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Opinion

Use of Phages in Combination with Antibiotics

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Abstract

Antibiotic-resistant bacterial infections area significant concern for public health. Phage therapy has been proposed as a promising alternative to antibiotics, but an increasing number of studies suggest that both antimicrobial agents in combination are more effective in controlling pathogenic bacteria than either alone. We advocate the use of phages in combination with antibiotics and present the evolutionary basis for our claim. Also, we identify compelling challenges for the practical application of phage–antibiotic combined therapy [1].

Introduction

The absence of production and introduction of new and more effective antibiotic / antibacterial drugs in clinical practice during the post-antibiotic gold era has seen an increase in the appearance of resistant pathogenic bacterial infections that pose a significant problem in global human health. The current situation is that in 2011, about 722,000 patients had an infection while in the hospital for acute treatment in the United States, 205 Americans die of infections acquired in the hospital (infections acquired in HAI hospital, nosocomial infections) every day. Antimicrobial is the immediate threat of reducing detection and development of new antibiotics [2 ,3].

Tichoic wall acids are found only in certain bacteria for positive bacteria (eg staphylococci, streptococci, lactobacilli, and bacillus spp); To date, they have not been found in Gram organisms. Teichoic acids are polyol phosphate polymers, with either ribitol or glycerol linked by phosphodister bonds. Substituent groups on polyol chains can include D-alanine (linked ester), N-acetylglucosamine, N-acetylgalactosamine, glucose; the alternative is typical of a particular bacterial species and can be used as a specific antigenic agent. Tychoic acids are covalently linked to peptidoglycan. These highly negatively charged polymers of the bacterial wall can be used for cation-sequestering [4].

Natural phage treatments rely on causing bacterial death through ruptured cells. However, rapid bacterial lysis may result in the release of endotoxin and inflammatory mediators into the surrounding environment with side effects. In contrast, phages can be designed as bacteria by deleting genes responsible for lysis (eg endolysin). Some of the tail phages, known as mild, can undergo a latent life cycle after ligation, called lysogeny, and provide synthetic genetic networks with desirable antimicrobial properties. Here, the viral genome is integrated into the bacterial chromosome as a photograph or remains free of cytoplasm, then replicated alongside the bacterium to conditions in favor of reactivation to manufacture virions. Although conditioned pajas are generally avoided in natural phage treatments, they have been used to provide artificial gene networks that can disrupt cell cell communication between bacteria involved in biofilm formation. Or work as adjuvants to antibiotics, such as by suppressing DNA repair mechanisms or over expressing sensitive proteins. The draw backs of these approaches are that the merged phages will be intangible, which can be claimed to suffer without complications in the treatment of the use of bacteriologic phages. Which are encoded by flooding, also carries the risk of providing a variety of benefits to their bacterial hosts, which are often included within MORON elements and suggest that conditioned FAFs have a symbiotic relationship with bacteria rather than mere parasites as lytic, [5].Patients are infected every year, and the Institute for Health Improvement (IHI) estimates that more than 5,000 patients die each year as a result. While most patients are treated successfully, uniquely if the infection is identified early, hospital stays are often extended by an average of 9.1 days, accounting for costs in excess of approximately $ 20,000 per patient. The burden of the total cost of the American health care system against MRSA infections

