Int J Basic Sci Med. 2024;9(1):36-44 Review Article

http://ijbsm.zbmu.ac.ir/

doi 10.34172/ijbsm.46661

Targeted Therapy for Intracellular Bacterial Pathogens

Ciamak Ghazaei[1*]

[1]Section of Microbiology, Department of Animal Sciences, College of Agriculture and Natural Resources (Mugan), University of Mohaghegh Ardabili, Ardabil, Iran

ARTICLE INFO
Article History:
Received: February 5, 2024
Accepted: March 7, 2024
ePublished: March 27, 2024

***Correspondence to**
Ciamak Ghazaei,
Email: ciamakghazaei@yahoo.com

Abstract
The spectrum of diseases caused by intracellular bacterial pathogens (IBPs) is broad, ranging from life-threatening conditions such as tuberculosis to other infectious-transmitted diseases. Conventional antibiotic treatment faces challenges due to antibiotic resistance, host cell toxicity, and limited drug penetration. Despite the excellent ability of these perilous pathogens to modulate host cell biology, localize in, and multiply through targeting the key virulence factors, cell brings about auspicious maneuvers to combat pathogenic diseases and alleviate their significant global burden. Modulation and identification of molecules, pathways, and responses are the initial steps of targeted therapy, varying from disease to disease. This article explores the cutting-edge advancements in targeted therapy approaches. Innovations such as nanoparticle-based drug delivery systems, phage therapy, immunomodulation, and gene editing, which hold a promising future for overcoming the limitations of traditional treatment, are also discussed. Efficient delivery systems, drug optimizations, and inch-perfect distribution and retention of therapeutic agents are some of the determining factors in the success of targeted therapy for bacterial pathogens. The article also presents a novel application wherein filamentous phages are loaded as targets in nanocarriers for therapeutic purposes. The present challenges faced by the researchers, along with future directions for this field of medical science, are also outlined. Overall, the scope of this article involves the various strategies involved in targeted therapy, drug modulations, and limitations faced in our current approaches.
Keywords: Disease, Intracellular bacterial pathogen, Targeted therapy, Drug optimization, Drug modulation

Please cite this article as follows: Ghazaei C.Targeted therapy for intracellular bacterial pathogens. Int J Basic Sci Med. 2024;9(1):36-44. doi:10.34172/ijbsm.46661.

Introduction

Intracellular bacteria are a group of microorganisms capable of residing and replicating within the cells of their host organisms. Classic examples of these deadly bacteria are *Mycobacterium tuberculosis*, *Salmonella*, *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, and *Listeria monocytogenes*.[1] These bacteria have a unique mechanism to invade and survive within the eukaryotic cells and are marked as the common causes of severe leading infections. The intracellular environment offers protection against external toxic agents and enables intracellular bacterial pathogens (IBPs) to escape the immune system. Consequently, intracellular bacteria have had to adapt to the adverse conditions found in subcellular compartments, such as the presence of lytic enzymes, acidic pH, and limited energy sources,[2] with the help of evolving mechanisms to modulate host cell biology in order to reside and colonize in deeper tissues. Antibiotics have so far been the primary treatment option against these pathogens, despite the growing concerns related to antimicrobial resistance and the fact that two-thirds of known antibiotics cannot penetrate and accumulate within mammalian cells.

The need for alternative approaches for the effective treatment of infections caused by IBPs is thereafter a primary concern. IBPs are resilient and keep the therapy challenge activated by the development of antibacterial resistance, the formation of biofilms, and the ability of certain pathogens to invade and localize within the host cell. Poor penetration of IBPs into host cells is the reason for inadequate bacterial clearance, leading to chronic and untreated infections. Therefore, to overcome the challenges of different cellular barriers, approaches adopted for treating these stubborn infections include the development of nanoparticles (NPs), antimicrobial peptides, and antisense oligonucleotides, which control gene expression through targeting RNA through the novel use of a topical antibacterial polymer, polyhexamethylene biguanide.[1] Moreover, there are advances in new technologies such as phage engineering, nanotechnology, biofilms, gene editing and therapy, RNA therapeutics, organelle-specific targeting, and optogenetics. These are a

few measures to hold the potential for revolutionary and advanced treatment by targeting cellular processes with greater precision and efficacy.

Impacts of Intracellular Bacterial Pathogens on Human Health

Infectious diseases have always been a threat not only to humans but also to plants and animals worldwide. A range of diseases and infections that pose a considerable risk to life and health are caused by IBPs. The ability of these IBPs to elude immune responses and thereby exhibit persistence creates complications for the complete eradication of infections caused by them. The ability of intracellular bacteria to spread to other cells without being detected and killed by the host immune cells is quite challenging. Bacterial transfer can occur when the host cells exchange plasma membrane proteins and cytosol through a process called trogocytosis, allowing bacteria to move from infected cells to uninfected cells, thus leading to a variety of diseases ranging from sexually transmitted diseases derived from chlamydia to life-threatening infections such as tuberculosis and listeriosis.[2,3]

When bacterial infections are observed quite often, the use of conventional antibiotics is essential, but the capacity to develop resistance against IBPs will still be there. Some bacteria are now resistant to even the most powerful antibiotics available, which is alarming. Moreover, taking antibiotics interferes with our gut's normal flora, which further deteriorates the condition of the body.[4] Table 1 provides IBPs and their associated diseases.

