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Finding Alternatives to Conventional Antibiotics in Face of Multidrug Resistance (MDR) Bacteria Crisis

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**Finding Alternatives to Conventional Antibiotics
in Face of Multidrug Resistance (MDR) Bacteria Crisis.**

An honors thesis presented to the
Department of Biological Sciences,
University at Albany State University at New York,
in partial fulfillment of the requirements
for graduation with Honors in Biological Sciences
and
graduation from The Honors College

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May 2021

Abstract

With a growing concern of healthcare crises like the current COVID-19 pandemic, the healthcare sector is in a dire need of finding solutions to the increasing multidrug resistance in microbial organisms. In the 2019, the *UN Ad hoc Interagency Coordinating Group on Antimicrobial Resistance* stated that if ignored, multidrug resistance organisms (MDROs) could cost 10 million lives each year by 2050. However, this is not a problem of the “future” per say, it is rather of the past, the present, and the future. According to the World Health Organization (WHO), approximately 700,000 people die of multidrug resistance (MDR) each year. Considering the present situation, the misuse of antibiotics in trying to combat the COVID-19 pandemic will likely accelerate antibacterial resistant genes (ARGs) spread across the globe. Hence, this is another wake up call for the urgent need of actions against antimicrobial resistance. For this literature review, I analyzed multiple scientific studies on various antibiotic alternatives that can treat bacterial infections including MDR. Due to time constraints, this library thesis only focuses on bacteriophages, CRISPR-Cas based antibacterial, nanoparticles, and anti-plasmid & plasmid curing agents. These therapeutics are proposed to have better outcome in the management of ARGs’ spread than the conventional antibiotics.

Keywords: *Antibacterial Resistant Genes (ARGs), Multidrug Resistance Organisms (MDROs), Antibiotic alternatives*

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Introduction

In the 2019, a groundbreaking report by the *UN Ad hoc Interagency Coordinating Group on Antimicrobial Resistance* stated that, if ignored, multidrug resistance organisms (MDROs) could cost 10 million lives each year by 2050. Some key factors in the development of MDR can be attributed to uncontrolled usage and dispensing of antibiotics, meanwhile, the rapid spread can be related to globalization which has increased the movement of people and goods that may be carriers of the pathogens with antibacterial resistant genes (ARGs).

The mounting numbers of MDROs is currently a threat to the global healthcare sector. Recent statistics by World Health Organization (WHO), reported about 700,000 deaths caused by drug-resistance, of which, 230,000 cases were related to multidrug-resistant tuberculosis (WHO, 2019). The horrifying concerns do not stop within the healthcare sector only, the shared consequences expand to the global economic sector as well. For example, each year in the United States, methicillin-resistant *Staphylococcus aureus* (MRSA) causes about 19,000 deaths, 360,000 hospitalizations, and costs about \$3-4 billion in healthcare (Martens & Demain, 2017). In addition, places with limited resources such as SubSaharan Africa, East Asia, and South America make it harder to predict how much danger we could be facing given the insignificant data available. For instance, a study that was conducted in Uganda to test “*antimicrobial drug resistance (AMR) patterns from blood cultures at a tertiary hospital,*” showed that AMR pathogens were resistant to first-line antibiotics at a higher rate than in the high-income countries that have active surveillance systems (Kajumjula et al., 2018). Also, Southeast Asia has been identified with risks of the emergence and the spread of AMR (Chereau et al., 2017).

As the current global pandemic evolves, the misuse of antibiotics to combat the covid-19 virus will likely accelerate the prevalence of antibacterial resistance genes (ARGs) across the world.

However, this is not just a “present or future problem,” it is rather of the past, the present, and the future. Recent studies like modern phylogenetics have shown evidence that antibiotics existed in ancient times and have revealed antibiotic resistance as a natural phenomenon found to have also been present prior to the antibiotic golden era. For example, serine β -lactamases were found to be ancient antibiotic strains that existed more than 2 billion years ago, with plasmid-encoded β lactamases appearing millions of years ago (Bush, 2018). However, there is no doubts that the mass production of antibiotics in the 20th century has massively contributed to the overwhelming MDR crisis.

A brief history of antibiotic discovery

Before the discovery of antibiotics, bloodletting, plant remedies, naturally occurring chemicals, and phage therapies were frequently used in treating bacterial infections. For example, dating back to 1550 BC, ancient Egyptians used honey lard and lint to treat wounds. Furthermore, the usage of moldy bread to treat infections, especially wound infections, was common in Egypt, China, Greece, and other nations. At the time, it was believed that these remedies “influenced the spirits or gods responsible for the illness or suffering” (Michigan State University, 2011).

Despite all these multiple treatment options, it was still costly or ineffective to successfully cure various infections such as pneumonia.

Remarkably, 1928 brought a great breakthrough of the first penicillin discovery. Alexander Fleming was a professor of bacteriology at the St. Mary’s Hospital in London. While sorting

through his petri dishes, he noticed an unusual plaque in one area where mold was growing on a staphylococcus bacteria petri dish. Followed by intensive work with the help of his colleagues, Fleming published his discovery in 1929. However, it was not until a decade later that Howard Florey, Ernst Chain, and their colleagues at the Sir William Dunn School of Pathology at Oxford University began the immense work of purification, understanding the chemistry, and testing effectiveness of penicillin. In 1941, Albert Alexander became the first patient to receive penicillin to treat his life-threatening infection. Nevertheless, unable to produce enough doses, Albert died a few days later due to a shortage of penicillin supply.

The dire need for large-scale production of penicillin was overshadowed by World War II. Florey and his colleagues decided to travel to the United States in search of penicillin mass production opportunities. Although the journey was not a smooth sail, the first commercial plant for largescale production was opened in 1944 by Pfizer. By March 1945, penicillin was available to customers in almost all pharmacies. In the same year, penicillin structure was determined to be a four-membered highly labile beta-lactam ring, fused to a thiazolidine ring. A Nobel prize was awarded to Alexander Fleming, Howard Florey, and Ernst Chain for their penicillin research. At that time, penicillin was for sure the “miracle cure”. By 1949, penicillin had become accessible to the public with 100,000 units costing less than 10 cents. Without restriction in place to regulate the usage of this drug, the future would soon face the consequences.