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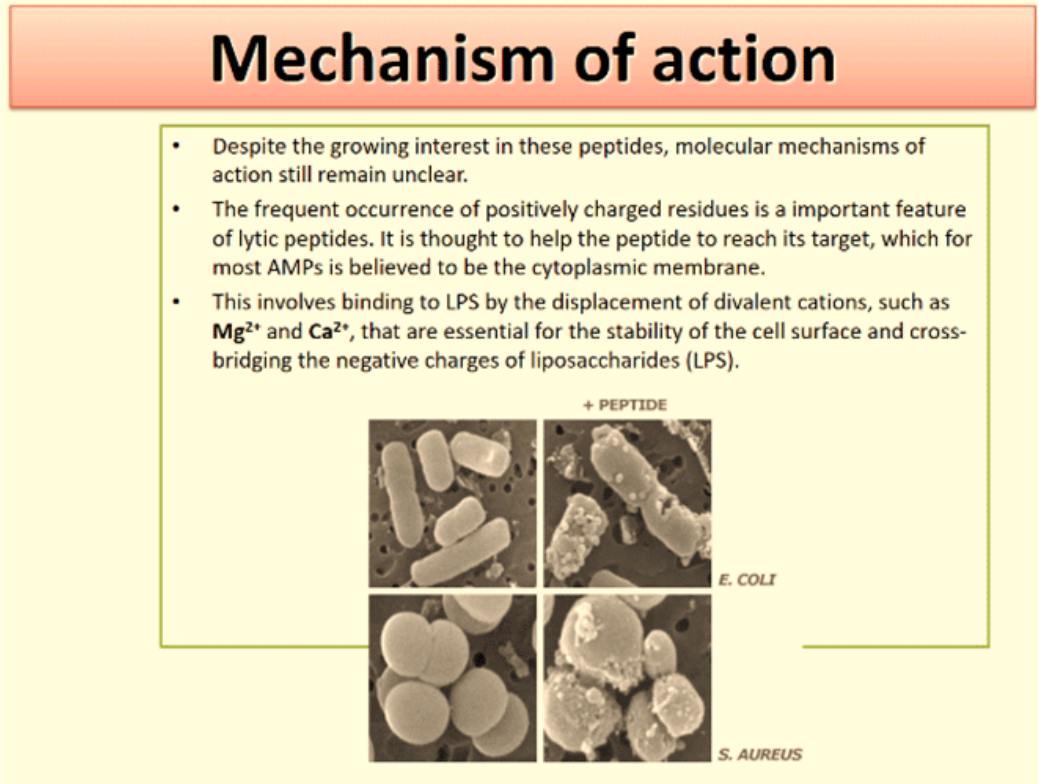
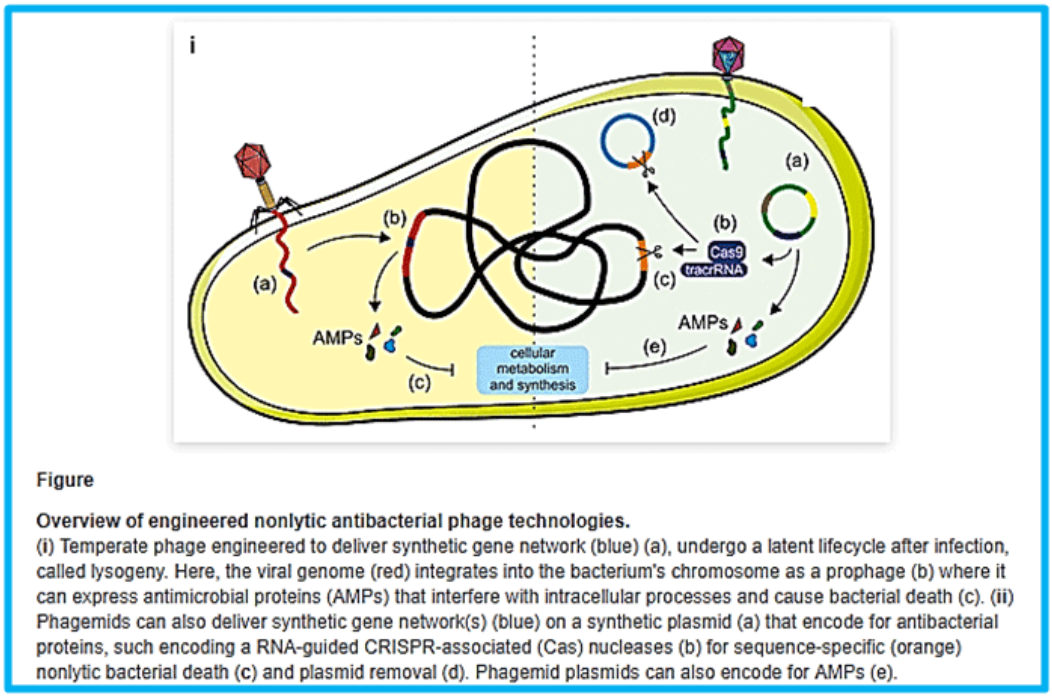


Figure 01:



Figure

Overview of engineered nonlytic antibacterial phage technologies.
(**i**) Temperate phage engineered to deliver synthetic gene network (blue) (a), undergo a latent lifecycle after infection, called lysogeny. Here, the viral genome (red) integrates into the bacterium's chromosome as a prophage (b) where it can express antimicrobial proteins (AMPs) that interfere with intracellular processes and cause bacterial death (c). (**ii**) Phagemids can also deliver synthetic gene network(s) (blue) on a synthetic plasmid (a) that encode for antibacterial proteins, such encoding a RNA-guided CRISPR-associated (Cas) nucleases (b) for sequence-specific (orange) nonlytic bacterial death (c) and plasmid removal (d). Phagemid plasmids can also encode for AMPs (e).

Figure 02:

(*Stethylococcusus aureus* and methicillin resistance [6]), is a bacterial disease that is spread by direct contact with an infected person or by touching a contaminated surface. MRSA is estimated at more than $2.5 billion annually. Gram-positive and Gram-negative bacteria exist all over the place (a common post-surgery infection in blue table below) [7, 17] But pose unique threats to patients hospitalized with weakened immune systems. Bacteria with positive bacteria cause enormous problems and are focused on many eradication efforts, but Gram harmful bacteria are developing dangerous resistance and are classified by the US Centers for Disease Control and Prevention (CDC) as a threat. For this reason, the need for current technologies to kill bacteria, both positive and negative gram, are vital to make hospitals safer for everyone. Two types of bacteria can cause a deadly infection and must be eliminated in the presence of the other. The treatment of those serious microbial infections in clinical use is often complicated by antibiotic resistance. However, there is an immediate need for innovative ideas in design and application of antimicrobial agents since bacteria gram-negative bacteria [8,9], develop strains that are intrinsically resistant to many antibiotics (persister) [10] strains, that are practically indifferent to all applied antimicrobial drugs. Moreover, in the last decades, only two antibiotic classes with a unique mechanism of action (an example is Teixobactin [11,12], a new cell wall inhibitor) have been published, and none of them are effective against persister Gram-negative microbes. (The Top Ten Most vicious Bacteria on Earth [13]).

