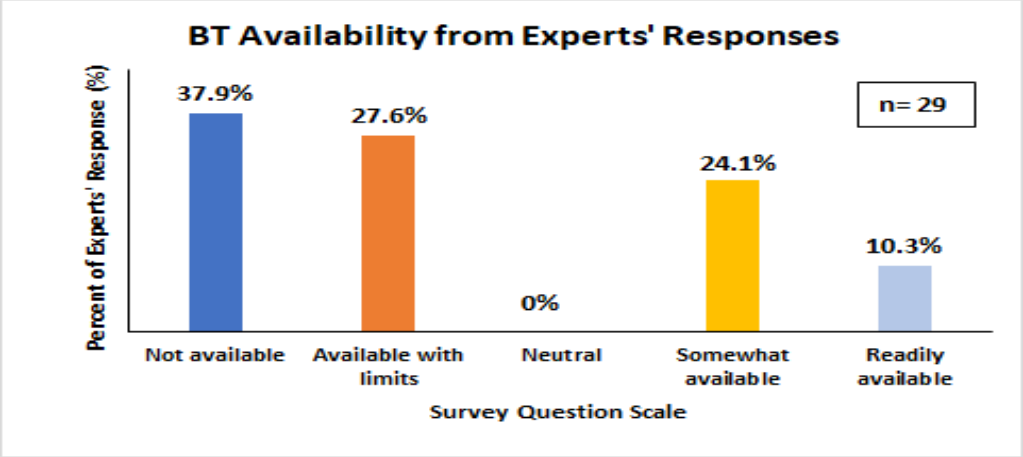


Figure 3.4 Graphs of the availability of bacteriophage therapy

Discussion

An online survey was conducted on phage experts to gauge their professional observations and recommendations for the field. The respondents included insight from physicians, academic and industry researchers from different parts of the world. The variables of years of experience, age and education were significant because it meant that the survey participants were credible and suitable. Two of the respondents were excluded from analysis mostly because they answered as having zero years of bacteriophage experience and skipped most of the pertinent questions, both scaled and open-ended. According to the survey responses for the demand of bacteriophage therapy, the answers were fairly scattered but trended towards increasing. The other scaled questions were for frequency and availability. The availability of bacteriophage therapy directly impacts the frequency of use since worthy patients can only use what is available and vice versa. Most answers for both frequency and availability demonstrate low values. When the demand grows larger, the accessibility of bacteriophage therapy will need to catch up. Therefore, our assessments deduce that this topic will eventually need to be addressed and protocols established in order to meet a growing demand. According to survey participants, the current eligibility criteria for countries who have the means to perform bacteriophage therapy are mostly for life-threatening infections. Some countries do not have an established eligibility criterion either because their countries have low AMR incidence and do not warrant a bacteriophage movement, or they simply lack the means. The perceived barriers involve the absence or inadequacy of infrastructure and regulations surrounding bacteriophage therapy which might account for the slow progression of the field and bacteriophage accessibility. Moreover, the lack of knowledge or understanding that not all viruses necessarily cause disease and that highly specific viruses used as a form of treatment can serve as a barrier for the general public. We believe that the perceived barriers greatly affect the

inclination of human clinical trials. According to survey responses for professional recommendations, they declared that there must be a continuation of lab and human compassionate testing, funding for BT programs, general public awareness through news, various media, and hospital education and finally, there must be policy regulation. Due to the field's novelty, guidelines outlining various therapy aspects are still open to error and debate. Policies and procedures may be decided over modes of phage administration, pharmacology, sterile phage preparation methods and sterility testing. The limitations of our survey method were low sample size and nonexistent or limited representation from certain countries. Because of low sample size, it is difficult to say how the responses represent the status of bacteriophage internationally. Every country has different public health experiences and healthcare policies that could possibly affect AMR rates. We can only make mild assumptions or conclusions within the confines of the survey responses. Our results show that no matter how unfamiliar or controversial bacteriophage therapy is, global health communities are being forced to consider it. The online survey furthered our understanding about the future direction of bacteriophage therapy based upon key informants' experiences.