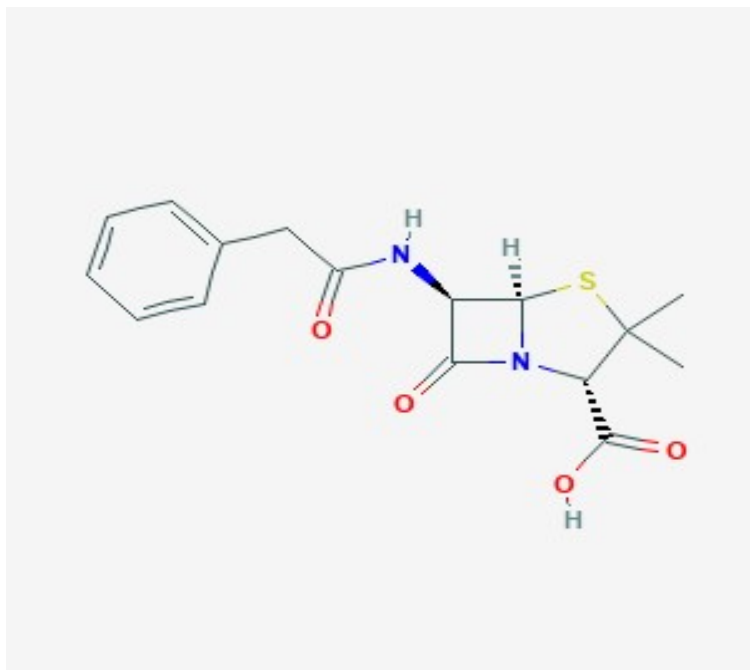


Figure 1

2D structure of penicillin

Despite the cost-effectiveness and great outcome of antibiotics, bacteria always look for ways to survive and reproduce which is a characteristic shared among all living organisms. Therefore, bacteria were deemed to develop drug resistance. Even before the concern of drug resistance has surfaced, Fleming had already offered warnings in 1945 of what could go wrong if the miracle drug were to be misused. According to the America Chemical Society (n.d.), Fleming mentioned in his lecture that the danger of penicillin would be underdosing rather than overdosing or being poisonous to patients.

The time may come when penicillin can be bought by anyone in the shops. Then there is the danger that the ignorant man may easily underdose himself and by exposing his microbes to non-lethal quantities of the drug make them resistant (Fleming, 1945)

Proceeding his predictions, the first penicillin resistance strain was discovered in 1947 followed by many other resistant strains that came along with the discovery of other antibiotics.

Antibiotic mechanism in bacteria

For decades, antibiotics have been powerful weapons to treat bacterial infections. Majority of the known antibiotics have three bacterial targets: the cell wall and membrane synthesis, translational machinery, and DNA replication machinery.

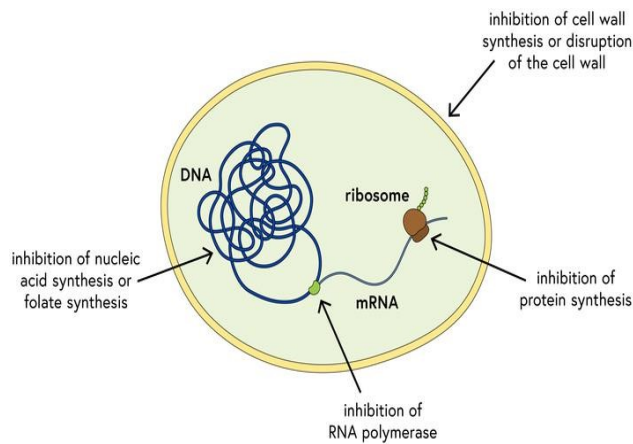


Figure 2

Schematic diagram of antibiotics targets

Antibiotic resistance mechanism

Bacteria have developed resistance mechanisms against each antibiotic mechanism. For example, bacteria hydrolyze and modify drugs to inactivate antibiotics, and alters target receptors through genetic mutations and post-translational modifications, thus, rendering antibiotics ineffective. Additionally, increased efflux pumps and cell wall structure modification aid in decreasing penetration of antibiotics into the cell. This is a huge problem because, without a minimum inhibitory concentration, bacteria have high chances of surviving and developing ARGs

for the drugs they are exposed to. This characteristic has been found prevalent in biofilms and is associated with increased risks of ARGs.

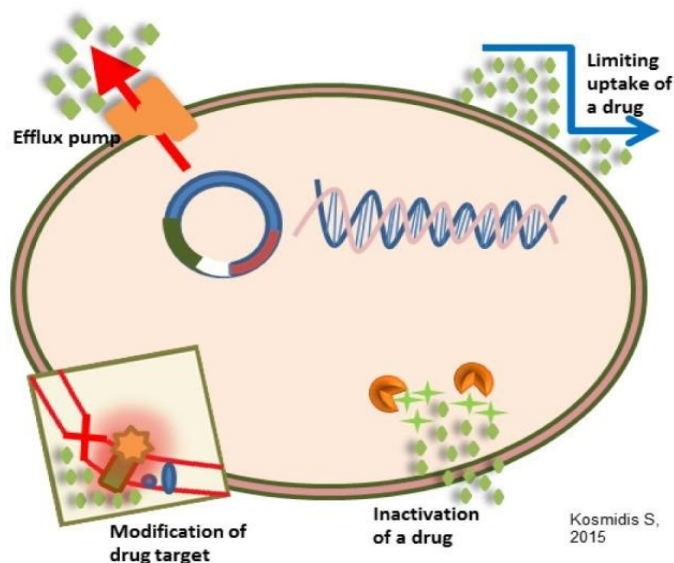


Figure 3

Schematic sketch demonstration the four main categories of drug resistance mechanisms

Types of antibiotic resistance

Antimicrobial resistance can be categorized as native or acquired. The *native AMR* is further classified as intrinsic or induced. *Intrinsic resistance* is the innate ability of a bacteria to resist antibiotics due to structural and functional characteristics such as the lack of target for antibiotics.