Intracellular Bacterial Pathogens: Invasion and Replication

Invasion

The journey of IBPs into host cells represents a critical moment in the pathogenesis of infectious diseases in which bacteria navigate a landscape of molecular interactions to invade and weaken host defenses. The initial step in bacterial invasion is adherence, which is performed by specialized cells called adhesins. These adhesins vary in composition and structure and are identified by receptors, made up of glycolipids or glycoproteins, present on host cell surfaces. Examples of host cell receptors are cadherins, selectins, and various glycosaminoglycans. Additionally, anchorage can be provided by structures such as bacterial appendages, including pili, fimbriae, or flagella. Adherence to the host cell promotes cellular signals that facilitate bacterial penetration. In bacteria, there are two trusted mechanisms for the invasion of the host (i.e., the zipper and trigger mechanisms). Through the zipper mechanism, bacteria can induce cytoskeletal changes that facilitate the formation of membrane protrusions, such as lamellipodia and filopodia.[15] These changes create a zipper-like structure around the pathogen, which allows it to be engulfed by the host cell through phagocytosis. This mechanism is often found in bacteria such as *Listeria monocytogenes* and some strains of *Escherichia coli*.[16]

The trigger mechanism, however, initiates signaling actions in host cells through interactions with specific receptors on the host cell surface.[17] It involves the injection of bacterial effector proteins into the host cell using specialized secretion systems, namely, the type III secretion system, or the type IV secretion system, and the like. The receptors can include various proteins such as integrins, Toll-like receptors, and pattern recognition receptors. By binding to these receptors, bacterial pathogens promote their invasion and manipulate the immune responses of the host by hijacking cellular processes.[18] This mechanism is commonly observed in *Salmonella typhimurium*.

In summary, the trigger mechanism manipulates the host cell by activating the signaling pathways, while the zipper mechanism entails the engagement of specific host cell receptors for the invading purpose. However, understanding these mechanisms for developing targeted therapeutic mediations in detail is highly crucial. Ultimately, the invasion is performed by phagocytosis or endocytosis. Types of endocytosis that can be utilized by host cells to internalize pathogens include clathrin-mediated endocytosis, caveolae-mediated endocytosis, and macropinocytosis. Nonetheless, some IBPs directly penetrate the host cell to internalize without involving endocytosis. The whole point of internalization is to build a conducive environment for the bacteria to hijack the host machinery and promote its own survival and replication.

Table 1. Diseases Associated With Intracellular Bacterial 3Pathogens

Bacteria	Host	Associated Disease	Localization Inside Host	References
Salmonella enterica	Macrophages	Typhoid and paratyphoid	Modified phagosome	5,6
Mycobacterium tuberculosis	Macrophages	Tuberculosis	Phagosome and cytosol	7-9
Chlamydia species	Conjunctiva and genital epithelial cells	Ocular and genital infections	Vacuole	10
Listeria monocytogenes	Epithelial cells	Listeriosis	Cytosol	11
Staphylococcus aureus	Keratinocytes, bovine mammary epithelial cells, and osteoblasts	Skin infections, mastitis, and osteomyelitis	Endosome and cytosol	12,13
Escherichia coli	Bladder epithelial cells and mammary epithelial cells	Urinary tract infections, and mastitis	Vacuole	14

Replication

The essential concept of the IBPs is that microorganisms that multiply within cells are fundamentally different in their biology and pathogenesis from those that are unable to do so. However, intracellular bacteria do have something in common with viruses, which is the requirement of a suitable host for systematic replication and proliferation. Furthermore, by looking at the criteria of the targeted host cells, it can be found that these pathogens commonly target cells of the same type. It is all fulfilled when the metabolic conditions of host cells fail to satisfy the high nutrient demand of a pathogen in their inactive state of metabolism. To overcome this hurdle for succeeding proliferation, these pathogens readjust the machinery of the host cells. The site of replication of IBPs is the vacuole or cytosol.[19] Due to easier access to nutrients in the cytoplasm, higher IBP replication is observed in the cytosolic region than in any other part of the host cell. Once phagocytized, bacteria often modify these compartments to control access to nutrients and thus dodge host immune mechanisms. Inside the cytosol, IBPs are at a huge advantage as a nutrient-rich environment offers a perfect escape from the host's immune cells, which facilitates robust replication. Although studies have shown that any sort of damage to the host cell tonoplast facilitates to enter of bacteria into the cytosol the cytosol and endangers the ability of these bacteria to colonize.[20] Meanwhile, bacteria develop intricate strategies to overcome obstacles in their way of replication. Mechanisms observed in this regard include the prevention of phagocytosis (Yersinia species), alteration of phagocytosis (Salmonella species), escape from the phagosome by lysing the tonoplast (Listeria and Shigella species), blockage of the fusion of lysosomes with phagosomes (Legionella species), and acidification of phagolysosomes (*Mycobacterium tuberculosis*).[21]

Compared to facultative intracellular bacteria, little is known about the interactions and physiology of obligate bacteria. One of the reasons for this knowledge gap is the challenge of studying bacterial signaling networks in vivo conditions. Interestingly, some studies have shown that the purification of obligate intracellular bacteria must proceed from host cells before most transformation methods are to be executed because chemical reagents do not typically possess the ability to pass both the pathogen membrane and host; for example, *Rickettsia prowazekii* has a replication time of 8–12 hours in the cell membrane, which is 2–3 times longer than that of *L. pneumophila*.[22]

Mechanism of Action of Antibiotics and Antimicrobial Agents

Antibiotics, or antimicrobial agents, a formidable weapon against bacterial infections, offer a diverse approach to evading bacterial pathogens. Antibiotic drugs have different modes of action against various species of intracellular bacteria; related examples are provided as follows:

Inhibition of Cell Wall Synthesis

Antibiotics considered as β-lactams (carbapenems, penicillin and its derivatives, and cephalosporins) and glycopeptides, such as vancomycin, inhibit the biosynthesis of peptidoglycan (PG), allowing cell vulnerability to autolysis and osmotic pressure.[23,24] Consequently, bactericidal antibiotics are able to inhibit the synthesis of the cell wall. The β-lactam drug class targets penicillin-binding proteins to inhibit cell wall synthesis by inhibiting the last stage of PG synthesis, thus disrupting the terminal transpeptidation mechanism. PG is a mesh-like network present in the cell walls of both gram-positive and gram-negative bacteria. Considering that animal cells lack PG, the mechanism of action is the best choice in nature . Penicillin G, amoxicillin, and cephalosporin C are examples of proceeding cell wall inhibition and are known to belong to the β-lactam drug class. Glycopeptides such as vancomycin block cell wall synthesis by attachment to the D-Ala-D-Ala terminal of the elongating peptide chain when the cell wall is being synthesized, causing a disturbance in the terminal transpeptidation mechanism and inhibition of cell wall synthesis. A structurally weak cell wall is susceptible to osmotic lysis, which leads to the rupture of the cell membrane and, ultimately, bacterial cell death.