CHAPTER 4

DISCUSSION

For our capstone project, we were interested in learning about bacteriophage therapy as a way of ameliorating multidrug resistant bacterial infections. Our methods included a simple literature review of laboratory and human articles and a survey. Many *in vitro* and *in vivo* studies have taken place for many decades and several clinical cases have been conducted in the last few years. Lab research employs sciences to develop methods or equipment that might be useful when used in a clinical context. It involves the study of animals, cells and genes to understand the pathophysiology of disease. The significance of the lab literature results provided a variety of test methods and evidence proving bacteriophage effectiveness *in vitro*. We featured laboratory articles on clinically significant ESKAPE organisms, a narrow focus of MDROs as there are more multidrug resistant bacterial strains, fungi and parasites in existence. The lab studies paved the way as a testing ground for human testing. Clinical human research is important for discovering new treatments for disease. The human literature articles presented different kinds of human infections and most proved phage therapy efficacious with rare exceptions. Clinical trials demonstrate what works and what doesn't work in humans that cannot be learned in laboratory or animal studies. At the present time, bacteriophage therapy is an odd hybrid of sorts which is both for research use only (RUO) and compassionate therapeutic intervention. Human compassionate case studies are currently but slowly being performed given that its accessible and they meet eligibility criteria. The significance of the literature findings show primarily that bacteriophage is effective at reducing and eliminating multidrug resistant organisms and therefore, is a worthwhile recourse. These findings are consistent with previous lab and human studies. From the phage expert surveys, we had an opportunity to ask key informants about various aspects of the field and

evoke discussion. We ultimately analyzed 29 responses from 13 different countries with a range of professional experience and higher education. According to experts' responses, the scaled topics of bacteriophage availability, frequency of clinical use and demand were low, but that demand was gradually rising. Open-ended topics for eligibility criteria were compassionate use, perceived barriers were lack of funding, research labs, rigorous clinical trials and regulatory framework and recommendations were funding, conduct clinical trials, raise public awareness and regulation. Phage experts are in favor of bacteriophage therapy, but they caution there must be a qualified governing unit that makes decisions about policies and procedures. As multidrug resistant infections gain more attention and momentum, hopefully there will be an acknowledgement and a natural progression to meet the demands by clinical institutions.

The major theme running through our capstone project was the topic of perceived barriers. Our original hypothesis statement was there are perceived barriers and the research question was "What are the perceived barriers to clinical research for bacteriophage therapy?" We proved the theory that perceived barriers exist and revealed what they are according to the literature review and survey. Most of laboratory articles said further research is needed and most of the human articles states that large-scaled human clinical trials must be performed to build robust data for future human application. The answers for perceived barriers by experts were many and diverse but the bulk of them were lack of funding, clinical trials and regulation. These responses would be categorized as a component of the politics surrounding bacteriophage therapy.

Refer to many research papers, there are strengths and limitations to consider. The strengths of this capstone paper are that diverse sources (literature and survey) were employed to inform on the topic of bacteriophage. The limitations for both the lab and human articles were a limited number of articles. Laboratory experiments pre-2010, human compassionate case studies pre-2009

and articles that experienced suboptimal results were not represented here. There was an apparent lack of scientific human articles which suggests a gap in the literature that is under-explored. We also had not accounted for possible article bias. The limitations for the survey was a low sample size of 29 participants which cannot accurately represent a global picture of each survey item. We also did not receive responses from countries with high rates of AMR like India or China which could have changed the results. Limitations are a natural part of research and ours were a learning experience.

The perceived barriers to clinical research are possibly the reason for the slow and cautious pace of implementation. But perhaps it should be. When it comes to treating human subjects with a new therapy, the scientific and medical community should not be hasty. There could be unanticipated consequences with bacteriophage in the future much in the same way we had not anticipated complications with antibiotics and resistance. Maybe researchers and clinicians hope to learn from past mistakes and put in the right protocols from the beginning and ensure adherence.

Human compassionate case studies show that we have officially crossed the threshold of a new therapy. The process of creating a therapeutic framework will vary from country to country but overall it begins with a governing body that makes decisions. In the United States the governing body for medicine is the Food and Drug Administration and other countries should have an FDA-equivalency. They will need to establish concrete eligibility criteria. In the short term, it may be a small list but in the long term, the criteria may become longer and more specific. Also, participating bacteriophage research labs must create a bacteriophage library. Once bacteriophage is tested and identified, it can be frozen and archived for potential future use. The nature of bacteriophage administration is such that in the event there is a critical need for it, it must already be available (found) or that it can be found in a rapid manner. Sometimes it’s a race against time

to save a person's life. Once eligibility criteria and phage libraries are organized, large-scale clinical trials can be done even if subjects are scattered throughout the country. Currently, the FDA approved phage therapy trials in the U.S. at the Center for Innovative Phage Applications and Therapeutics (IPATH) located in our home city of San Diego (Voelker, 2019). Physician researchers at the University of California San Diego (UCSD) School of Medicine will conduct the trial in collaboration with AmpliPhi Biosciences Corporation, a San Diego–based biotechnology company (Voelker, 2019).