Intrinsic resistance can be associated with all the resistance mechanisms and is present in all bacteria. Meanwhile, *induced resistance* is caused by exposure to non-lethal quantities of drugs. The *acquired AMR* can be obtained through genetic material acquisition routes or genetic mutations on chromosomal DNA (Reygaert, 2018).

As antibiotics continue to become less effective, the good old and new antibiotic alternative agents could be the solution to the growing AMR crisis. Although studies on this topic have been around for decades, the known agents are yet to be approved for usage due to insufficient data. Therefore, more in-depth studies should be carried to further explore this topic. In this library thesis, I reviewed multiple publications on various antibiotic alternative therapeutics including bacteriophages, CRISPR-Cas based antibacterial, nanoparticles, anti-plasmid and plasmid curing agents, antimicrobial peptides, and proteins (AMPs), quorum sensing inhibitors (QSI), peptide nucleic acid (PNA), and zinc finger nucleases (ZFNs). Due to time constraints, this library thesis only discusses bacteriophages, CRISPR-Cas based antibacterial, nanoparticles, and anti-plasmid & plasmid curing agents. These therapeutics are proposed to have better outcome in the management of MDR spread than the ordinary antibiotics.

Phage Therapy

Phage therapy is an old practice that uses bacteriophage to treat bacterial infections. There many advantages of using phage therapy. Bacteriophages are host specific and precise. This makes it easier to target bacteria without killing other bacteria that are beneficial to humans. Additionally, unlike other antimicrobials, phages can self-maintain and adapt to the strategies of their prey. They can penetrate areas with poor circulation and even cut through thick biofilms, hence, delivering the minimum inhibiting concentration in the infected areas. Furthermore, literatures show that combination phages and other antimicrobial agents increases their effectiveness in fighting bacteria.

Although phage therapy is currently used as a last resort treatment against AMR like methicillin resistant and vancomycin-resistant strain, etc., there are many success stories of its application.

For example, a study conducted by Fish et al (2018) “*Resolving Digital Staphylococcal Osteomyelitis Using Bacteriophage—A Case Report,*” showed resolution of *Staphylococcal osteomyelitis* in a patient with diabetic foot ulcers who had refused getting her toe amputated and/or being put on long-term antibiotic. A highly purified Eliava Institute commercial *staphylococcal bacteriophage* was administered once a week for a course of seven weeks. The follow-up reports of the patient showed a complete resolution of osteomyelitis, (Fish et al., 2018).

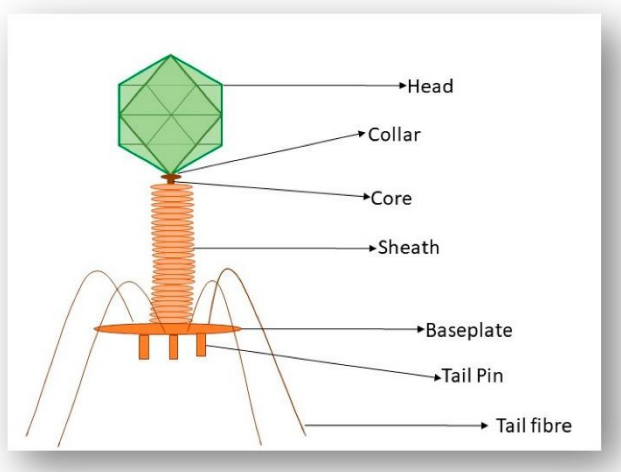


Figure 4

T4 phage, an example of bacteriophages

Brief history

In 1896, Ernest Hanbury Hankin was the first to report something in the water in Ganges and Yamuna rivers in India that exhibits antibacterial action. In 1915, Frederick William Twort discovered a small agent that could infect and kill bacteria, but he was unsure whether it was the

stage of life in bacteria that causes it to lysis, an enzyme, or a virus. In 1917, Felix d'Herelle discovered the phages. "In a flash, I had understood what caused my clear spots was, in fact, an invisible microbe ... a virus parasitic on bacteria" (Wakefield, 2000). Currently, it is known that some phages infect bacteria and others infect archaea. Of the nineteen families of phages classified by the International Committee on Taxonomy of Virus (ICCTV), nine families infect bacteria, nine families infect archaea, and one family infects both bacteria and archaea. Like all viruses, phages are simple organisms made of nucleic acids of either double or single-stranded RNA or DNA surrounded by a protein capsid (Stewart, 2018; Wakefield, 2000).

Before the discovery of antibiotics, phage therapy was widely used in medicine but lost popularity in the western medicine in the 1990s' antibiotic golden era. The recent drug resistance has sparked interest in revisiting phage therapy practices. However, this area of research is facing many challenges including lack of funding for clinical trials. Despite all, some phages have been found effective in human and have been approved for usage. For example, phage Sb-1, a staphylococcal phage that was first isolated in the Eliava Institute. Sb-1 is effective against *Listeria*, and it has been approved for use in the U.S. Other numerous virulent phages are currently being studied. Some of which can treat multiple strains. For example, phage ϕ 812 has been found capable of targeting hundreds of strains of *S. aureus* and ϕ MR11 was found capable of rapidly and completely lysing MDR *S. aureus* under growing conditions (Moghadam, 2020; Rashel, 2007).