Inhibition of Protein Synthesis

Drug class aminoglycosides and tetracyclines attack protein synthesis by targeting the 30S subunit, and examples include streptomycin, gentamicin, neomycin, tetracycline, and doxycycline. In aminoglycosides, this is achieved by binding with high affinity with the A-site on the 16S ribosomal RNA (rRNA) of 30S rRNA. Thus, it causes misreading of codons when aminoacyl-transfer RNA is transferred and incorrect amino acid compilation into the polypeptide chain.[25] Consequently, the erroneous protein is produced, which generates noxa to the cell membrane when released. Tetracycline passively diffuses the cell membrane through the porin channel and blocks protein synthesis by inhibiting tRNA binding to the messenger RNA-ribosome complex. It binds reversibly to the 30S ribosomal subunit to achieve protein synthesis blockage.

The antibiotics favorable for 50S subunit inhibition are macrolides, chloramphenicol, and oxazolidinones by their own action mechanisms. Macrolides such as azithromycin bind to the 50S subunit of 23S RNA to achieve their goal. The mechanism holding behind it activates to block transpeptidation and translocation in order to block protein synthesis, resulting in the separation of proteins before maturation and the formation of incomplete peptide chains. Chloramphenicol is a class of antibiotics that is lipid-soluble and can easily pass

through the membrane of bacteria. Afterward, it binds reversibly to the L16 protein of the 50S subunit of the ribosomes of bacteria, causing the inhibition of peptide bond formation, thus blocking the transfer of the amino acid by the blockage of peptidyl transferase activity.
Oxazolidinones such as linezolid and tedizolid behave as antibacterial agents by disturbing the translation of bacterial proteins. They attach to the 50S subunit site of the bacterial 23S ribosomal RNA, thus interfering in the development of a functional 70S initiation complex, which is essential for bacterial replication. This will prevent bacteria from multiplying.[24]

Inhibition of Cell Membrane Functions

This mechanism targets bacteria with a lipid bilayer of cell membranes. Polymyxins exhibit cationic detergent-like activity; they interact with lipopolysaccharides in Gram-negative bacteria, resulting in the disruption of the integrity of the outer membrane.[24]

Inhibition of Nucleic Acid Synthesis

The chief target processes of this mechanism are DNA and RNA, which are achieved by antibacterial drugs (e.g., rifamycin and fluoroquinolones). Rifamycin strongly attaches to the polymerase subunit present inside the DNA/RNA pathway and causes the blockage of the bacterial DNA-dependent RNA polymerase. In this way, the elongation of RNA is inhibited and cannot take place. Bacterial RNA polymerase enzymes are structurally different from eukaryotic RNA polymerase enzymes and are responsible for selective toxicity behavior against bacterial cells.[24]

On the other hand, quinolones inhibit DNA synthesis by blocking two enzymes, namely, DNA gyrase and topoisomerase IV; these two are termed basic type II topoisomerases. They both target and attack in the same manner, resulting in one double-stranded DNA molecule shifting through another. This leads to the initial strand's inferior rank when it has afterward turned. Quinolones have a formidable affinity for the DNA gyrase A subunit. They disturb the way strands split and reseal and lead to the negation of normal cell division. The role of topoisomerase IV is to nick and separate the daughter DNA strand after DNA replication. This is the foremost duty occurring in Gram-positive bacteria. Drugs possessing a higher affinity for this enzyme can cause greater potency against Gram-positive bacteria.[24]

Blockage of Metabolic Pathways by Competitive Inhibition:

Trimethoprim has a synergistic effect on sulfonamides. It is an antifolate antibacterial agent that blocks bacterial dihydrofolate reductase, considered an important enzyme that catalyzes tetrahydrofolic acid formation. Nonetheless, during this process, it inhibits the synthesis of bacterial DNA and, consequently, bacterial life.

Sulfonamides stop the multiplication of bacteria by acting as competitive inhibitors of p-aminobenzoic acid in the metabolism cycle of folic acid. Bacterial sensitivity is similar for various sulfonamides, and if resistance belongs to one sulfonamide, it refers to resistance to all. Most sulfonamides are absorbed orally and readily.

