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Chapter 6

Outer Membrane Permeability Enhancer

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ABSTRACT

Bacterial resistance poses a significant threat to global healthcare systems and requires innovative solutions to combat this growing problem. In this context, the concept of outer membrane permeability enhancers has emerged as a promising strategy. The gram-negative bacteria's outer membrane acts as a robust defense mechanism, limiting the effectiveness of antibiotics. The introduction of outer membrane permeability enhancers aims to address this challenge by enhancing

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the permeability of the outer membrane, thus facilitating the entry of antibiotics into bacterial cells. In this chapter, the authors explore the intricacies of outer membrane permeability enhancers like EDTA, polymyxins, aminoglycosides, and tris or tris (hydroxymethyl) aminomethane and their potential impact on bacterial resistance. The outer membrane's composition, comprising lipopolysaccharides, proteins, and phospholipids, creates a formidable barrier that many antibiotics struggle to penetrate.

INTRODUCTION

Antimicrobial drug resistance is a pressing global health concern that threatens our ability to treat bacterial infections effectively. One major contributor to this problem is the emergence and spread of multidrug-resistant Gram-negative bacteria, which possess formidable outer membranes that serve as a barrier against many antibiotics. The outer membrane of Gram-negative bacteria is composed of a lipid bilayer, lipopolysaccharides (LPS), and protein channels known as porins. This complex structure limits the entry of therapeutic agents, making it challenging to combat infections caused by these resistant pathogens.

This chapter exploring innovative strategies to overcome the outer membrane barrier and enhance the effectiveness of antibiotics. One such strategy involves the use of Outer Membrane Permeability Enhancers (OMPEs), which are compounds designed to increase the permeability of the Gram-negative bacterial outer membrane. OMPEs represent a promising approach to tackling drug resistance by improving the delivery of antibiotics and other therapeutic agents to their intracellular targets.

The development and application of OMPEs are rooted in an understanding of the unique features of the Gram-negative bacterial outer membrane and the mechanisms by which it resists drug penetration. This introduction will explore the key aspects of OMPEs in the context of antimicrobial drug resistance, including their mechanisms of action, potential applications, and significance in addressing the growing threat of antibiotic-resistant Gram-negative infections.

ANTIBACTERIAL DRUG RESISTANCE

Antibacterial drug resistance is the ability of bacteria to survive and grow in the presence of antibiotics that have potency to kills them or stop their growth. Antibacterial drug resistance can make difficulty in the treatment of microbial infections, increase the risk of complications and spread, and lead to higher medical costs and mortality. Antibacterial drug resistance caused by the misuse and overuse of antibiotics, as

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well as poor infection prevention and control practices. Some examples of infections that are becoming resistant to antibiotics are pneumonia, tuberculosis, gonorrhea, and salmonellosis (Chiang et al, 2018).

There are many factors that contribute to the emergence and spread of antibacterial drug resistance, such as the misuse and overuse of antibiotics in human and veterinary medicine, the lack of infection prevention and control measures in health care settings and communities, the poor sanitation and hygiene practices in some regions, and the genetic diversity and adaptability of bacteria (Liu et al, 2022).

There are also many strategies to prevent and combat antibacterial drug resistance, such as:

1. Rational use of antibiotics based on prescription and diagnosis.
2. Development of new antibiotics and alternative therapies.
3. Implementation of surveillance and monitoring systems to track resistance patterns and trends.
4. Promotion of infection prevention and control measures, such as vaccination, hand washing, and isolation,
5. Education and awareness of health professionals and the public about the responsible use of antibiotics.

MECHANISMS INVOLVED IN ANTIMICROBIAL RESISTANCE

Antimicrobial resistance (AMR) is a pressing global concern characterized by various mechanisms. These include genetic mutations, horizontal gene transfer, drug efflux pumps, enzymatic inactivation, target site alterations, biofilm formation, adaptive resistance, antibiotic modification, overuse/misuse of antibiotics, and a shortage of new antibiotics. These mechanisms collectively undermine the effectiveness of antimicrobial drugs and pose a significant challenge to public health. Combatting AMR requires responsible antibiotic use, robust infection control measures, innovative drug development, and international collaboration.