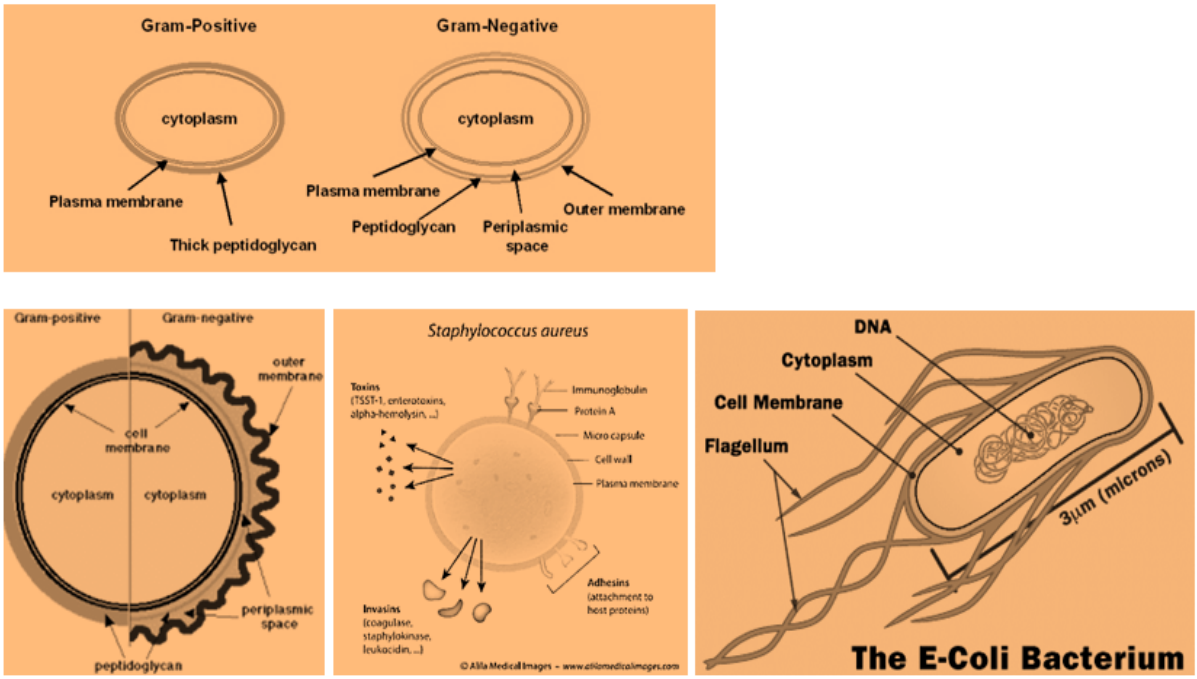


Figure 03:

Discussion

Antibiotic abuse reduced their effectiveness in controlling pathogens and led to an increase in the number of c-resistant antimicrobial resistant microbes. As an alternative to antibiotics, bacteriophages have become a topic of operation with the emergence of multidrug resistant bacteria, which pose a threat to public health. Recent studies have shown that bacteriophages can be used indirectly to identify pathogenic bacteria or directly as biocontrol agents. Furthermore, they can be used to develop new molecules for clinical applications, vaccine production, drug design, and nanomedicine using a phage display.The Use of phages in as anti-infective in therapy can be hugely successful based on the selectivity of the bacteriophages toward bacteria. Also, the mechanism of an action directed at the genetic materials in the nuclei of the microbial cells is different from the cell wall disruption characteristic to most antibacterial agents, mainly antimicrobial peptides[14] and their surrogates. However, Hospital-acquired infections are; like post-surgical infections are characterized by their multi-infectors blend. We can see more than five infectors in the post-surgical infection in the Ethiopian clinic. Here we can find many sorts of infectors, Gram-negative like Klebsiella spp. And Gram-positive S. aureus, treatment of such wounds will apply a mixture of phages, whereas today's treatment can apply one broad band anti-infective agents (delivered as a pill, injection, infusion) for all microbes listed [15].