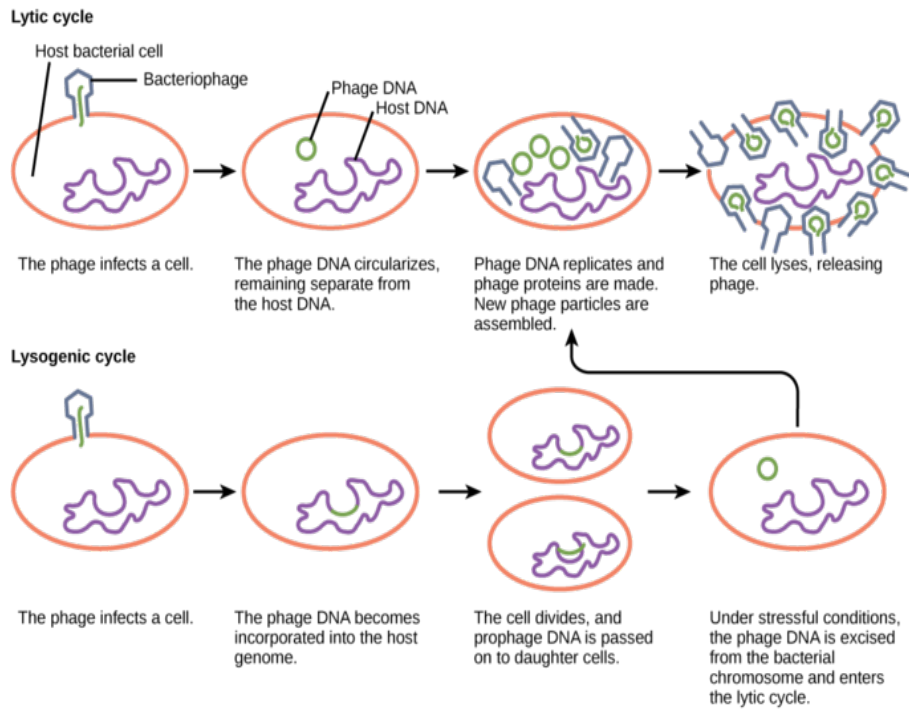


Figure 5

Schematic sketch of lytic cycle and lysogenic cycle

Mechanism

The process by which a phage transfers its genome into a bacterium is known as transduction. Once the genetic material is inserted in the bacteria, they take full advantage of the bacterial mode of reproduction to make more copies of themselves. The phages then weigh the benefit of participating in one of these two cycles:

In the *lytic cycle*, the virulent phage infects a bacterium, multiple in large numbers then lyses. In the *Lysogenic cycle*, a temperate phage infects a bacterium and inserts its DNA in the bacterium chromosome. The phage DNA (now referred to as prophage) is then transcribed along with the bacterium DNA. When phages are in the prophage stage, they can be reactivated by DNA

damage agents like UV light. Also, some small fraction of the prophages in a population spontaneously lyses without external factors. The decision of which cycle the phages will choose depends on which will maximize its survival and reproduction rate.

Limitations

Although the reintroduction of phage therapy could be a great weapon against AMR, there are some concerns about this method of therapeutic. The most prevalent question being the degrees to which phage therapy could contribute to antibiotic resistance. Sometimes, during the transduction process, the phages pick up DNA from their previous host and incorporate it into their new bacterial host. The incorporated DNA could be possessing ARGs, which could be adopted by the new infected bacteria, thus, also becoming resistant to those antimicrobial agents. Another limitation of this method is the bacteria's CRISPR -cas system; the adaptive system by which a bacterium stores the phage DNA from previous infection and use it to develop a defense mechanism against phage DNA of the next infection. In case the phages' Anti-CRISPR proteins fails to interact with bacteria's CRISPR-Cas systems, the bacteria develop resistance for the invading phage DNA in the future. Therefore, this could decrease the effectiveness of this therapeutic method. Additionally, despite bacteriophages not being able to infect eukaryotic cells, sometimes they can be detected by the human immune system which can cause an overreaction in defense. Therefore, further investigations are needed to address these concerns (Dastjerdeh et al., 2016).

CRISPR-Cas Based Antibacterial Therapy

The clustered regularly interspaced short palindromic repeats – CRISPR-associated (CRISPR-Cas) system is a bacteria's adaptive immune system. It works similarly to RNA

interference (RNAi) in eukaryotic cells by identifying and neutralizing the invading phage DNA like the previous infection. Recent surge in antibiotic resistance have raised interest in understanding how the CRISPR-Cas system can be used to fight AMR. Targeting the CRISPR array of the bacteria with CRISPR-Cas based antibacterial can lead to antibiotic sensitivity or cell death due to the introduction of irreversible chromosomal lesions. For example, there is a newly engineered.

CRISPR pill which is under investigation. This pill contains “genome-editing power tool CRISPR that instructs harmful bacteria to shred their genes to bits” (Fan, 2017). Another study conducted by Bikard et al. (2014) aimed at reprogramming Cas9 nuclease as a “*sequence-specific antimicrobial, a tool that would allow selective killing of one or more bacterial species within a heterogeneous population.*” In this study, they constructed a phagemid pDB121::mecA to target methicillin resistance gene *mecA* and used it to treat the clinical isolate of *S. aureus* USA300 Φ . Their results observation showed that *S. aureus* USA300 Φ decreased from 50% before treatment to 0.4% after treatment of pDB121::mecA phagemid in a 1:1 mixture of USA300 Φ and RN Φ cells. In addition, another study conducted by Kang and his colleagues. also demonstrated that Cas9 (sgRNA targeting *mecA*)-bPEI (branched polyethyleneimine) inhibited MRSA strains' growth (Gholizadeh, 2020; Gomma et al., 2014; Kang et al., 2017). Therefore, this further proves the promising outcomes of using CRISPR-Cas to treat MDR bacteria. However, CRISPR-Cas therapeutic are new in medicine. More studies and clinical trials are needed to further elucidate the effectiveness of this treatment method in human medicine.

Brief history

CRISPR was first identified as repeated sequences interspersed with spacer sequences in *E. coli* by a Japanese researcher, Yoshizumi Ishano and his team from Osaka University in 1987.

This incident was totally by accident. In the following years, other strains spacers were discovered in different strains of *Mycobacterium tuberculosis* by researchers led by D. van Embden in the Netherlands in 1993.