Potential Vulnerabilities in Intracellular Bacterial Pathogens

The extracellular pathogen is a type of microorganism that cannot survive inside the phagocyte after it has been engulfed, but intracellular pathogens that reside inside the host cells are called phagocytes. Intracellular bacteria naturally pose particular challenges to therapeutic targeting and often make their targeted control difficult. Unlike extracellular pathogens, which can be directly targeted by circulating antibiotics or immune mechanisms, intracellular bacteria have evolved sophisticated strategies to evade host defense mechanisms.[25]

Understanding the molecular targets in intracellular bacteria is critical for developing targeted therapies. The approach of targeted therapy can be utilized to the fullest only when the targets are totally perfect and the drug delivery is efficient. By identifying the essential components of bacterial physiology, such as enzymes, structural proteins, and regulatory factors, researchers can uncover pathways that facilitate bacterial spread.[25] It is important to note that the drugs and agents to be used in targeted therapy should be designed to hit on specific vulnerabilities in bacteria. These vulnerabilities may include bacterial proteins, enzymes, adhesins, metabolic pathways, or secretion systems that hinder the processes of bacterial adherence to the host, bacterial survival mechanisms, and ultimately the bacterial spread.[26]

Strictly speaking, targeting bacterial enzymes is as effective as it sounds. Enzymes, being highly specific, target particular pathways in bacteria such as DNA replication, transcription, translation, and cell wall synthesis, regulating bacterial growth and proliferation. By selectively obstructing the enzymes required by bacteria, respective processes can be hindered effectively. *R. prowazekii* secretes phospholipases that determine the localized and controlled degradation of the host cell membrane and directly enters the cytoplasm through the membrane lesions.[27] In this case, disintegrating bacterial phospholipases using belactosin A, AACOCF3 (arachidonyl trifluoromethyl ketone), and ethylenediaminetetraacetic acid can help achieve the inability of *R. prowazekii* to infect its host.[28] Targeting virulence factors, such as adhesins, surface proteins, and secretion systems, can attenuate bacterial pathogenicity. Quorum sensing systems in bacteria regulate gene expression and coordinate behaviors. Disrupting signaling molecules such as acyl-homoserine lactones

in Gram-negative bacteria or oligopeptides in Gram-positive bacteria has been proven to decrease bacterial cell density. Another key target found in the IBPs is the toxin-antitoxin (TA) system. The discovery of this system is unfolding the significant roles attributed to bacterial invasion and pathogenicity. Small molecules or peptides that disrupt TA interaction or stabilize the antitoxin, thereby neutralizing the toxin's activity, can be explored in this regard.[29] Impinging bacterial TA systems facilitates intrusion into biofilm formation, leading to clearance for the host's immune cells to invade the breached site.

In general, the multifaceted approach provided by therapeutic targeting can be made fruitful by identifying potential virulence factors and signals in bacteria and disrupting them to enhance phagocytic immune responses or manipulating host cell metabolism to restrict bacterial replication and survival. The whole idea is to understand the intricate mechanisms by which intracellular bacteria establish infection and hijack host immune responses, and the more we thrive in pointing out the vulnerabilities, the more the researchers can develop targeted strategies to effectively disrupt these processes.

Recent Advances in Targeted Therapy

Recent years have witnessed remarkable revolutions in the field of targeted therapy, fundamentally changing the dynamics of medical treatment. Although the essence of targeted therapy is simple and the implications are profound, it has broadened our horizons by providing a new and versatile approach to harnessing the powers of the immune system. Some notable innovations are provided as follows:

Phage Therapy

Successful killing of IBPs can also be achieved by the use of engineered bacteriophages. Correct expression of cell-penetrating peptides (CPPs) helps locate the bacterial target. Clearance of intracellular bacteria is promoted when these phages enter the bacterial cell, promote cell lysis, and finally release progeny phages to kill the rest of the bacteria. Phage therapy also carries the potential for synergistic effects by combining antibiotics and immunomodulators.[30] Moreover, phage therapy has helped in the invention of another class of antimicrobials called endolysins.[31] These are basically bacteriophage-derived PG hydrolases causing lysis of the host bacteria. They rapidly kill the Gram-positive bacteria by degrading the externally exposed PG by external attacks. Their major advantages include high specificity for their target bacteria, nontoxicity to eukaryotic cells, and a very low risk of resistance development due to their highly conserved PG target bonds.

Nanoparticle-Based Drug Delivery Systems

NP-based drug delivery systems offer auspicious approaches for targeting IBPs. Targeted delivery of drugs using nanotechnology not only improves biodistribution but also the retention of drugs at the site of infection. Commonly used NPs for this purpose include liposomes, polymeric NPs, dendrimers, and inorganic NPs.[32] Functionalization of these NPs with targeting ligands or peptides that recognize intracellular receptors by trafficking pathways enhances their specificity for intracellular bacteria.[33] NPs also allow multiple antimicrobials, or phages, to be delivered simultaneously in a single capsule. Nanoemulsion is another technique for drug delivery that leads to an increase in solubility, stability, and slow release of the encapsulated loaded molecules.[34] In addition, what makes nanotechnology stand out in the field of targeted therapy is the high surface area, enhanced reactivity, and controllable size of the nanocarriers, improving drug solubility and modulating drug release characteristics.[34]

Immunomodulation

It plays a critical role in the targeted therapy of intracellular bacteria by governing the immune response to tackle these pathogens effectively. It encompasses important actions, including the stimulation of innate immune cells, T-cell activation, proliferation, and effector functions for the prevention of tissue damage, and promotion of bacterial clearance. Immunomodulation involves the use of antimicrobial peptides (AMPs) and CPPs to exert direct bactericidal effects. AMPs have evolved to provide extensive protection against various pathogens. In addition, they are effective against bacteria that are resistant to conventional antibiotics. CPPs penetrate biological membranes and transport target agents to the interior of the cell. AMPs and CPPs can cross the host cell membrane without destroying its integrity.[35] In addition to modifications, these peptides can be combined with antibiotics, resulting in synergistic outcomes. For instance, AMPs containing all-D amino acids, together with anti-tuberculosis drugs (isoniazid and rifampicin), enhance the efficacy of these antibiotics against *Mycobacterium tuberculosis*.[35]

Drug Repurposing and Combination Therapies

Drug repurposing involves utilizing approved drugs that target host functions required for microbial infections, thus offering a promising alternative to developing novel antimicrobials. Using a combination of antibiotics and non-antibiotic compounds (potentiators) that could inhibit bacterial resistance or enhance antibiotic activity offers an effective approach to confronting multidrug-resistant bacteria. This is a cost-effective and time-efficient strategy. Some examples of drugs that have been repurposed for intracellular bacterial infections are as follows:

- Ibrutinib: A host-directed tyrosine kinase inhibitor

that significantly impairs intracellular bacterial survival, particularly against *Staphylococcus aureus* infections.[36, 37]

- Dasatinib: A tyrosine kinase inhibitor that inhibits the replication of the dengue virus and reduces bacterial load and pathology in mouse lungs infected with certain bacteria.[37]
- Crizotinib**:** A host-directed tyrosine kinase inhibitor that has shown efficacy in impairing intracellular bacterial survival, particularly against *S. aureus* infections.[37]

Challenges and Limitations

The limitations of the current therapies include the development of antibiotic resistance, limited access to drugs, and the potential for host cell toxicity. Despite these challenges, researchers are actively working on developing new drugs and strategies to overcome them and improve targeted therapy for intracellular bacteria. Some key challenges and limitations are listed below:

Antibiotic Resistance

It poses a significant challenge in the targeted therapy of intracellular bacteria. Resistance mechanisms used by bacteria may involve efflux pumps, alterations of drug targets, or enzymatic inactivation of antibiotics. Antibiotic resistance genes are transferred through spontaneous mutations by the acquisition of resistance traits. For instance, the acquisition of β-lactamases (hydrolytic enzymes) causes β-lactam antibiotics to be effective in breaking them down.[38]

Intracellular Residence

Intracellular bacteria often reside within host cells at some locations, which make it difficult for therapeutic agents to approach them effectively. This environment provides them protection from the immune system and allows them to persist even after the clearance of extracellular bacteria. Another factor that needs to be enlightened is that the IBPs, once inside the host cell, enclose themselves in vacuoles, or phagosomes. Drug penetration in these structures can be hindered due to their altered pH, enzymatic activity, and the like. *Salmonella enterica* secretes particular effector proteins, preventing the fusion of its phagosome with a lysosome.[39]

Limited Drug Penetration

Therapeutic drugs have limited penetration into host cells, resulting in suboptimal concentrations of the drug within the intracellular compartments where the bacteria are residing. Bacteria have developed mechanisms in this regard; thus, they form biofilms, especially in *S. aureus*, hindering the action of antimicrobial agents.[40] Some conventional antibiotics and therapeutic agents face difficulty crossing the cell membrane of host cells. Therefore, the presence of efflux pump systems in some bacteria actively removes drugs from within, thereby reducing intracellular drug concentrations.

A unique challenge arises when a drug is highly specific. The aminoglycoside antibiotics enter cells through endocytosis. They bind to a receptor called megalin, which is abundant in the renal proximal tubule. The targeted interaction of aminoglycosides with megalin leads to their accumulation in the kidneys, which can cause nephrotoxicity in patients.

Persistence and Relapse

Persistence has been an evolving survival strategy in bacteria. The best approaches showcased by bacteria in this regard are as follows:

- Formation of endospores
- Formation of exospores
- Formation of viable but non-culturable cells
- Formation of persister cells[41]

Generally, bacteria enter a dormant state using any of the above-listed strategies, and when the therapeutic agents have been removed, the surviving bacteria start to regrow. This refers to the relapse of the infection. Additionally, studies have shown that the treatment of bacteria with high doses of antimicrobials leads to the formation of persister cells.[41] Depletion of antimicrobial agents from the target site resuscitates these persistent cells and makes conditions conducive to their proliferation.

Endospores are commonly derived from bacteria. Exospores are produced in the eukaryotic cells of fungi, cyanobacteria, and algae. Endospores are produced inside the mother cell, while exospores are produced toward the mother cell end and released as buds.

Host Cell Toxicity

Unintended harmful effects on the host cell in the face of targeted therapy are invincible. Although the development of drugs and agents that minimize harm to the host is among the top priorities, this challenge cannot be fully eliminated; some extent of the side effects will always remain. Poor distribution, or metabolism, of the therapeutic agent may result in systemic toxicity. However, improvements in this regard can be brought about through the optimization of drug delivery systems. Often, the therapeutic agent is not sufficiently selective for the target and results in interactions with the host cells. Targeted therapies may also induce excessive or dysregulated immune responses in the host, which can be avoided by careful immunomodulation.[41] On the whole, addressing host cell toxicity as a critical issue is meant to bring developments in the field of targeted therapy.

Future Directions and Potential Advancements

With the emergence of robust challenges such as antimicrobial resistance, the need for more effective and

precise treatment approaches has become paramount. There is a growing need to develop strategies that can enhance treatment efficacy and minimize adverse effects.

Genome Editing

The introduction of gene editing has been a breakthrough in the field of medical science. Clustered regularly interspaced palindromic repeat (CRISPR)-based gene editing stands out among all the available techniques. The CRISPR-Cas system offers an interesting option for the development of next-generation antimicrobials to combat infectious diseases, especially those caused by antimicrobial-resistant pathogens.[42] The specific programmability of CRISPR-Cas systems makes them superior to conventional antibiotics. These systems can also be utilized to identify potential therapeutic targets in intracellular bacteria. Researchers can identify genes required for intracellular survival, virulence, or antibiotic resistance by infecting host cells with bacteria carrying CRISPR-Cas systems. The role of CRISPR in improving treatment outcomes is immense, as it enhances the efficacy of antimicrobial therapy by sensitizing IBPs to antibiotics.