Mutation of Target Proteins: Microbes can undergo genetic mutations that alter the structure of the target proteins where antibiotics are going to bind (Figure 1). Mutation of target proteins plays an important role in the development of antimicrobial drug resistance. When microorganisms, such as bacteria or fungi, undergo genetic mutations in the genes that encode the target proteins of antimicrobial drugs, it can lead to reduced drug effectiveness or complete resistance. For example, linezolid is a new antibiotic that binds to a unique site on the bacterial ribosome and inhibits protein synthesis. It is effective against gram-positive bacteria, including vancomycin-resistant *enterococci* (VRE) and methicillin-resistant *Staphylococcus*

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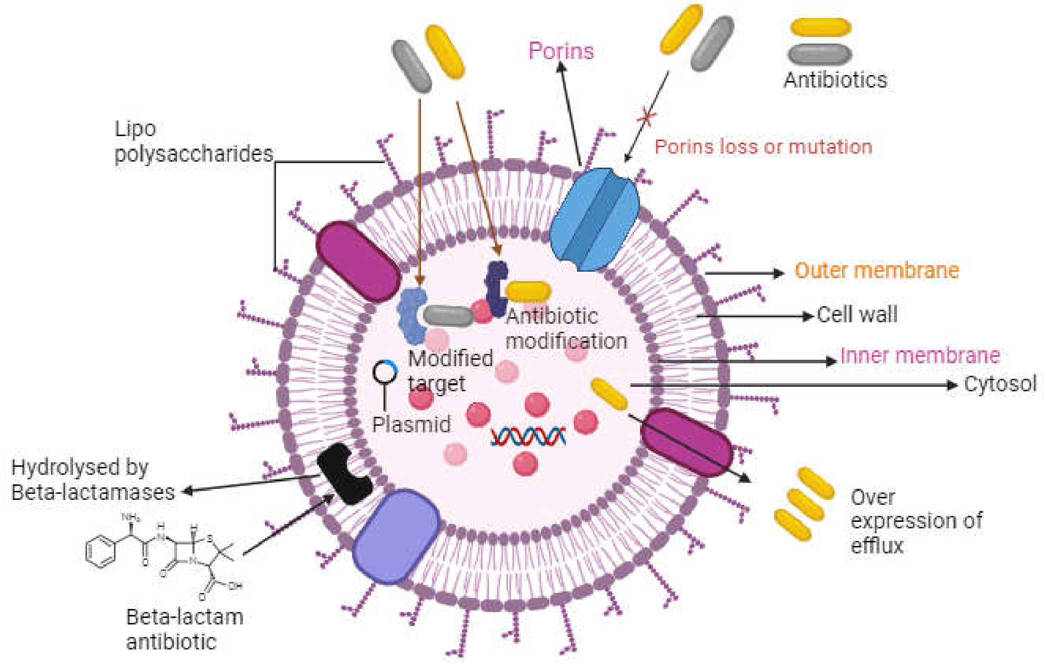
Figure 1. Diagram depicting the mechanism involves in antimicrobial resistance including mutation of target proteins, enzyme production, efflux pumps, and altered permeability



aureus (MRSA). These mutations often result in structural changes in the target proteins, preventing the drugs from binding effectively and inhibit their intended functions. (Ghai, 2023) This adaptation by microorganisms is a major challenge in the treatment of infections, as it can render previously effective antibiotics or antifungal agents ineffective. Understanding the mechanisms and patterns of mutation in target proteins is essential for developing new antimicrobial strategies and combating the growing threat of drug-resistant infections. Researchers and healthcare professionals must continually monitor and adapt their approaches to address these mutations and maintain the efficacy of antimicrobial therapies (Foster, 2017).

Enzyme Production: Enzyme production is one of the mechanisms that microbes use to develop resistance to antimicrobial drugs (Figure 1). Some microbes can produce enzymes that either modify or degrade the drug, making it ineffective. For example, some bacteria can produce β-lactamases, which are enzymes that break down the β-lactam ring of penicillins and cephalosporins. Other bacteria can produce aminoglycoside-modifying enzymes, which are enzymes that add chemical groups

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to aminoglycosides, such as streptomycin and gentamicin, and prevent them from binding to the bacterial ribosome (Alexander et al., 2023).

There are different ways that microbes can acquire the genes for enzyme production. Some microbes have these genes on their chromosomes and can pass them on to their offspring through vertical gene transfer. Other microbes can obtain these genes from other microbes through horizontal gene transfer, such as plasmids, transposons, or bacteriophages (Khare et al, 2021).

Enzyme production is a major challenge for the treatment of infections caused by drug-resistant microbes. Therefore, scientists are developing new strategies to

Efflux Pumps: An antimicrobial drug needs to be inside a bacterial system in high concentrations for a longer period in order to kill or stop the growth of bacteria. Microbes may possess efflux pumps that actively pump out antibiotics and other foreign particles from their cells (Figure 1). This process reduces the concentration of the antibiotic within the microbe cell, making it less effective. Some efflux pumps specifically pump out particular antibiotics such as macrolides, lincosamides, streptogramins, and tetracyclines, whereas multiple drug resistance pumps pump out a variety drugs (Webber, 2003).