In the early 2000, researchers Francisco Mojica and Ruud Jansen described the role of CRISPR-Cas in the adaptive immune system of prokaryotes. The role of the Cas protein as a nuclease was later discovered by Makarova and colleagues. In 2012, researchers George Church, Jennifer Doudna, Emmanuelle Charpentier, and Feng Zhang made a major revelation by discovering the usage of CRISPR-Cas system as a cut-and-paste tool that can be used to remove or introduce new genes as well as silence or activate genes (Ishino et al., 2018). In 2020, researchers Emmanuelle Charpentier and Jennifer Doudna were awarded a Nobel Prize chemistry 'for the development of a method for genome editing' using CRISPR-Cas system.

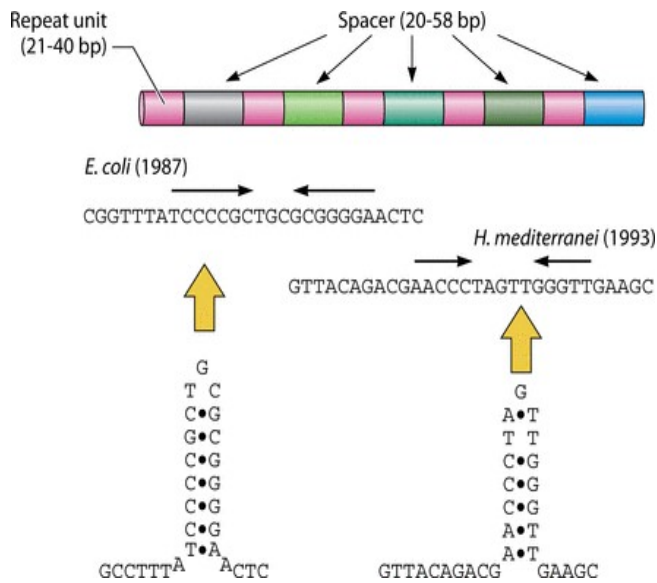


Figure 6

Schematic structure of CRISPR showing the repeated sequences

CRISPR-Cas 9 mechanism

The Cas9 system operates in three stages: adaptation, expression, and interference. In the *Adaptation stage*, the bacteriophage inserts its DNA in the bacteria. The bacteria cuts pieces of phage DNA and incorporate it in its CRISPR locus forming a new CRISPR array. During the *expression stage*, Cas proteins and accessory factors worked together to transcribe RNA from the spacers of the CRISPR locus (precrRNA) which is then cleaved into mature crRNA. In the *Interference stage*, crRNA along with Cas proteins specifically detect the invading DNA, cleave it, and generate a double-strand break (Gholizaden, et al., 2020; Rath et al., 2015).

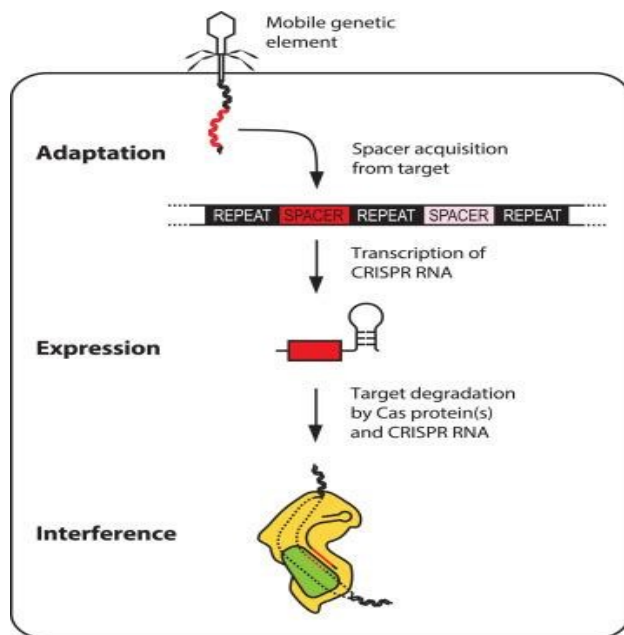


Figure 7

Three stages of CRISPR-Cas system: Adaptation, Expression, and Interference

CRISPR-Cas based antibacterial mechanism

The mechanism by which the CRISPR-Cas antibacterial therapeutic work in combating AMR is very fascinating. This method turns bacteria's own defense mechanism CRISPR-Cas

system against themselves. The RNA-guided nucleases are engineered with the capability of targeting and destroying bacterial strains, including multidrug-resistant (MDR) pathogens. To deliver these nucleases, a mode of delivery is needed to reach the target. Currently under investigations are polymer derivative CRISPR nanocomplex, bacterial carrying plasmids transmissible by conjugation, and bacteriophages.

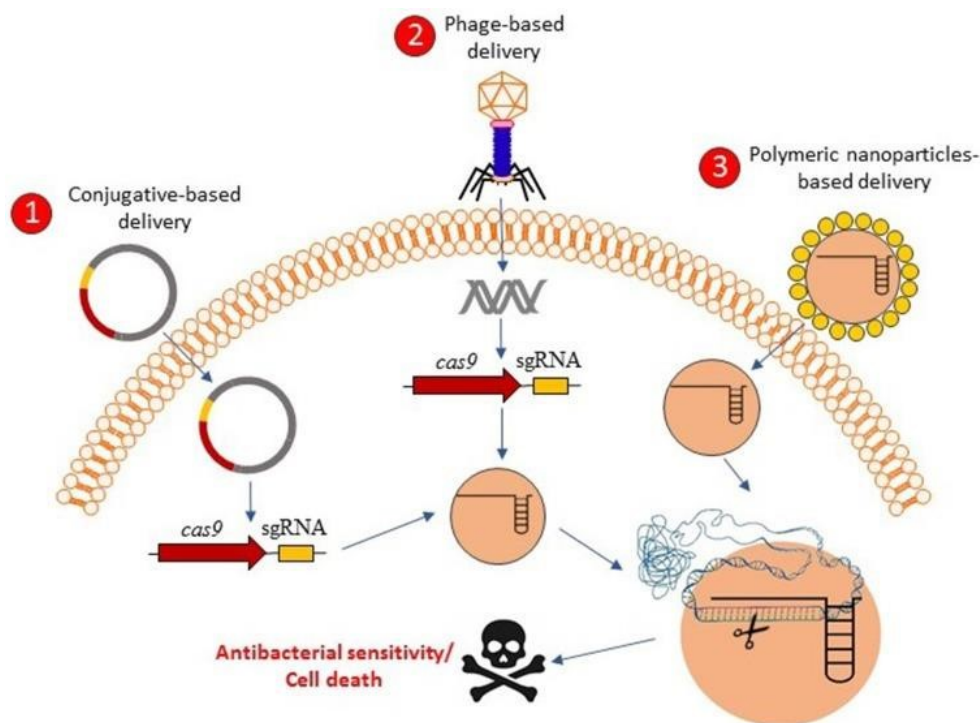


Figure 8

Schematic of delivery methods of CRISPR-Cas antibacterial

The benefit of using the above delivery methods include, targeting specific bacteria of interest. This reduces the indiscriminate killing of commensal bacteria. The combined effects of engineered RNA-guided nucleases and the delivery carriers provides hope of combating the accelerated evolution of drug resistance.