Modifying Traditional Antibiotics

Modifying the traditional antibiotics that are to be used in the targeted therapy can enhance their effectiveness, leading to an improvement in pharmacokinetics and reducing cell toxicity. This approach can also prove to be a major breakthrough in combating bacterial resistance. Modifications in the C-7 and C-9 positions of the tetracycline D-ring produced omadacycline,[43] enabling it to overcome the common tetracycline resistance mechanism exhibited by bacteria. Another successful antibiotic derivative, cefiderocol, was obtained by conjugating a catechol-type siderophore to the typical cephalosporin core. This modification resulted in an excellent ability to penetrate the outer membrane of Gram-negative bacteria more effectively than traditional cephalosporins.[44] By utilizing the power of molecular engineering, it is possible to rejuvenate the existing antibiotics and hope for more effective and auspicious treatments.

Improved Delivery Systems

Integrating nanotechnology in the development of new drug delivery systems is a promising approach. Drug-loaded NPs can encapsulate poorly soluble drugs, improving their solubility and dissolution rates. It also allows precise targeting at the site of infection and minimizes the risk of cell toxicity. Nanotechnology can also facilitate the co-delivery of multiple therapeutic agents simultaneously. This multi-targeted therapy can further eliminate the risk of resistance in bacteria. NPs can overcome biological barriers, such as the blood-brain barrier or mucosal barriers. This will enable the delivery of antimicrobial agents to previously inaccessible sites.[45] A successful example of currently used drug-loaded NPs is Doxil. Liposomal doxorubicin (Doxil) uses liposomes to target cancer cells, reducing the risk of cell toxicity and enhancing the drug supply to the target tumors compared to conventional doxorubicin.[46]

Bacteriophages and Endolysins

The working and further developments in phage therapy for IBPs are almost ceased when broad-spectrum antibiotics are utilized. Phages can rapidly evolve and adapt to overcome bacterial resistance mechanisms. This flexibility in phages, as compared to antibiotics, is expected to make them potentially effective against multidrug-resistant bacteria. Engineered endolysins are auspicious to improve stability, binding affinity, and activity against target bacterial strains. Bacteriophages and endolysins can also be delivered using nanocarriers, which will protect them from enzymatic degradation and provide more efficient penetration.

Microbiome-Based Therapies

The human microbiota serves as an effective barrier against pathogenic bacterial infections. The microbiota influences many of the functions of the epithelium of the human system. Distinct species of the microbiome directly impact the CD4+ T helper cells to differentiate and help harvest energy by facilitating the fermentation of dietary fibers in the intestine.[47] However, disturbances to the microbiota, particularly due to the exploited use of antibiotics and antimicrobial agents, increase the risk of colonization by IBPs. Prebiotics, probiotics, live biotherapeutics, and postbiotics are taken into consideration when discussing microbiome-based therapies. These substances, when administered in recommended amounts, confer health benefits to the host. Thus, keeping in view the principle behind fecal microbiota transplantation, technology can be applied to enable microbiota transplantation, helping in transferring beneficial microbes or microbial products from healthy donors to individuals with disturbed microbiota. This may hold potential for modulating immune responses against IBPs in specific contexts. Similar to every other transplant, the immune status of the recipient should be carefully studied to shape the engraftment and efficacy of microbiome therapy.[48]

Vaccines

Precaution is always better than cure. The future approaches being followed by pharmaceutical companies in vaccine discovery and production include reducing the need for multiple doses of a vaccine and improving the manufacturing processes of vaccines.[49,50] More than 260 vaccines are currently in the development stages for the

prevention or treatment of infections and diseases. There are several types of vaccines, termed inactivated vaccines, polysaccharide, live-attenuated vaccines, and messenger RNA vaccines, as well as subunit, recombinant, and conjugate vaccines.

Conclusion

In the battle against infectious diseases, targeted therapy stands as a promising approach. Bacterial pathogens continue to develop resistance to antibiotics. The complexity of host-pathogen interactions will never let the ongoing research stop. With time, it is necessary to refine our therapies and develop novel strategies. Moreover, it is essential to deeply understand the mechanisms of bacterial pathogenesis and the evolving dynamics of targeted therapy in order to further advance our research and develop more effective interventions. With improved diagnostics, narrow-spectrum drugs will demonstrate a promising future by accurately eliminating IBPs. More sophisticated use of molecular engineering in the development of carriers, drugs, and combination therapies will positively allow precise sensing, finer drug delivery, and improved control of toxicity. However, focusing solely on therapies and treatments is not effective. Vaccines and other preventive measures should be taken as well. Awareness should be raised regarding the lethality caused by IBPs. Regardless of what we have developed until now and what is still pending, we stand in the face of challenges, including bacterial resistance, cell toxicity, and bacterial persistence. However, we should strive toward more precise, effective, and budget-friendly treatments to ultimately improve global public health and save countless lives.

Competing Interests

There is no conflict of interests.

Ethical Approval

Not applicable.