Frequency of aerobic bacteria from postoperative wound infection at Ayder Teaching and Referral Hospital, Mekelle, Ethiopia (January to June 2012)

No.	Gram	Microorganism	Frequency N (%
1	G+	*S. aureus*	40 (34.2)
2	G-	*Klebsiella* spp.	29 (24.8)
3	G+	CoNS[a]	18 (15.4)
4	G-	*Proteus spp.*	15 (12.8)
5	G-	*P. aeruginosa*	11 (9.4)
6	G-	*E. coli*	6 (5.1)
7		*Citrobacter* spp.	4 (3.4)
		Total	123 (100)

Table:

Frequency of Aerobic Bacteria from Post-Operative Wound Infection at Ayder Teaching and Referral Hospital, Mekelle, Ethiopia (January to June 2012) [16]

In recent years there has been a remarkable interest in the old concept of using phage as a therapeutic tool. There are no doubt reports of decreased efficacy both in antibiotics and in the interest of pharmaceutical companies in the development of new agents that feed the drive of antibiotic alternatives. Although they are not without their constructive critics, approaches of all phage may well be an invaluable anti-infectious tool in certain therapeutic applications. However, we and others imagine an alternative use for phage that, although less heralded [16], focuses on their specific antibacterial components, rather than infectious virion, as an active kill agent. With emerging lysins as the most promising antibacterial candidate, defined by their specific strong-limeric activities, we continue with lysin development. Whether it is used topically or systemically, in humans or in agriculture, as purified proteins or as expressed by engineered animals or in bacterial secretion systems, a growing body of data validates the potential benefits of lysine. Even beyond the ears, the phage is a professional parasite of bacteria, and as such, employ an arsenal of agents to paddle the host function and structure. It just makes sense that a comprehensive search for new antibacterial agents will try mine this vast viral reservoir of antibacterial functions. If it is at all possible that "large portions of the amount" for phage, this work is justified [17].Indian researchers published on the test of bacteriophage versus antimicrobial agents for the treatment of murine burn treatment [18]. The widespread use of antimicrobial agents in hospital settings has led to the emergence of multidrug-resistant organisms of low virulence such as Klebsiella causing serious opportunistic infections [19]. Beside this, concerns about problems such as high cost of treatment and inability to restore initial appearance of skin have resulted in research on newer agents for the treatment of burn wounds. Bacteriophages or simply phages can be the best answer to antibiotic resistance in the treatment of bacterial infections [20]. These phages are economical, safe, self-replicating and effective bactericidal agents. In our earlier studies, we have reported the efficacy of phage therapy in treating various infections when injected systemically In the study, the efficacy of topical application of silver nitrate and gentamicin was checked and compared with that of a well-characterized Klebsiella-specific phage, Kpn5, for treating K. pneumoniae B5055 induced burn wound infection in BALB/c mice.Phage isolation. Klebsiella-specific phage Kpn5 was isolated from a sewage sample. Its utility in treating K. pneumoniae B5055 induced burn wound infections on i.p. the injection has been established (Kumari et al., 2009). In this study, phage Kpn5 was evaluated for topical treatment of burn wound

infection. Hydrogel preparation is also possible as an ointment to treat wounds. There are approaches used in the published literature on the formulation and stabilization of phage for storage and encapsulation of bacteriophage in micro- and nanostructured materials using freeze drying (lyophilization), spray drying, in emulsions e.g. ointments, polymeric microparticles, nanoparticles and liposomes. As phage therapy moves forward towards Phase III clinical trials, Researchers are looking at promising new approaches for micro- and nanoencapsulation of phages and how these may address gaps in the field.[21].This study highlights the importance of silver nitrate, gentamicin and phage Kpn5 for controlling burn wound infections caused by nosocomial pathogens such as K. pneumoniae. A single application of phage Kpn5 was found to be superior to multiple applications of silver and gentamicin in the treatment of burn wound infection caused by K. pneumoniae B5055 in BALB/c mice. Studies using these compounds in combination to treat burn wound infection are warranted. This will not only increase the survival of the infected animals, but such a strategy will also keep a check on the development of resistant mutants, a problem frequently encountered by clinicians. In a recent study, we have demonstrated such an effect on treating biofilms of K. pneumoniae B5055 with a combination of ciprofloxacin and phage [22]. Reports [23] the Suggest that live pajim can be used to treat fatal infectious diseases caused by harmful bacteria such as Escherichia coli, Pseudomonas aeruginosa, Acinetobacter baumannii, Clevisilla pneumonia, Vibrio vulni fi cus and Salmonella spp, and bacteria with positive bacteria , Such as Artococcusphacium and Staphylococcus aureus. The non-genetically modified phage and gene display system is also useful for the treatment of Helicobacter pylori and P. aeruginosa, respectively. In addition to the phage particles in each case, it was also reported that the efficacy of hydrolysalpeptogenes (pysogenes), streptococcus pigogenesis, Bacillus anthracis and group B streptococci. All phage lysins studied so far exhibit instant bacterial activity immediately when applied exogenously. Furthermore, phage-encoded inhibitors of peptidoglycan synthesis (antibiotic proteins), search methods for new antibacterial agents using information genome informatics, and vaccines utilizing phages or their products are being developed. Phage treatment will compensate for complications of inevitable chemotherapy such as the emergence of multidrug resistance or bacteria to replace. However successful, Phage therapy seems to be extremely efficient in infections that are a result of one resistant type of infector. However, in cases where infections are caused by many microbes, the therapeutic efficacy of bacteriophage and the antibiotic Agent (A pharmaceutical substance) individually and in combination [24] to treat colibacilli may be more effective. Another clinical trial was conducted to evaluate the combination of anoxicin-induced antibiotic and muscle-activated bacteriophage. The two treatments individually provided effective treatments for E. coli infection, but the synergy between the two treatments led to the absolute protection of the birds, suggesting a significant value of the combination therapy[25].Synergy in anti-infective treatment Most antibiotics are used in human as antibacterial agents. They are natural products, developed by one species of bacterium (bacteria or fungi) as chemical weapons, often during overcrowding, to kill other bacteria in a neighboring microcvironment environment. Over the past 60-70 years most antimicrobials have been discovered by screening soil samples for natural products such as killing bacteria, including known pathogens, first on culture plates and then on animal infections.