Limitations

This therapeutic is still at the developmental stage and limited information is known about its efficacy and effectiveness. Also, there are some concerns regarding CRISPR-Cas antibacterial modes delivery. For example, the possibilities of the bacteriophages to cause overreactions in human immune system. Therefore, more studies are needed to confirm their effectiveness, as well as the side effects they can cause in animal models and in human research subjects.

Nanotherapy

Nanoparticles (NPs) or ultrafine particles are particles of matter that have at least one dimension with a diameter of 1–100 nm or whose basic unit in the three-dimensional space is in this range. NPs' small size enables better interaction with cells due to a larger surface area-to-mass ratio and versatile and controllable application. NPs' small size also facilitates the delivery of drugs to a specific location in the body, because it allows them to stay in the blood and circulate in the organism until they reach their target. Additionally, unlike the conventional antibiotic, NPs work by a direct contact with the bacteria without the need to penetrate the cell. This makes most of the resistance mechanisms irrelevant. The growing excitement about this novel therapeutic is because bacteria are less prone to developing resistance to its mode of actions.

There is evidence that NPs can serve as effective weapons against MDR infections of both Gram-positive and Gram-negative bacteria. For example, ZnO NPs was found to inhibit *Staphylococcus aureus*. Meanwhile Ag NPs was found to possess inhibition activities against *Escherichia coli* at a particular concentration level (Kadiyala et al., 2018). Furthermore, many NPs such as Au-based NPs, Ag-based NPs, and Fe₃O₄ NPs have shown the ability to prevent or overcome biofilm formation which harbors many MDR strains. For example, a studied conducted by Wang et al. (2017), showed that “a concentration of nanosilver as low as 0.05% can

significantly reduce the number of arthroplasty surgery-related infections, including methicillin-resistant *S. aureus* (MRSA), *S. aureus*, *S. epidermidis*, and *Acinetobacter baumannii* infections.” (Wang et al., 2017). However, more studies are needed to fully elucidate how this novel therapeutic will help us fight the growing MDR crisis.

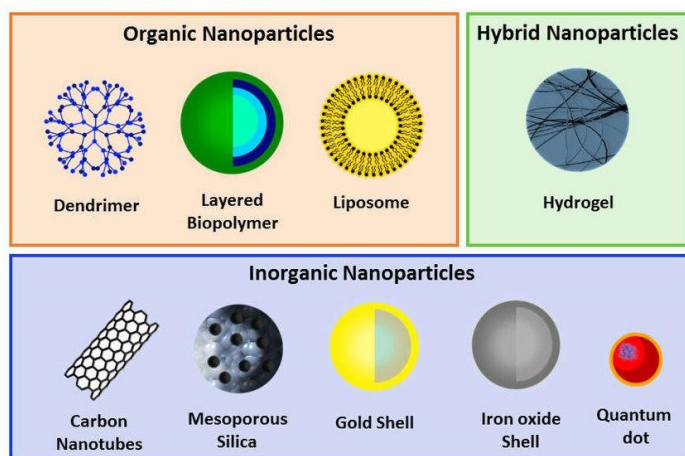


Figure 9

Schematic representation of different types of nanoparticles (NPs)

Brief history

In the 1900, Max Plank and Albert Einstein came up with the theoretical proposing the existence a range of tiny particles. In 1902, structures smaller than 4 nanometers were detected. In the following years, the development of microscopes with better resolutions made it possible to demonstrate nanoscale structures, and, to position and manipulate them in a controlled way. This new and exciting field in science was first referred to as nanotechnology in the 1974 by Norio Taniguchi. The intensive amount of research was done which demonstrated various application of NPs. In the early 1990, NPs modified for the first time were used to transport DNA fragments and genes into cells using antibodies. However, it was not until 1991 that the term “*nanomedicine*”

was allegedly used for the first time by K. Eric Drexler, Chris Peterson and Gayle Pergamit in their book *Unbounding the Future*. The term was later established in the book *Nanomedicine* by Robert A Freitas which was published in 1999 (Krukemeyer et al., 2015). Since then, nanomedicine has been used in different therapeutic including cancer treatments. The current AMR crisis has now motivated researchers to put the NPs characteristics to use by developing NPs antibacterial drugs. Current studies show hope in this therapy.

Mechanism

NPs drug can enter the body by inhalation, oral ingestion, intravenous injection, and contact with the skin. Once inside, NPs interact with bacteria membranes using electrostatic attraction, van der Waals forces, and receptor-ligand, and hydrophobic interactions. If the conditions are compatible, NPs enter the cells via different methods: “Macropinocytosis, phagocytosis, Clathrin-mediated endocytosis, clathrin-caveolin independent endocytosis, and caveolae-mediated endocytosis” (Foroozandeh, 2018). NPs' physicochemical properties including size, charge, zeta potential, surface morphology, and crystal structure play significant roles in their action against bacteria.