Bacteriophage therapy is a field that has become resuscitated because of the growing concern of multidrug resistant infections. It will not immediately reduce or solve the problem of antimicrobial resistance, but it will have an active role in its alleviation. The time until antibiotic alternatives become fully authorized is uncertain and cPT seems to be limited to geographic, experimental centers. In the meantime, cPT is filling in the gaps between failing current antibiotics and incoming new antibiotics. The most pressing concern right now is that large scale human clinical trials must generate efficacy data that will lead to eventual licensed phage products. As it stands, bacteriophage therapy is being used ever-cautiously on those who fall through the cracks.

CHAPTER 5

CONCLUSION

Multidrug resistance is a phenomenon that will escalate whilst there are limited therapeutic options available. Research scientists continue to perform laboratory testing and the clinical community has taken action by permitting bacteriophage therapy for emergency-only human infections. Throughout most of the world, there is limited literature regarding clinical phage therapy data. More compassionate testing is being done during the time frame of this capstone project, but results have not been made available to the general public yet. This gap in the literature is currently being addressed and is an opportunity for further research in a field that is running out of options regarding MDROs. From the published laboratory science articles, human compassionate case study articles as well as our conducted bacteriophage field expert surveys, the evidence points towards a promising resolution using bacteriophage against antimicrobial infections. Our research demonstrated that many laboratory studies have been done, human clinical therapy is slowly being performed and that phage experts believe it is a viable option to antimicrobial resistance, but that careful efforts, funding and clear regulations are required. Furthermore, we uncovered perceived barriers to clinical research. The laboratory and human cases emphasize that more testing must be conducted to build on the rigor of trust and effectiveness. The online survey participants offered many perceived practical and regulatory barriers such as lack of funding and willing research laboratories, scientific unknowns, the pharmaceutical lobby and the lack of rigorous clinical trials data outlining pharmacology, safety and efficacy. Phage therapy is not a new concept, but it has yet to be fully accepted and enter mainstream therapeutics. It was our hope that this capstone project paper dispensed significant information and confirmation that bacteriophage therapy deserves consideration for treatment of

multidrug resistant infections worldwide. It was our hope to learn about how bacteriophage therapy is entering the treatment pipeline and how it affects the present era of infectious disease. We hope to follow the science into the future and bear witness to its progression and potential contributions.

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Appendices

Phage expert survey email template:

Dear Sir or Madam,

Allow us to introduce ourselves. We are Olympia Alvarez and Taehoon Ha, two Master of Public Health students at California State University, San Marcos. For our capstone project, we hope to collect phage experts' opinions and information regarding the current science of bacteriophage and MDROs through an email survey.

In compliance with IRB guidelines, we must inform that you are not obligated to participate in the survey but we hope you will consider it. If you choose to participate, please click on the link below and kindly answer the survey questions. Furthermore, if you feel inclined, please pass along this email to any colleagues that you believe are willing to contribute and may bring insight and value to our project. We hope to enhance our learning and understanding from the survey responses.

Thank you for your time and attention in this matter. Respectfully,

Olympia Alvarez and Taehoon Ha

Link → Phage Field Expert Survey "https://www.surveymonkey.com/r/B6K8BK2"

Phage expert survey questions:

1. What country do you work in?
2. How long have you worked in the field of bacteriophage (research or academics)?
3. In your country, how high is the demand (by patients/physicians) for bacteriophage therapy for the purpose of multidrug resistant infections? (Very high, High, Neutral, Low, Very Low)
4. In your country, how frequent is bacteriophage therapy clinically administered to human patients? (Very frequently, Frequently, Occasionally, Rarely and Never)
5. In your country, how readily available is bacteriophage therapy? (Readily available, Somewhat available, Neutral, Available with limitations, Not available)
6. In your country, what are the eligibility criteria for bacteriophage therapy?
7. In your professional opinion, what are the practical and regulatory barriers to clinical research?
8. What are your professional recommendations for the field?
9. Please give your age.
10. What is your highest level of education?

Link → Phage Field Expert Survey "https://www.surveymonkey.com/r/B6K8BK2"

Table 1. Laboratory articles (annotated bibliography)