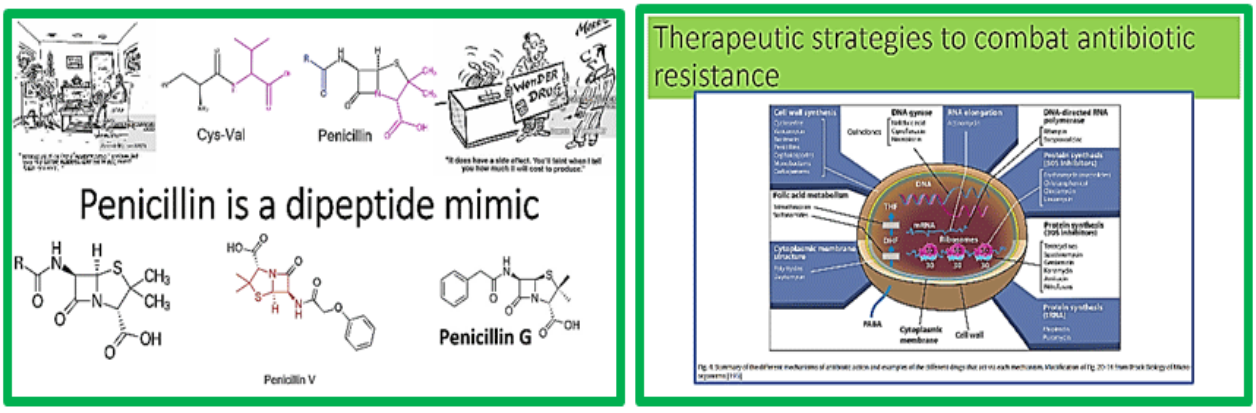


Figure 04:

Penicillin analogs are very often used as anti-infective agents. They usually disrupt the membrane of bacterial cells and thereby curing infections. The Cell defends itself by acticating some emzymes to decompose the lactam unit of the penicillins. In such circumstance, agents that inhibit the unwanted enzymatic activity are administered to protect the active penicillin agents. This is a symbiotic synergy as reflected in Augmentin.

Augmentin

Amoxicillin/clavulanate potassium

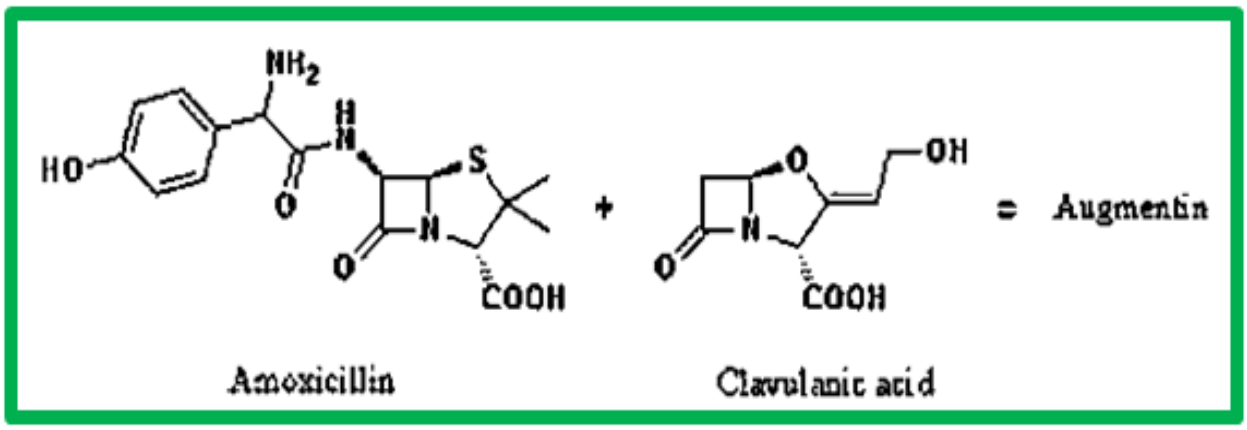


Figure 05:

Mechanism of Action: Amoxicillin inhibits bacterial cell wall synthesis, while clavulanate inhibits bacterial beta-lactamase.

