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HUNT FOR NON-ANTIBIOTIC ANTIBACTERIAL APPROACHES: A POSSIBLE GAME CHANGER?

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ABSTRACT

A considerable technological advancement in the present decade has immensely improved healthcare system, contributing to longer lifespan of humans, but at the same time, witnessed some of the deadliest aftermath of antibiotic resistance. Multi-drug resistant (MDR) pathogenic bacteria are nowadays evolving at an alarming rate, which has already led to several hundreds of deaths worldwide. Although natural alternatives to antibiotics do exist in nature, the biggest limitation is implementing them as treatments for humans. These limitations are mainly due to high cost of production, lower efficacies and unforeseen side effects. Several genetic engineering techniques are recently seeing success in humans as treatments for diseases caused by such MDR-bacteria. The objective of this review is to discuss on some potential non-antibiotic approaches to resist multi-drug resistance in bacteria.

Keywords: antibiotic resistance, genetic engineering techniques, multi-drug resistant, non-antibiotic approaches, pathogenic bacteria

INTRODUCTION

Massive advancements in healthcare and medicine have led to longer lifespan of mankind in general. One of these achievements includes the discovery of antibiotics by Sir Alexander Fleming which enabled mankind to overcome several contagious diseases. But due to unrestricted usage of antibiotics all across the globe, bacteria have become increasingly resistant to them, which thus pose an inescapable threat to mankind. The Centers for Disease Control and Prevention (CDC) reported the burning issue of antibiotic resistance in their AR (Antibiotic Resistance) Threat Reports of 2013 **[1]**. They reported the emergence and spread of various drug-resistant bacterial strains like *Clostridium difficile, Neisseria gonorrhoeae, Pseudomonas aeruginosa, Staphylococcus* spp., *Campylobacter* spp., *Acinetobacter* spp. and many more **[1]**. Bacteria are constantly evolving and developing new mechanisms to defend themselves against antibiotics **[1, 2]**. Rising cases of infectious diseases due to these new and re-emergent multi-drug resistant (MDR) pathogenic bacteria is a global peril, and researchers all around the world are trying to look for the alternative treatments against these notorious pathogens **[3]**. This review aims to bring together several such novel antibacterial approaches that can act as substitutes for antibiotics.

1. NATURALLY OCCURRING ALTERNATIVES/TRADITIONAL APPROACHES

A) Virus- and Bacteria-based approaches:

- **Phage Therapy:** The evolving MDR bacteria question the efficacy and usage of antibiotics to treat infections currently **[4]**. Phages can select their susceptible host amidst a mixed bacterial population via specific interactions between the cognate bacterial cell surface proteins and tail fiber proteins on the phage. This leads to the injection of their genetic material into the host cell and by hijacking the host cell machinery, they can propagate, ultimately lysing the host cell, and completing the lytic phase. The progeny phages, thus released, can now initiate a new round of infection **[5]**. Therefore, targeting pathogenic bacteria, without the expense of commensal bacteria, by bacteriophages can be an effective treatment against antibiotic resistance **[6]**. Recently, investigations in animal models have provided positive results using phage therapy where gut-

derived sepsis caused by *Pseudomonas aeruginosa* in mouse models rescued approximately 67% of the mice by oral administration of phages as compared to the control set **[7]**. Successful application of phage therapy was effective for several human pathogens including *E. coli, Proteus* spp., *P. aeruginosa*, *Staphylococcus aureus*, *Salmonella* spp. and *Enterococcus* spp. **[8]**. Phage therapy offers a few advantages over the traditional antibiotic method, the most important one being the species and strain specificity **[9]**. The specificity of phages enables targeted killing of pathogenic bacteria as opposed to the broad-spectrum antibiotics that causes hideous secondary effects such as *C. difficile* infections (CDIs) and antibiotic-caused diarrhea due to microbiota dysbiosis **[9]**. But by targeted killing of only one strain of bacteria, it is a less effective therapy than antibiotics against infected burn wounds **[10]**. Fortunately, phages are equipped with extracellular polymeric substance (EPS)-depolymerase, an enzyme that destabilizes the polysaccharide coat of the biofilm-forming bacteria, allowing ultimate access of phages to the cells and bringing about cell lysis **[11]**. Despite the multifaceted advantages, phage therapy suffers from the inherent disadvantage of the release of endotoxins and pyrogenetic substances from the lysed cells, which need to be removed to ensure an efficient therapy **[4]**.