References

1. Soni J, Sinha S, Pandey R. Understanding bacterial pathogenicity: a closer look at the journey of harmful microbes. Front Microbiol. 2024;15:1370818. doi:10.3389/fmicb.2024.1370818
2. Santucci P, Greenwood DJ, Fearns A, Chen K, Jiang H, Gutierrez MG. Intracellular localisation of *Mycobacterium tuberculosis* affects efficacy of the antibiotic pyrazinamide. Nat Commun. 2021;12(1):3816. doi:10.1038/s41467-021-24127-3
3. Straif-Bourgeois S, Tonzel JL, Kretzschmar M, Ratard R. Infectious disease epidemiology. In: Ahrens W, Pigeot I, eds. Handbook of Epidemiology. New York, NY: Springer; 2019:1-79. doi: 10.1007/978-1-4614-6625-3_34-1.
4. Becattini S, Taur Y, Pamer EG. Antibiotic-induced changes in the intestinal microbiota and disease. Trends Mol Med. 2016;22(6):458-478. doi:10.1016/j.molmed.2016.04.003
5. Gorvel JP, Méresse S. Maturation steps of the *Salmonella*-containing vacuole. Microbes Infect. 2001;3(14-15):1299-1303. doi:10.1016/s1286-4579(01)01490-3
6. Brumell JH, Tang P, Zaharik ML, Finlay BB. Disruption of the *Salmonella*-containing vacuole leads to increased replication of *Salmonella enterica* serovar typhimurium in the cytosol of epithelial cells. Infect Immun. 2002;70(6):3264-3270. doi:10.1128/iai.70.6.3264-3270.2002
7. Armstrong JA, Hart PD. Response of cultured macrophages to *Mycobacterium tuberculosis*, with observations on fusion of lysosomes with phagosomes. J Exp Med. 1971;134(3 Pt 1):713-740. doi:10.1084/jem.134.3.713
8. Rohde KH, Veiga DF, Caldwell S, Balázsi G, Russell DG. Linking the transcriptional profiles and the physiological states of *Mycobacterium tuberculosis* during an extended intracellular infection. PLoS Pathog. 2012;8(6):e1002769. doi:10.1371/journal.ppat.1002769
9. Watson RO, Manzanillo PS, Cox JS. Extracellular *M. tuberculosis* DNA targets bacteria for autophagy by activating the host DNA-sensing pathway. Cell. 2012;150(4):803-815. doi:10.1016/j.cell.2012.06.040
10. Kumar Y, Cocchiaro J, Valdivia RH. The obligate intracellular pathogen *Chlamydia trachomatis* targets host lipid droplets. Curr Biol. 2006;16(16):1646-1651. doi:10.1016/j.cub.2006.06.060
11. Gaillard JL, Berche P, Mounier J, Richard S, Sansonetti P. In vitro model of penetration and intracellular growth of *Listeria monocytogenes* in the human enterocyte-like cell line Caco-2. Infect Immun. 1987;55(11):2822-2829. doi:10.1128/iai.55.11.2822-2829.1987
12. Brouillette E, Grondin G, Shkreta L, Lacasse P, Talbot BG. In vivo and in vitro demonstration that *Staphylococcus aureus* is an intracellular pathogen in the presence or absence of fibronectin-binding proteins. Microb Pathog. 2003;35(4):159-168. doi:10.1016/s0882-4010(03)00112-8
13. Fraunholz M, Sinha B. Intracellular *Staphylococcus aureus*: live-in and let die. Front Cell Infect Microbiol. 2012;2:43. doi:10.3389/fcimb.2012.00043
14. Dikshit N, Bist P, Fenlon SN, et al. Intracellular uropathogenic *E. coli* exploits host Rab35 for iron acquisition and survival within urinary bladder cells. PLoS Pathog. 2015;11(8):e1005083. doi:10.1371/journal.ppat.1005083
15. Kamaruzzaman NF, Kendall S, Good L. Targeting the hard to reach: challenges and novel strategies in the treatment of intracellular bacterial infections. Br J Pharmacol. 2017;174(14):2225-2236. doi:10.1111/bph.13664
16. Kansau I, Berger C, Hospital M, et al. Zipper-like internalization of Dr-positive *Escherichia coli* by epithelial cells is preceded by an adhesin-induced mobilization of raft-associated molecules in the initial step of adhesion. Infect Immun. 2004;72(7):3733-3742. doi:10.1128/iai.72.7.3733-3742.2004
17. Swanson JA, Baer SC. Phagocytosis by zippers and triggers. Trends Cell Biol. 1995;5(3):89-93. doi:10.1016/s0962-8924(00)88956-4
18. Mukhopadhyay S, Herre J, Brown GD, Gordon S. The potential for Toll-like receptors to collaborate with other innate immune receptors. Immunology. 2004;112(4):521-530. doi:10.1111/j.1365-2567.2004.01941.x
19. Knodler LA. *Salmonella enterica*: living a double life in epithelial cells. Curr Opin Microbiol. 2015;23:23-31. doi:10.1016/j.mib.2014.10.010
20. Mitchell G, Chen C, Portnoy DA. Strategies used by bacteria to grow in macrophages. Microbiol Spectr. 2016;4(3):10.1128/microbiolspec.MCHD-0012-2015. doi:10.1128/microbiolspec.MCHD-0012-2015
21. Ernst RK, Guina T, Miller SI. How intracellular bacteria survive: surface modifications that promote resistance to host innate immune responses. J Infect Dis. 1999;179 Suppl

