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Modified Bacteriophage to Kill Multidrug Resistant Bacteria

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Abstract:
Phage therapy is an important alternative to antibiotics in the current era of multidrug resistant pathogens. Scientists have engineered bacteriophages, to use CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats), which is the gene-editing system to kill specific bacteria. These viruses infect only specific species or strains of bacteria, so they have less of an impact on the human body's normal flora than antibiotics do.

Introduction:
Throughout much of the twentieth century, antibiotics have been a primary defense against bacterial disease. Unfortunately, inappropriate and excessive use of them is threatening their efficacy, thus many of the bacterial pathogens have evolved into multidrug-resistant (MDR) forms. It develops when bacteria mutates or acquires a resistance gene, and they're called "*superbugs"*. The rapid emergence of those superbugs is occurring worldwide.(1) However, there are deferent various ways to combat this crisis, and scientists are discovering new techniques to fight superbugs, and one of them is the *phage therapy*. Phage therapy relies on the use of naturally occurring bacteriophages to infect and lyse bacteria at the site of infection. Current research on the use of phages and their lytic proteins, specifically against multidrug-resistant bacterial infections, suggests phage therapy has the potential to be used as either an alternative or a supplement to antibiotic treatments.(2)

Discussion:
Phages are simple, diverse, non-living biological entities consisting of DNA or RNA enclosed within a protein capsid. They are incapable of reproducing independently, and are ultimately dependent on a bacterial host for survival.
Bacteria have evolved numerous mechanisms to resist infection by lytic phages, and phages have an equally impressive diversity of mechanisms for breaking this resistance. For bacteria, this can include alteration or loss of receptors and integration of phage DNA into the clustered regularly interspaced palindromic repeats/CRISPR associated system (CRISPR/Cas) system, while for phage this can include recognition of new or altered receptors and anti-CRISPR genes.
Innovations in the gene-editing tool CRISPR/Cas have created opportunities for phage therapy. One example of which is the use of bioengineered phage to deliver a CRISPR/Cas programmed to disrupt antibiotic resistance genes and destroy antibiotic resistance plasmids. These phages may be applied to hospital surfaces to reduce frequency and spread of antibiotic resistance genes.
Animal studies have shown promising results for multidrug-resistant *E. coli* O25:H4-ST131, *Vibrio parahaemolyticus*, *S. aureus*, and *A. baumanii*.
One of the phage lytic enzymes that are found in the majority of phage species is the peptidoglycan cell wall hydrolase called endolysin (lysin). Lysin is capable of bacterial cell lysis; it's fast acting, potent, and inactive against eukaryotic cells.
Lysins have successfully saved mice from bacteremia caused by multidrug-resistant *A. baumannii*, *Streptococcus pneumoniae*, and MRSA, among others. Combining phage lysins and antibiotics may be more effective at eliminating infections than by using antibiotics alone.(2)

There is an indication that phages are capable of restoring antibiotic sensitivity in antibiotic-resistant bacteria, as in the case of multidrug-resistant *P. aeruginosa*. Individuals with cystic fibrosis, severe burns, surgical wounds and/or compromised immunity are particularly at risk for *P. aeruginosa* infections, especially acquired in hospitals. *P. aeruginosa* infections are known to be difficult to manage. The most problematic mechanism of resistance to antibiotics is drug efflux *via* multi-drug efflux (Mex) systems, which extrude different antibiotics that permeate the cell. Mex systems contain three components that function *via* active transport to move numerous molecules out of the cell: an antiporter that functions as a transporter (e.g., MexB, MexY), an outer membrane protein that forms a surface-exposed channel (e.g., OprM), and a periplasmic membrane fusion protein that links the two proteins (e.g., MexA, MexX). Scientists predicted that phage binding to surface-exposed OprM of the MexAB and MexXY systems of MDR *P. aeruginosa* would exert selection for bacteria to evolve phage resistance, while impairing the relative effectiveness of these efflux pumps to extrude chemical antibiotics. We obtained samples from six natural sources and enriched for phages that could infect *P. aeruginosa* strains PA01 and PA14, two widely used MDR *P. aeruginosa* models. This effort yielded 42 isolated phages that infected both strains of MDR *P. aeruginosa*. To test if any of these phages could bind to OprM of MexAB and MexXY efflux systems, they used a transposon knockout collection of bacterial mutants derived from *P. aeruginosa* strain PA01. Results showed that one of the 42 phage isolates failed to infect the *oprM* knockout strain, but successfully infected wildtype PA01 and all other tested knockout mutants. This phage was originally isolated from a freshwater lake sample. Phages drive the bacteria to evolve increased phage resistance by suffering increased sensitivity to chemical antibiotics. Thus, this approach to phage therapy should be doubly effective; success is achieved when phage lyses the target bacterium, and success is also achieved when bacteria evolve phage resistance because they suffer increased sensitivity to antibiotics.[3]

Another study aimed to isolate multi drug resistant bacteria from patients with septic wounds and then isolate and apply bacteriophages *in vitro* as alternative therapeutic agents.

Pus samples were aseptically collected and analyzed by gram staining. Results suggested that gram-negative bacteria were more predominant than gram-positive bacteria in septic wounds; most of these isolates were resistant to ampicillin, amoxicillin, penicillin, vancomycin and tetracycline. Bacteriophages isolated from sewage demonstrated perfect lytic activity against the multi-drug resistant bacteria causing septic wounds. *In vitro* analysis of the isolated bacteriophages demonstrated perfect lysis against the MDR-bacteria, and these isolated phages may be promising as a first choice for prophylaxis against wound sepsis. Bacteriophages have bactericidal activity against the pathogenic bacteria responsible for diseases, and they may quickly reduce bacterial loads.

Results demonstrated that both MDR-gram-positive bacteria (*S. aureus*) and gram-negative bacteria (*P. aeruginosa*, *E.coli* and *K. pneumoniae*) obtained from septic wounds were susceptible to bacteriophage lysis, and these phages deserve further study for optimization under *in vivo* conditions. In the future, phage therapy will be a reliable way to treat MDR-bacterial infections, although much work is needed to understand all the mechanisms involved, including use of phage cocktails for multiple bacterial infections.[4]

Conclusion:

Bacteriophages are naturally occurring killers of bacteria. They can be effective antibacterial agents due to their specificity against a particular bacterial species and lack of impact on other microflora. Multi-drug resistance has become a major problem for the treatment of pathogenic bacterial infections. The use of phage therapy is an attractive approach to overcome the problem of drug resistance in several pathogens that cause fatal diseases, and can also restore antibiotic sensitivity to some MDR bacteria.

References:

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