- **Lysins:** Endolysins (more simply lysins) are phage-encoded enzymes capable of hydrolysing the host bacterial cell walls **[12]**. Phage lysins appear to be an appealing resource for avoiding some of the existing problems in antibiotic therapy. Exogenously injected lysins disrupt peptidoglycan cross-links in the bacterial cell walls, resulting in strong bactericidal actions against sensitive bacteria **[12]**. These enzymes have several unique characteristics, like they do not elicit a negative immunological response and bacterial resistance is unlikely to develop **[12]**. These lysins are effective against particularly Gram-positive pathogenic bacteria like *S. aureus, Streptococcus pneumoniae, Streptococcus pyogenes*, and in limited cases against

Mycobacterium tuberculosis **[13]**. Certain lysins have been isolated, among which PlyPa03 and PlyPa91 are effective against Gram-negative bacteria like *P. aeruginosa* **[14]** and *Acinetobacter baumannii* **[15]** also. TSPphg, a new phage lysin discovered in extremophilic *Thermus* phage, exhibits bactericidal action against both Gram-positive and Gram-negative pathogenic bacteria, particularly antibiotic-resistant *Klebsiella pneumoniae* strains **[16]**.

- **Antimicrobial Peptides (AMPs):** Antimicrobial peptides (AMPs), usually 12-50 amino acids in length, contain a net positive charge that causes bacterial cell lysis **[4]**. Present reports suggest that some AMPs can interact synergistically with conventional antibiotics, reducing chances of selection of resistant clones under selective pressures **[17]**. Several AMPs under clinical trials include nisin A, gramicidin, polymyxin and melittin **[18]**. The success of AMPs is still not convincing due to unfavourable pharmacokinetic profiles (as in the case of Friulimicin B) **[19]**, safety and efficacy issues (as in the case of murepavadin) **[20]**. Bacteriocins are ribosomally-translated AMPs synthesized by both Gram-positive and Gram-negative bacteria against another group within a population, which can serve as alternatives to antibiotics. Bacteriocins may be broad-spectrum or narrow spectrum **[21]**, but the advantage of bacteriocins over other AMPs is mainly due to their ability to survive harsh environmental conditions like UV-rays, extreme changes in pH and temperature **[21]**. Some bacteriocins have shown promising activity *in vivo*, including Nisin-F produced by *Lactobacillus lactis* F10 which when administered intranasally prevented respiratory infections in rats infected with *S. aureus*, irrespective of the immunological status **[22]**. Due to their small-size, low toxicity, specificity of targeted killing of pathogenic strains and biodegradability, bacteriocins are a promising alternative to antibiotics **[21]**. Short synthetic peptides known as Innate Defence Regulators (IDRs), another class of AMPs, regulate the innate immune signalling pathways, and bacteria are unable to develop

resistance against them **[23]**. SGX94, a lead clinical compound derived from IDR class of compounds, has broad-spectrum activity against various Gram-negative and Gram-positive bacterial infections by regulating the host cytokine levels, thereby modulating inflammatory responses **[23]**.

B) Plant-based approaches: Secondary metabolites obtained from plants can also serve as antimicrobial agents. Phenols and phenolic acids might be useful additions to the antibacterial arsenal **[24]**. Pyrogallol-based chemicals such as resorcinol, catechol, gallic acid and ferulic acid (hydroxycinnamic acid), are more powerful than others, in degrading the bacterial cell wall **[24]**. These cause cellular contents to seep out, and are more potent against Gram-positive bacteria **[24]**. Flavonoids also contribute to killing bacteria by virtue of their inhibitory effect on DNA gyrase and subsequently hampering replication **[25]**. They also disrupt cell membrane of bacteria and metabolism **[25]**. Alkaloids **[25]**, terpenoids **[26]** and tannins also have potential antibacterial effects **[25]**.

C) Coral-based approaches: Certain secondary metabolites derived from corals also have antibacterial activities against *S. aureus, Staphylococcus epidermidis, Bacillus cereus, E. coli, Tetragenococcus halophilus, Pseudomonas putida, Vibrio parahaemolyticus* and *Nocardia brasiliensis* **[25]**.