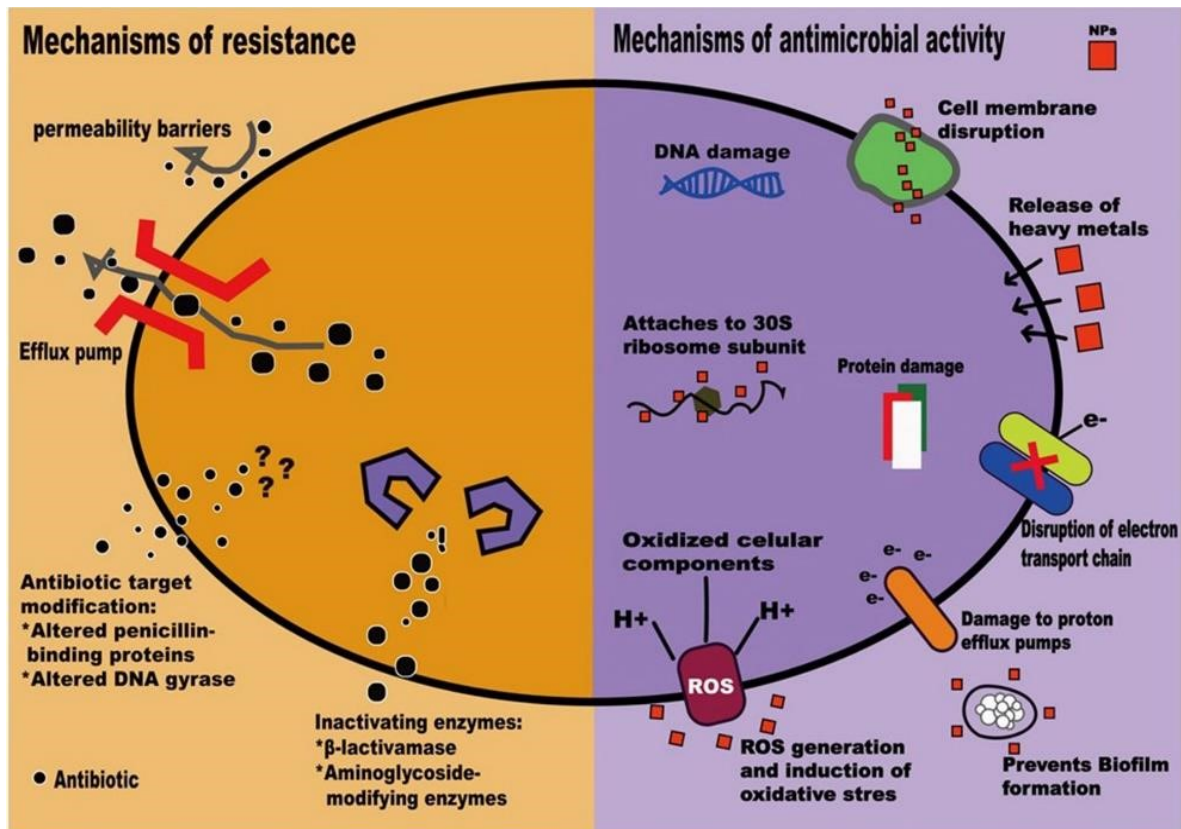


Figure 10

Schematic of mechanism of NPs antibacterial as compared to mechanism of resistance for conventional antibacterial

When NPs enter the intracellular of a bacterium, they interact and damage the cell's basic component such as DNA, lysosomes, ribosomes, and enzymes which influences the shape of the cell and disrupts functions of the cell membrane, thus, leading to cell death. Additionally, NPs also induces oxidative stress which can disrupt and change the permeability of the cell membrane. This can lead to increased influx of toxic drug. Therefore, scientists are exploring how NPs could potentially utilized as cofactors or carriers of other antibacterial drugs.

Limitations

NPs' mode of actions and effectiveness can be affected by many environmental factors including aeration, pH, and temperature. One of the concerns about the usage of NPs is limited information of the potential toxicity and the level of hazard they pose to human. Currently known, high levels of ROS can potentially be destructive to eukaryotic cells. Another concern is the potential transfer of ARGs by plasmid conjugation among same species and across genera. For example, Qui et al., showed that *nanoalumina* (5 mmol/L) promoted the conjugative transfer of plasmid RP4 between bacteria of the same genus, more specifically from *E. coli* to *E. coli* by 200-fold. *Nanoalumina* also significantly promoted the conjugative transfer of plasmid RP4 from Gram-negative bacteria to Gram-positive bacteria by more than 50-fold (Qui et al., 2012; Wang et al., 2017). Given the complexity levels of these concerns, thorough investigations should be carried out to evaluate the effectiveness and side effects of NPs based antibacterial under different conditions, as well as their contribution to the transfer of ARGs.

Anti-plasmid and Plasmid Curing Therapy

A plasmid is a small extrachromosomal DNA molecule in the cytoplasm of a bacterium. It can replicate independently of the chromosomes. Many AMR genes are on the bacterium plasmid which eases the spread of drug resistance. Studies have suggested giving patients plasmid curing agents before surgeries can prevent the associated infections. (Buckner et al., 2018). Although plasmid curing does not guarantee complete AMR eradication, it can reduce ARGs spread in many sectors of life such as the agriculture sector which is associated with a large spread of AMR from human and animal waste fertilizers (Meek et al., 2015; Rahube et al., 2016). Also suggested by Buckner and colleagues, “plasmid curing could be used to remove ARGs from

bacteria in sewage before being released into the environment.” Studies on plasmid curing have been around for decades. However, there are many concerns about their toxicity and side effects. This has prevented them from being used in medicine.

Brief history

Plasmid curing is a process that has been around for decades. Research for potential plasmid curing agents gained momentum in the 1970s. By the 1980s, many researchers reported possible agents, however, majority of them were toxic and/or had side effects which made them less effective for medical use. Due to the rising in AMR, there is a resurgence of interest in plasmid curing as preventative therapeutic. The advances in technology have provided better options for the plasmid curing agents such as the CRISPR/Cas-based plasmid curing system.