Therapeutic Effect: Amoxicillin is bactericidal in susceptible microorganisms.

Clavulanate protects amoxicillin from enzymatic degradation.

Pharmacokinetics: Well absorbed from the GI tract. Protein binding: 20%. Partially metabolized in the liver. Primarily excreted in the urine. Removed by hemodialysis. Half-life: 1-1.3 hr (increased in impaired renal function). Augmentin antibiotics appear to be a safe stroke treatment and proper adjunct treatment for tPA (Tissue Plasminogen Activator - TPA).While resistance to overuse antibiotics has become a significant health concern in the country, scientists have noted that the use of minicycline in stroke will not contribute to the problem, as it will only be given for a few days. In fact, the researchers found that the drug remained active in the body in stroke patients older than younger patients who received it for other reasons, meaning that patients would require only a single dose for three days. She and Hess investigated the potential of secondary-stroke leverage for a decade [26].The rapid proliferation of antibiotic-resistant pathogens has spurred the use of drug combinations to maintain clinical efficacy and combat the evolution of resistance. Drug pairs can interact synergistically or antagonistically, yielding inhibitory effects larger or smaller than expected from the drugs' individual potencies. To comprehend how antibiotics, work and, concomitantly, why they stop being effective requires a brief look at the targets for the main classes of these antibacterial drugs. As summarized in Box 1, there are three proven targets for the main antibacterial drugs [27]:

1. bacterial cell-wall biosynthesis;
2. bacterial protein synthesis; and
3. bacterial DNA replication and repair.

The molecular mechanisms [28] by means of which peptide antibiotics interfere with the synthesis of bacterial DNA, biosynthesis of proteins, cell biosynthesis, and membrane integrity are varied, but have historically been understood by one antibiotic, one inhibitory mechanism. The new antibiotic mechanisms of the peptide have been recently discovered, and the mechanisms of peptide antibiotics involved in synergistic relationships with antibiotics and proteins have been more clearly defined. Nan apparent response to selective pressures, organisms produce antibiotics that combine elegantly with fungus multiple shares and everyday interactions peptide antibiotic planning to improve the success of the antimicrobial. There are synergies and dual mechanisms of antibiotic peptides [29].

Bifunctional enzymes containing both transtidase and translocosilase are the target sites for killing bacteria by the patcylins and Cephalosporins containing beta-lactam, which are used as pseudo-asyl acetate substrates of the active sites of transpeptides (also called penicillin or PBPs). The ring was opened, very deacylated penicillylated transpeptidases very slowly, thus conquering the active enzyme sites, not allowing normal cross linking of peptide chains in the peptidoglycan layer and leaving it weakly mechanical and susceptible to lysis over changes in osmotic pressure. In addition to penicillin and cephalosporins, the vancomycin family of glycolic peptide antibiotics also target the peptidoglycan layer within the cell wall cell. But instead of directing the enzymes involved in the peptide cross, vancomycin bonds increase the peptide 6 substrates, thus preventing it from reacting with trans pathy or transglycosylase. The effect is the same: failure to make peptidoglycan cross links leads to a weaker wall that predisposes the treated bacteria to lysis killing of the cell wall layer. In the spontological form of the antibodies of vancomycin, five hydrogen bonds are produced for the D-Ala-D-Ala dipeptide terminal of each associated peptidoglycan profile chain (Figure 1c), which is the high affinity of the antibiotic to its target, both in partial cross linked walls in neutral lipids. Because lactams and vancomycin work on substrate