2. BIOTECHNOLOGICAL ALTERNATIVES

- **Bioengineered Antimicrobial Peptides:** Current research focuses on the modifications of existing AMPs or developing new AMPs by 'homology modeling' to use them against biofilms, persister cells and drug-resistant bacteria **[27]**. Avidocin CD, a modified bacteriocin could prevent CDIs while maintaining the indigenous gut microbiota in an unaltered state **[28]**. Bioengineering several bacteriocins like Enterocin A, Pediocin PA-1 and nisin variants increased efficiency in a variety of pathogens **[21]**.
- **CRISPR/Cas9:** The novel approach of RNA-guided DNA cutting can be exploited to eliminate the pathogenic MDR bacteria selectively **[29]**. Phase 1b human trials in patient volunteers with chronic urinary tract infections (UTIs) were reported to be a success in February 2021 by Locus Biosciences **[30]**. CRISPR-Cas3 also made phage more effective in killing

E. coli by direct administration into bladder by catheter in Phase 1 trials **[30]**.

- **Vaccines:** While antibiotics generally operate by a single mode of action - either by disrupting the bacterial cell membrane or by inhibiting the translational machinery **[31]**, vaccines often target multiple epitopes of the same antigen, or can even target multiple antigens, reducing chances of resistant clone evolution. Several vaccines are now under investigation against a variety of pathogens including *C. difficile, E. coli, S. aureus, N. gonorrhoeae, P. aeruginosa, K. pneumoniae, Shigella* spp., invasive non-typhoidal *Salmonella, Salmonella enterica*, Group A *Streptococcus* and *M. tuberculosis* **[32]**. Pioneering novel vaccine technologies including reverse vaccinology, use of adjuvants and designed bacterial outer membrane vesicles have the potential of targeted killing of MDR-pathogenic microbes **[32]**.
- **Nanotechnology:** Nanotechnology is a scientific and engineering technology conducted at the nanoscale, and is recently seeing applications in the medical field as well. Nanoparticles can create an effective nanostructure for delivering the antimicrobial agent, efficiently targeting the bacterial community **[33]**. Meanwhile microbial pathogens cannot develop resistance to them, and they can enter bacterial cells easily **[33]**. The nanoparticles disrupt the bacterial outer membrane, inhibit the exchange of ions, and trigger apoptotic pathways **[34]**. Silver, gold, copper, iron, and zinc are the metals most often utilized in metal-based nanoparticles **[35]**. Oxides of these metals have potent bactericidal activity and can be used to kill several bacterial species **[34, 35]**. Silver-nanoparticles cause oxidative stress by formation of reactive oxygen species (ROS) **[36]**. They also induce protein malfunctions and DNA damage in bacteria, resulting in cell death **[36]**. Gold nanoparticles are stable and biocompatible, and they may be easily altered to improve their antibacterial characteristics by changing their structure and size, or by adding chemicals **[37]**. Gold nanoparticles can also increase the antibacterial properties of loaded

antibacterial medicines by acting as drug transporters **[37]**. Photothermal effects of modified gold nanoparticles can be utilized to destroy bacteria by photothermal therapy **[37]**. The production of ROS (Reactive Oxygen Species) by zinc and copper nanoparticles disrupts bacterial growth. Zinc nanoparticles, especially ZnO-based nanoparticles, have been developed and evaluated for use in healthcare and medicine due to their antibacterial activity **[38]**. Their capacity to kill microorganisms, including pathogens, has been shown to be effective in *Listeria monocytogenes*, *E. coli*, *S. aureus*, *P. aeruginosa, Campylobacter jejuni* and *S. enterica* **[38]**. Copper nanoparticles have shown promise as antibacterial agents against common human pathogenic bacteria like *E. coli* and *S. aureus* **[39]**. Antibacterial activity of iron was demonstrated by Fe_3O_4-PEG magnetic nanoparticles (MNPs) against two bacterial strains: Gram-negative *E. coli* and Gram-positive *S. aureus* **[40]**. The modified MNPs killed bacteria by inducing DNA fragmentation, although the effects were more pronounced in *S. aureus* than in *E. coli* **[40]**. At effective concentrations which are used to kill bacterial cells, most metal oxide nanoparticles have minimal toxicity toward humans, which is a merit for employing them on a large scale **[34]**.