Mechanism

Plasmid curing has different agents including antibiotics e.g., rifampicin, detergents e.g., sodium dodecyl sulfate (SDS), biocides e.g., triclosan, natural products e.g., Plumbagin, phage therapies, other plasmids, CRISPR/Cas etc. A complete mechanism of plasmid is still unclear; however, researchers have been evaluating the success of plasmid curing based on the efficacy level of reversing “plasmid-mediated antibiotic resistance and/or by physical loss of the plasmid(s)” (Buckner et al., 2018). A study conducted by Lopatkin et al. (2017) suggested removing plasmid from a bacterium by inhibiting plasmid conjugation.

Plasmid conjugation inhibition

Studies have shown that conjugative transfer of plasmids by type IV secretion systems increases AGRs spread. Having a mechanism to inhibit conjugation will have a huge impact in controlling the AMR crisis. I reviewed a study conducted by Casu et al. (2017) in which they

developed a small molecule 239852 to prevent the dimerization of TraE. TraE is “an essential component of the type IV secretion system involved in a variety of functions including conjugation of pKM101.” Using X-ray structures, they obtain molecules 1E6 (2-furoic acid) and 4H10 (2-chloroisonicotinic acid).

One 1E6 molecule bound in the inhibitor-binding surface groove and another bound to an α -helical region at the dimerization site of VirB8-like proteins on TraE. Molecule 4H10 bound adjacently to the inhibitor-binding surface groove. Due to their proximity, 1E6 and 4H10 were combined to form molecule 239852 (2-(2-furyl) isonicotinic acid) to yield higher affinity. They confirmed that new molecule 239852 binds to the previously described inhibitor binding surface groove. They also looked at another molecule 105055 (4-(1H-pyrrol-1-yl) pyridine-2-carboxylic acid). Another new molecule 105055 was found to bind to the α -helical region of TraE close to the dimerization site of VirB8-like molecules. Their study findings showed that combination molecules 239852 and 105055 can inhibit dimerization of the protein and significantly reduced conjugative plasmid pKM101 by 45% as compared to the control. Furthermore, adding the known TraE inhibitor BAR072 to 239852 and 105055 mixtures, greatly decreased plasmid conjugation to 4%.

Other mechanisms for plasmid curing include “prevention or reduction of plasmid replication by agents integrating into DNA, breaking DNA or influencing plasmid supercoiling.” Furthermore, increasing the fitness cost associated with plasmid carriage can also lead to plasmid curing” (Buckner et al., 2018).

Limitations

There are many limitations of using this therapy. Many curing compounds especially those that were discovered early on have been found to be toxic. Additionally, the new agents such as the CRISPR/Cas-based plasmid curing system are still at the developmental stages, hence, more studies are needed to expand on the knowledge of these agents as well as expand libraries of curing agents available for use.

Discussion

The MDR in the bacterial population is currently on the rise. This is a big concern for the public health sector because conventional antibiotics that were once considered “miracle cure” are increasingly becoming more prone to resistance and have been associated with the development and the spread ARGs. Hence, alternative treatments are urgently needed to combat ARGs spread. In this literature review, I researched alternative antibacterial treatments. My aim was to find different antibacterial therapeutics that can reduce ARGs spread in the bacteria population. I found many intriguing options such as bacteriophages, CRISPR-Cas based antibacterial, nanoparticles, anti-plasmid and plasmid curing agents, antimicrobial peptides and proteins (AMPs), quorum sensing inhibitors (QSI), peptide nucleic acid (PNA), and zinc finger nucleases (ZFNs). Notably, many of these therapeutics are not entirely new and have been in place for decades before the antibiotic era. Unfortunately, all these therapeutics can also potentially lead to ARG development and/or spread. For example: plasmid conjugation, a mechanism common in many of these therapeutics like in CRISPR-Cas based antibacterial, is associated with potential transfer of ARGs among bacteria of the same species and across the genera. Based on this, it is hardly possible to develop a revolution-free therapy. However, the chances at which these alternative antibacterial therapeutics contribute to ARGs’ transfer happens at a lower frequency than in the conventional

antibiotics which makes them better options for medicine. Due to time constraint, this review only explores in detail: bacteriophages, CRISPR-Cas based antibacterial, nanoparticles, and anti plasmid and plasmid curing agents.

Phage therapy is an ancient method that has been in use for a while. Although it had lost its popularity in the western medicine due to the discovery of antibiotics, it has remained a big part in the eastern medicine. The usage of bacteriophages has resurfaced in the western medicine because of the growing antibiotic resistance. Despite the available limited information, phage therapy has shown to have great advantages over the conventional antibiotics including host specificity, the ability to self-maintain, as well as the ability to adapt to the defense mechanism of their prey.

On another hand, CRISPR-Cas based antibacterial therapy is new in medicine, because of the interesting mechanism by which these drugs would work, it has captured many people's eyes. The engineered CRISPR-Cas based nucleases work by instructing the bacteria's defense mechanism to destroy their own DNA. This is very important because destroying the CRISPR-Cas system decreases the chances of developing of ARGs for bacteriophages. It worth noting how the usage of bacteriophages defeats the initial mechanism by which bacteria utilizes CRISPR-Cas to recognize and destroy bacteriophages' DNA. Instead, this therapeutic utilizes phages to infect bacteria and convince them to destroy their own DNA (Gholizadeh et al., 2020).

Nanotherapy is also important to learn about. NPs antibacterial drugs have also shown great advantages over conventional antibiotics because of their physicochemical properties such as a small size, which enables greater surface interaction and the ability to stay in the blood without being filtered out until it reaches the target site. This helps to ensure the presence of the minimum

inhibiting concentration at the infection site which limits the bacteria's ability the development of ARGs.

The properties of these therapeutics, also make them some of the best options for the anti-plasmid and plasmid curing agents. Additionally, studies have suggested that a combination of these therapeutics or with antibiotics could further elevate their effectiveness as treatments for bacterial infections.

In conclusion, although curing an infected patient is an ideal goal in medicine, to fully address the rising MDR crisis, preventative measures such as plasmid curing must be employed especially in high-risk areas like in clinical settings and in water treatment systems. Therefore, more focus should be directed towards the development of noninvasive anti-plasmid drugs capable of removing ARGs by the removal of the plasmids from the bacteria of interest.

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