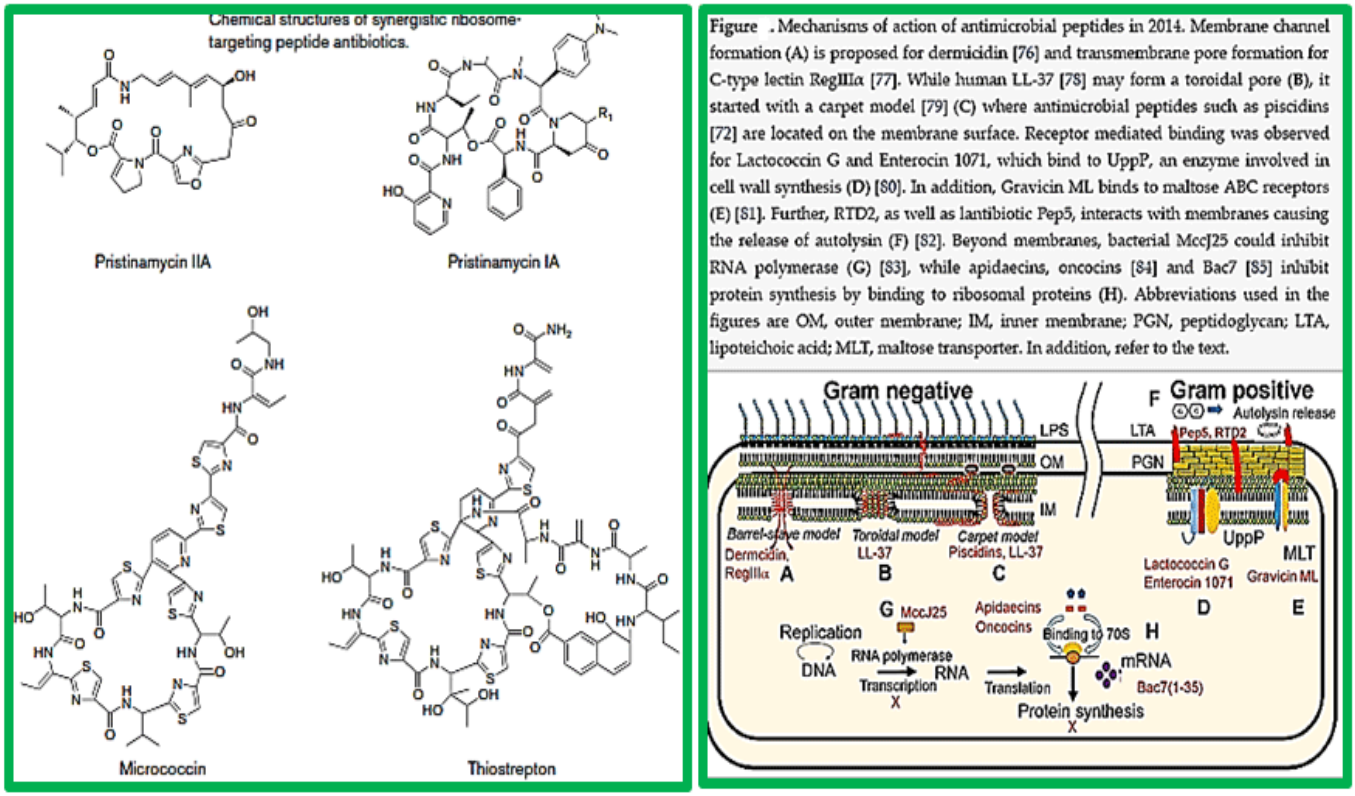


Figure 06:

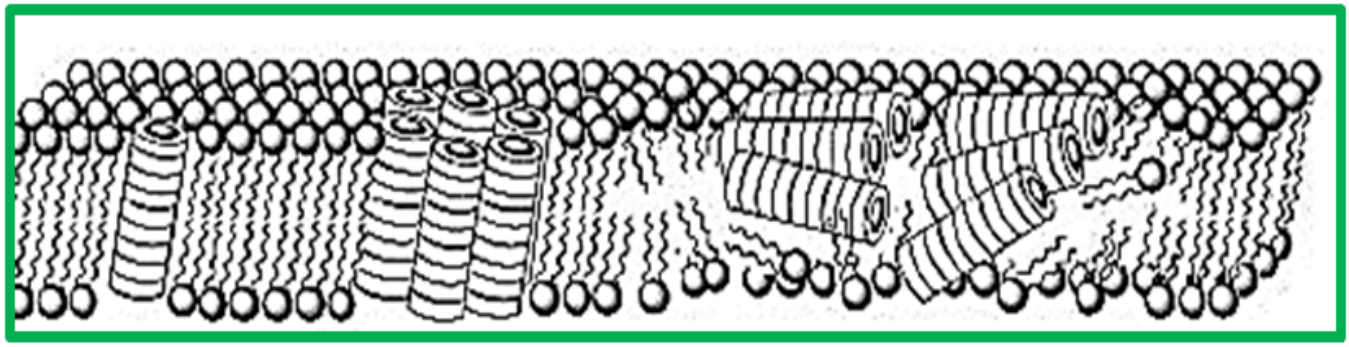


Figure 07:

substrate and enzyme substrate, they show synergy when used in combination. The spread of multidrug-resistant (MDR) strains of bacteria necessitate the discovery of new classes of antibacterial and compounds that inhibit these resistance mechanisms.The transport of drugs through, and into the bacterial cell envelope, active or passive, is a significant factor if the resistance of the microbes to anti-infective therapy. Reaching saturation

Saturation of the Bacterial Membrane with Anti-Infective Molecules (Coils in the Drawing)