No.	Article	Target MDRO	Phage/cocktail	Method	Effective?	Perceived Barriers
1	Cao et al., (2015)	*Klebsiella pneumoniae*	Phage 1513	Intranasal administration mice with pneumonia	Yes	Phage resistance must be better understood and studied
2	Fong et al,. (2017)	*Pseudomonas aeruginosa* (isolated from patients with CRS of CF and non-CF)	Pa 193 Pa 204 Pa 222 Pa 223	Biofilms grown in vitro were treated with cocktail	Yes (76% reduced at 48 hours)	Further evaluation of pf pre-clinical studies needed
3	Fukuda et al., (2012)	*Pseudomonas aeruginosa*	KPP12	Eye-drops given to mice with kerastitis	Yes	None given
4	Jasim et al., (2018)	*Acinetobacter baumanii (XDR, PDR)*	136 lytic phages (no names given)	In vitro In vivo (bacteremic mice)	Yes (> 1 log reduction)	None given
5	Khalifa et al., (2015)	*Enterococcus faecalis* *Enterococcus faecium* (root canal treatment)	EFDG1	In vitro testing against planktonic and biofilm	Yes	None given
6	Lehman et al., (2019)	*Staphylococcus Aureus (MRSA & VISA)*	AB-SA01	In vitro	Yes (94%)	None given
7	Lood et al., (2015)	*Acinetobacter baumanii (MDR)*	21 distinct lysins PlyF307	In vitro In vivo (bacteremic mice)	Yes (> 5-log-unit decrease)	The need for more testing on mammalian models before conducting human RCTs
8	Manohar et al., (2018)	*Escherichia coli* *Kleb. pneumoniae* *Enterobact. cloacae* (mixed infection)	ECP311 KPP235 ELP140	*Galleria mellonella* larvae model	Yes (100% survival rate)	More testing is required on more complex animal models

No.	Article	Target MDRO	Phage/cocktail	Method	Effective?	Perceived Barriers
9	Pallavali et al., (2017)	*P. aeruginosa* *S. aureus* *K.pneumo* *E. coli*	PA DP4 SA DP1 KP DP1 MDR EC3	Bacteria isolated from pus swab samples and tested in vitro	Yes	Extensive study required to understand all mechanisms involved
10	Taha et al., (2018)	*Klebsiella pneumoniae*	ZCKP1	Isolated from foot wound of a diabetic patient	Yes (>2 log CFU/mL reduction)	Ensure phages cannot transfer resistance to resident microbiota

Table 2. Human case study articles (annotated bibliography)

No.	Article (alpha)	Target MDRO	Phage or cocktail	Method	Effective?	Perceived Barriers
1	Letkiewicz et al., (2009)	*Enterococcus faecium*	Lytic phages from IIET (no name given)	Enterococcal phages prepared based on the ILET phage collection and administered via rectal.	Yes	Large-scale controlled clinical trials need to be conducted to verify efficiency and safety
2	Wright et al., (2009)	*Pseudomonas aeruginosa*	Biophage-PA (BC- BP- 01 to BC- BP- 06)	Phage liquids applied topically into the ear of 12 patients	Yes	PT needs further and larger trials including chronic otitis and other infections.
3	Fadlallah et al., (2015)	*Staphylococcus aureus*	*S. aureus* bacteriophage SATA-8505 (ATCC PTA-9476)	Administered Topically and intravenously for 4 weeks	Yes	clinical trials are warranted to assess the therapeutic potential of phages, particularly in antibiotic-resistant cases.
4	Fish et al., (2016)	*Staphylococcus aureus*	staphylococcal phage Sb-1	Topically to once weekly.	Yes	Further work for controlled clinical trials of staph phage for diabetic foot infections is needed.
5	Schooley et al., (2017)	*Acinetobacter baumannii*	Cocktail (ΦPC, ΦIV, and ΦIVB)	Phage cocktail administered through intracavitary and intravenous	Yes	More concerted efforts to investigate the use of therapeutic bacteriophages for

No.	Article (alpha)	Target MDRO	Phage or cocktail	Method	Effective?	Perceived Barriers
						MDROs.
6	LaVergne et al., (2018)	*Acinetobacter baumannii*	Cocktail of 5 *A baumannii* bacteriophages (no name given)	Administered through the surgical drain and intrathecally	N/A	Treatment stopped with patient's family consent
7	Aslanov et al., (2018)	*Klebsiella pneumoniae*	Commercial phage cocktail	Administered through oral for 5 days	Yes	an urgent need to investigate alternative preventive and treatment options
8	Nir-Paz et al., (2018)	*Klebsiella pneumoniae Acinetobacter baumannii*	Lytic phages (no name given)	Administered intravenously	Yes	Further clinical trials are warranted
9	Jault et al., (2019)	*Pseudomonas aeruginosa*	Cocktail of 12 natural lytic anti-P aeruginosa bacteriophages (PP1131)	Randomized eligible patients received standard care or phage therapy via a dressing	No	Further studies using increased phage concentrations and phagograms in a larger sample of participants are warranted.
10	Law et al., (2019)	*Pseudomonas aeruginosa*	AB-PA01 cocktail	Administered every 6 hours, IV for 8 weeks.	Yes	Further clinical trial is critical