3. MODIFICATIONS OF GUT MICROBIOTA

- **Use of Predatory Bacteria:** Grouped along with other classes of 'living antibiotics' that include phage therapy, probiotics and targeted killing of pathogens, predatory bacteria have gathered momentum over the last few years **[41]**. *Bdellovibrio* spp. and like-organisms (BALOs), belonging to the class of δ-proteobacteria, have been identified as potent biocontrol agents as they can prey upon a variety of Gram-negative bacteria including some MDR-human pathogens viz., pathogenic strains of *E. coli, Salmonella* spp., *Pseudomonas* spp. and *Legionella* spp. **[42]**. Hunting strategies of predatory bacteria can be subdivided into two major groups: epibiotic and endobiotic **[43]**. Epibiotic predation is further sub-divided into two categories: (A) Lone

predation, where some representative members include: *Bdellovibrio exovorus*, *Micavibrio* spp., and *Vampirococcus* spp. **[44]**; and (B) Communal predation, which involves actinobacterial species of the genus *Streptomyces* which are a special group that do not require direct contact with the prey for lysis **[44]** and a biofilm-forming group, *Myxococcus xanthus*, that require proximity to the prey cells to disrupt them by the secretion of antibiotics such asmyxovirescin and hydrolytic enzymes such MepA protease **[45]**. Endobiotic predation involves penetration into the cytoplasm or periplasmic space of the prey by the predator where it grows ultimately causing cell lysis **[44]**. *Bdellovibrio bacteriovorus* can reduce the dreaded opportunistic MDR-human pathogen *Klebsiella pneumoniae* in human serum and buffer, though the time required for reduction in prey numbers varied under different environments **[46]**. Though there can be re-growth of the pathogen after predation, predatory bacteria can serve as a promising 'living antibiotic' to combat the antibiotic-resistant pathogens **[46]**. *B. bacteriovorus* strain 109J did not show any effects on the viability of cells and did not induce any inflammatory responses on the human cell lines tested **[47]**. This highlighted on their non-pathogenic traits pointing out as a probable therapeutic **[47]**.

- **Use of Probiotics:** Probiotics are living organisms that provide health benefits to humans (WHO/FAO, 2002) **[48]**. Human gut microbiota comprises of several commensal bacterial and yeast species **[4]**. *Helicobacter pylori* are responsible mainly for causing ulcers in stomach and upper part of the small intestine **[49]**. Probiotics have been proven effective against *Helicobacter pylori* infections, resulting in increased host tolerance by promoting competition for adhesion **[49]**. Probiotics have also been effectively used against *K. pneumoniae*, an opportunistic pathogen; *C. difficile*; Group A rotavirus, the causative agent of infantile diarrhea, and also administered in COVID-19 patients to prevent any secondary infection **[50]**. Probiotics present a promising

alternative, because no adverse effects have been reported till date with their long-term usage **[50]**.

- **Fecal Microbiota Transplantation (FMT):** Fecal Microbiota Transplantation (FMT) is defined as the transplantation of stool from healthy individual (donor) to a patient (recipient) who is believed to suffer from diseases related to unhealthy gut microbiome **[51]**. It has been successful in treating recurrent *C. difficile* infections (rCDIs) **[51]**, and is currently being investigated for treating disorders related to gut dysbiosis. For successful application of FMT donor screening, standardized protocols for stool preparation and delivery must be focused on **[51]**. It has also been shown to be successful against clearing colonization with MDR-*E. coli, Salmonella* spp*., S. aureus, Acinetobacter* spp. and *Klebsiella* spp., but promising reports in human subjects is only very limited **[4]**.

CONCLUSION

The goal of this study was to look for feasible alternatives to antibiotics in the fight against MDR-bacteria. Clinical trials are ongoing for these novel antimicrobial agents, so that they can be safely applied to the field of medicine. Alternative non-antibiotic approaches are need of the hour to face the rapidly emerging threat of antibiotic resistance. Experts from diverse fields need to come together and develop novel strategies to address this challenge, restricting the unnecessary over-usage of antibiotics.

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CONFLICT OF INTEREST

The authors declare that there is no potential conflict of interest.

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