[30] is an essential factor in the agent's efficiency as an eradicating agent. in the Drug efflux, unidirectional pumping of cytotoxic drugs, is a significant mechanism of antimicrobial.multiresistance in bacteria. Although these efflux systems are usually chromosomally encoded, some are present on plasmids. Some of the efflux pumps are relatively well known: Emr and Acr system in Escherichia coli,

whose outer membrane protein seems to be the multifunctional To1C; the mex efflux system described in Pseudomonas aeruginosa and ABC-type in Gram-negative bacteria. Also the role of efflux in Gram-positive bacteria is reviewed including Bacillus, Staphylococcus and Streptomyces.Functional synergies between antimicrobial peptides and peptides against gram-negative bacteria. Antimicrobial peptides (AMPs) are integral components of innate immunity and are usually in combinations in which they can be synergized for a more significant or more potent activity. In the past, scientists have reported peptide imitations of AMPs with potent and selective antimicrobial activity. Using test-board assays, they show that peptoids and AMP can interact synergistically, with low refractive index concentrations as low as 0.16. These results indicate that microbial peptides and peptides are functionally and mechanically analogous [31]. The most widely used methods to treat bacterial infection are antibiotic therapies. However, traditional antibiotics are becoming less efficient because of the development of drug-resistant bacterial strains. [32] It is of great need to develop new and potent antibacterial materials. Reported antibacterial agents include antimicrobial peptides,[33] bacteriophages,[34] silver,[35] carbon-based materials,[36] and cationic polymers.[37] Positively charged cationic antimicrobial agents show an efficient membrane-damaging effect toward negatively charged bacteria, regardless of standard or drug-resistant bacteria.[38] With the development of antimicrobial agents, cationic compounds such as quaternary ammonium (QA), imidazolium, pyridinium, and phosphonium salts are extensively used for the biocidal properties against a broad spectrum of bacteria.[39] However, the antimicrobial efficacy would be highly reduced when cationic polymers are immobilized as antimicrobial coatings, where the diffusion of cationic polymers into cell membranes is highly hindered.[40] As a result, the development of novel antimicrobial agents is in constant demand to combat bacterial infections. The combination of photodynamic therapy (PDT) and cationic antimicrobial agents would provide one better way to treat infection cases caused different types of bacteria. [41] To our best knowledge, polycationic synergistic antibacterial agents with integrated QA salt and photosensitizer components are very challenging and have not been reported. [42].

Phage and antibacterial Synergy

Key to any successful new drug use is its discovery and the following characterization. For phage therapy, equivalent steps should be taken, including the determination of how to combine [43].Phage insulation is usually done in combination with initial host-range characterization, that is, for enrichment and isolation hosts. This is followed by in vitro assessment in conjunction with additional host range characterization (ie, involving a larger panel of potential hosts) and bioinformatics characterization (in silicon). An enzymatic development, if carried out, will usually follow host range and characterization of silicon. For promising pages, in situ characterization comes next, including animal models for future human treatments (in vivo characterization), or with other species for non-human treatments. Clinical tests can follow, including the treatment of non-human species. Alternatively, phages can be used for biological control of environments and biological control and therapeutic use of phages can be anti-bio-not only whole phages can be used for treatment or control, but so is enzybiotics. Further development towards the successful implementation of trade or of the public sector must generally comply with regulatory requirements. Multipurpose mixtures are known as phage cocktails. Article Review in this topic by Weber-Da Browska et al. Discusses the essential stages involved, including sources and methods of test isolation, selection of phage-progation hosts, characterization methods, selection criteria for therapeutic purposes, and limitations on prenatal procurement. The use of phages as antibacterial therapy is especially important for targeting these pathogens, which have limited antibiotic treatment options. Isolation on demand of the corresponding phages can be achieved through enrichment of samples from environmental reservoirs, as investigated by Mattila et al. Interestingly, the differences between phage isolation based on enrichment and urban wastewater vary considerably, with the best results for Pseudomonas aeruginosa, Salmonella, and the complex β-lactamase spectrum (ESBL) producing Escherichia coli and Klebsiella pneumoniae. The procedure is less useful for vancomyccus resistant to vancomycc and acinetobacter baumannii, while the isolation of new phages against resistance to methicillin staphylococcus aureus (MRSA) strains was highly ffi cult. Expecting, the latter may be due to the selection of an environmental buffer used for isolation against MRSA phage since, as Wang et al. To show, pig swine flu may be a better source for these bacteriophages.

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Concluding Remarks

The application of Phage antibacterial therapy is based on a targeted genetic mechanism of bacterial eradication. I many effective treatments in cases of a targeted resistant bacterial strand, like foot abscesses our burns, where mainly one resistant bacteria culture caused the infection, success was achieved based on the unique selectivity of the phages toward the specific bacterium. However, in cases of multiple bacteria-based infections, combined treatment was found as the most effective treatment, Phage, and broad-band antimicrobials agents. Broad-activity antibiotics do allow for the treatment of undiagnosed causative agents with some certainty of success. Conversely, even phages with the broadest bacterial spectrums still do not come close to those of broad-spectrum antimicrobials. However, phage narrow host ranges cannot be assumed to exist in nature. It is evident that the clinical application of bacteriophage-based therapy of resistant bacteria-based infections is only in its infancy. More efforts should be allocated to do the combined [44] phage-based anti-infective treatments a real-life saver medical practice.

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