2:S326-S330. doi:10.1086/513850
22. McClure EE, Chávez ASO, Shaw DK, et al. Engineering of obligate intracellular bacteria: progress, challenges and paradigms. Nat Rev Microbiol. 2017;15(9):544-558. doi:10.1038/nrmicro.2017.59
23. Kapoor G, Saigal S, Elongavan A. Action and resistance mechanisms of antibiotics: a guide for clinicians. J Anaesthesiol Clin Pharmacol. 2017;33(3):300-305. doi:10.4103/joacp.JOACP_349_15
24. Campbell EA, Korzheva N, Mustaev A, et al. Structural mechanism for rifampicin inhibition of bacterial RNA polymerase. Cell. 2001;104(6):901-912. doi:10.1016/s0092-8674(01)00286-0
25. Kuehl CJ, Dragoi AM, Talman A, Agaisse H. Bacterial spread from cell to cell: beyond actin-based motility. Trends Microbiol. 2015;23(9):558-566. doi:10.1016/j.tim.2015.04.010
26. Sharma AK, Dhasmana N, Dubey N, et al. Bacterial virulence factors: secreted for survival. Indian J Microbiol. 2017;57(1):1-10. doi:10.1007/s12088-016-0625-1
27. Feng W, Chittò M, Moriarty TF, Li G, Wang X. Targeted drug delivery systems for eliminating intracellular bacteria. Macromol Biosci. 2023;23(1):e2200311. doi:10.1002/mabi.202200311
28. Ghosh R, Pal S, Sarkar S, Dam S. Pathological aspects of microbial phospholipases. In: Chakraborti S, ed. Phospholipases in Physiology and Pathology: Academic Press; 2023:9-33. doi:10.1016/b978-0-443-21800-2.00017-8
29. Boss L, Kędzierska B. Bacterial Toxin-Antitoxin Systems' Cross-Interactions-Implications for Practical Use in Medicine and Biotechnology. Toxins (Basel). 2023;15(6):380. doi:10.3390/toxins15060380
30. Whatmore AM, Reed RH. Determination of turgor pressure in *Bacillus subtilis*: a possible role for K+ in turgor regulation. J Gen Microbiol. 1990;136(12):2521-2526. doi:10.1099/00221287-136-12-2521
31. Schmiel DH, Miller VL. Bacterial phospholipases and pathogenesis. Microbes Infect. 1999;1(13):1103-1112. doi:10.1016/s1286-4579(99)00205-1
32. Lin DM, Koskella B, Lin HC. Phage therapy: an alternative to antibiotics in the age of multi-drug resistance. World J Gastrointest Pharmacol Ther. 2017;8(3):162-173. doi:10.4292/wjgpt.v8.i3.162
33. Chehelgerdi M, Chehelgerdi M, Allela OQB, et al. Progressing nanotechnology to improve targeted cancer treatment: overcoming hurdles in its clinical implementation. Mol Cancer. 2023;22(1):169. doi:10.1186/s12943-023-01865-0
34. Hosseini SM, Taheri M, Nouri F, Farmani A, Morovati Moez N, Arabestani MR. Nano drug delivery in intracellular bacterial infection treatments. Biomed Pharmacother. 2022;146:112609. doi:10.1016/j.biopha.2021.112609
35. Zhao L, Islam MS, Song P, Zhu L, Dong W. Isolation and optimization of a broad-spectrum synthetic antimicrobial peptide, Ap920-WI, from *Arthrobacter* sp. H5 for the biological control of plant diseases. Int J Mol Sci. 2023;24(13):10598. doi:10.3390/ijms241310598
36. Nehdi A, Samman N, Mashhour A, et al. A drug repositioning approach identifies a combination of compounds as a potential regimen for chronic lymphocytic leukemia treatment. Front Oncol. 2021;11:579488. doi:10.3389/fonc.2021.579488
37. Bravo-Santano N, Behrends V, Letek M. Host-targeted therapeutics against multidrug resistant intracellular *Staphylococcus aureus*. Antibiotics (Basel). 2019;8(4):241. doi:10.3390/antibiotics8040241
38. Eisenreich W, Rudel T, Heesemann J, Goebel W. Persistence of intracellular bacterial pathogens-with a focus on the metabolic perspective. Front Cell Infect Microbiol. 2020;10:615450. doi:10.3389/fcimb.2020.615450
39. Bongers S, Hellebrekers P, Leenen LPH, Koenderman L, Hietbrink F. Intracellular penetration and effects of antibiotics on *Staphylococcus aureus* inside human neutrophils: a comprehensive review. Antibiotics (Basel). 2019;8(2):54. doi:10.3390/antibiotics8020054
40. Munita JM, Arias CA. Mechanisms of antibiotic resistance. Microbiol Spectr. 2016;4(2):10.1128/microbiolspec.VMBF-0016-2015. doi:10.1128/microbiolspec.VMBF-0016-2015
41. Azimi T, Zamirnasta M, Alizadeh Sani M, Soltan Dallal MM, Nasser A. Molecular mechanisms of *Salmonella* effector proteins: a comprehensive review. Infect Drug Resist. 2020;13:11-26. doi:10.2147/idr.s230604
42. Duan C, Cao H, Zhang LH, Xu Z. Harnessing the CRISPR-Cas systems to combat antimicrobial resistance. Front Microbiol. 2021;12:716064. doi:10.3389/fmicb.2021.716064
43. Gallagher JC. Omadacycline: a modernized tetracycline. Clin Infect Dis. 2019;69(Suppl 1):S1-S5. doi:10.1093/cid/ciz394
44. Ye J, Chen X. Current promising strategies against antibiotic-resistant bacterial infections. Antibiotics (Basel). 2022;12(1):67. doi:10.3390/antibiotics12010067
45. Waheed S, Li Z, Zhang F, Chiarini A, Armato U, Wu J. Engineering nano-drug biointerface to overcome biological barriers toward precision drug delivery. J Nanobiotechnology. 2022;20(1):395. doi:10.1186/s12951-022-01605-4
46. Aldughaim MS, Muthana M, Alsaffar F, Barker MD. Specific targeting of PEGylated liposomal doxorubicin (Doxil®) to tumour cells using a novel TIMP3 peptide. Molecules. 2020;26(1):100. doi:10.3390/molecules26010100
47. Hou K, Wu ZX, Chen XY, et al. Microbiota in health and diseases. Signal Transduct Target Ther. 2022;7(1):135. doi:10.1038/s41392-022-00974-4
48. Alam MZ, Maslanka JR, Abt MC. Immunological consequences of microbiome-based therapeutics. Front Immunol. 2022;13:1046472. doi:10.3389/fimmu.2022.1046472
49. Sharma K, Li-Kim-Moy J. COVID-19 vaccines in 2023. Aust Prescr. 2023;46(3):60-63. doi:10.18773/austprescr.2023.020
50. Tavares LP, Galvão I, Ferrero MR. Novel immunomodulatory therapies for respiratory pathologies. In: Kenakin T, ed. Comprehensive Pharmacology. Oxford: Elsevier; 2022:554-594. doi: 10.1016/b978-0-12-820472-6.00073-6.