Pumps are present in following species of bacteria:

1. *E. coli* and other Enterobacteriaceae against tetracyclines
2. Enterobacteriaceae against chloramphenicol
3. *Staphylococci* against macrolides and streptogramins
4. *Staphylococcus aureus* and *Streptococcus pneumonia* against fluoroquinolones

Horizontal Gene Transfer: Bacteria can transfer resistance genes horizontally to other bacteria. This transfer can occur through processes like conjugation, transformation, or transduction, enabling the rapid spread of resistance within microbial populations (Liu et al, 2022).

HGT is a major factor that contributes to the emergence and spread of antimicrobial drug resistance, especially in multidrug-resistant (MDR) bacteria. MDR bacteria, that are resistant to at least three classes of antibiotics, such as methicillin-resistant *Staphylococcus aureus*

(MRSA) and vancomycin-resistant *enterococci* (VRE). HGT can enable MDR bacteria to acquire new ARGs from other bacteria, making them resistant to more antibiotics. HGT can also facilitate the dissemination of MDR bacteria and their ARGs across different environments, such as hospitals, farms, and water sources (Sun et al, 2019).

Biofilm Formation: Microbes within biofilms (structured microbial communities) are often more resistant to antibiotics. The biofilm matrix can physically block antibiotics from reaching the bacterial cells, making them less susceptible to treatment.

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Biofilm formation is another mechanism that microbes use to develop resistance to antimicrobial drugs. Biofilms are surface-attached groups of microbial cells, surrounded by a self-produced extracellular matrix. Biofilms protect the cells from the action of antimicrobial agents, as well as from the host immune system and environmental stresses (Hall & Mah, 2017).

Some of the factors that contribute to the increased resistance of biofilm cells to antimicrobial drugs are:

1. Reduced penetration: The extracellular matrix of biofilms can act as a physical barrier that prevents or slows down the diffusion of antimicrobial agents into the biofilm. This can result in lower concentrations of the drugs reaching the target cells, especially in the deeper layers of the biofilm.
2. Altered metabolism: The cells in biofilms have different metabolic rates and gene expression profiles than planktonic cells. Some cells in biofilms may enter a dormant or slow-growing state, which makes them less susceptible to antimicrobial agents that target active processes, such as cell wall synthesis or protein synthesis.
3. Phenotypic variation: The cells in biofilms can exhibit phenotypic variation, which means that they can switch between different phenotypes or traits, such as motility, adhesion, or antibiotic susceptibility.
4. Genetic exchange: The cells in biofilms can exchange genetic material, such as plasmids or transposons, with other cells through horizontal gene transfer. This can result in the transfer of antibiotic resistance genes among different bacterial species or strains, making them resistant to one or more antibiotics.

Altered Permeability: Altered permeability is a mechanism that microbes use to develop resistance to antimicrobial drugs (Figure 2). It involves the modification of the microbial membrane, which acts as a barrier that prevents or reduces the entry or exit of antimicrobial agents. Altered permeability can affect the intrinsic resistance of microbes, as well as the acquired resistance due to mutations or gene transfer. Altered permeability in drug resistance is a complex and multifaceted challenge in the field of medicine. Understanding the mechanisms behind this phenomenon is crucial for developing effective strategies to combat drug-resistant diseases (Viveiros et al, 2007).

Altered permeability involves changes in the cell membrane that limit the entry of drugs into the cell. This alteration can occur through various mechanisms, including, porin proteins (loss or mutation in porins), and efflux pumps (over expression of efflux).

Some of the factors that can alter the permeability of microbes to antimicrobial drugs are:

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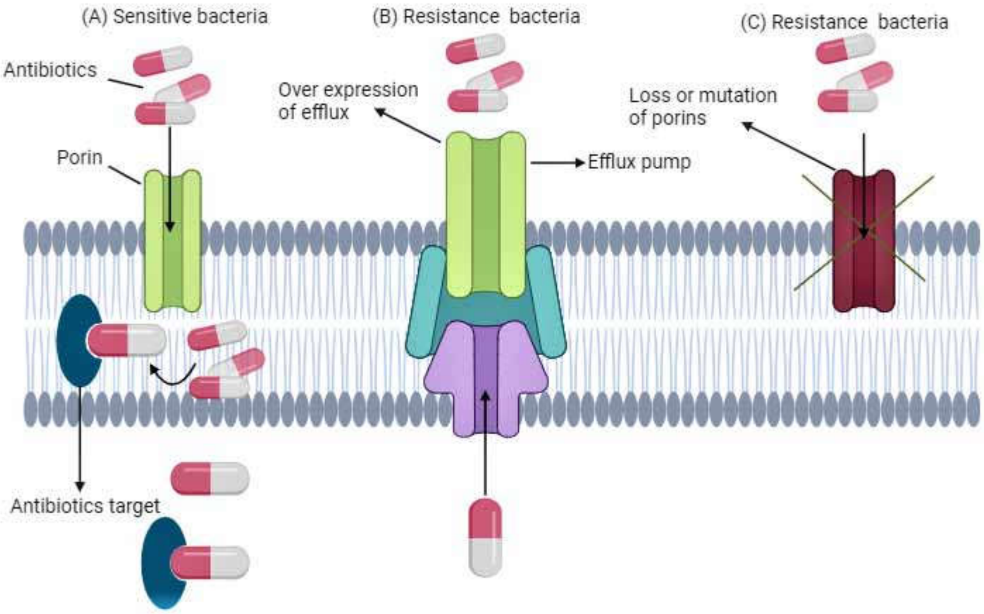
Figure 2. Diagram depicting the mechanism of antimicrobial resistance through the altered permeability



1. Porin proteins: Porins are channels that allow the passage of hydrophilic molecules, such as antibiotics, across the outer membrane of Gram-negative bacteria (Zhou et al, 2023). Some bacteria can reduce their permeability to antibiotics by decreasing the number or changing the structure of porin proteins, resulting in lower uptake or increased resistance. For example, mutations in porin proteins can cause resistance to aminoglycosides (Viveiros et al, 2007).
2. Efflux pumps: Efflux pumps are transporters that expel various substances, such as antibiotics, from the cytoplasm or periplasm of bacteria. Some bacteria can increase their resistance to antibiotics by increasing the number or activity of efflux pumps, resulting in higher efflux or reduced accumulation. For example, overexpression of efflux pumps can cause resistance to fluoroquinolones, beta-lactams, and tetracyclines (Webber, 2003).
3. Lipid composition: Lipids are the main components of the microbial membrane that affect its fluidity and permeability. Some bacteria can alter their lipid composition in response to environmental stress, such as antibiotic exposure, and modulate their membrane permeability to antibiotics. For example, some bacteria can increase the proportion of unsaturated fatty acids in their membrane, which can reduce the permeability to hydrophobic antibiotics (Viveiros et al, 2007).

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Altered permeability is a common and important cause of antimicrobial drug resistance, especially in Gram-negative bacteria that have a complex membrane structure. Therefore, scientists are searching for new ways to overcome this problem, such as:

1. Using permeabilizers, which are substances that can enhance the permeability of bacteria to antibiotics by disrupting or weakening their membrane. For example, some permeabilizers can interact with lipopolysaccharides or phospholipids in the outer membrane of Gram-negative bacteria and increase its permeability to hydrophilic antibiotics.
2. Using inhibitors of efflux pumps, which are substances that can block or reduce the activity of efflux pumps and increase the intracellular concentration of antibiotics. For example, some inhibitors of efflux pumps can bind to and inhibit the function of specific efflux pump proteins or regulators.
3. Using synergistic combinations, which involve administering two or more antibiotics that have different mechanisms of action and target different parts of the microbial cell. This can enhance the efficacy and reduce the resistance development of antibiotics. For example, some synergistic combinations can use one antibiotic to increase the permeability of bacteria to another antibiotic.

THE OUTER MEMBRANE BARRIER

The outer membrane barrier is an important characteristic of Gram-negative bacteria that helps to protects them from numbers of antibiotics and other harmful foreign substances. The outer membrane consists of a lipid bilayer with lipopolysaccharide (LPS) molecules on the outer surface and outer membrane proteins (OMPs) that span the entire membrane. OMPs are involved in various functions, such as nutrient uptake, signal transduction, and cell adhesion (Ghai & Ghai, 2018).

Certain molecules, such as small nutrients, can pass through porins – protein channels embedded in the outer membrane. These porins serve as gatekeepers, allowing specific molecules to enter while keeping larger, potentially harmful substances at bay. In the context of drug resistance, this selective permeability becomes a significant hurdle for antibiotics attempting to breach the membrane (Hancock, 1997).

However, some bacteria can alter their outer membrane structure and composition that helps to reduce the permeability of antibiotics and increase their drug resistance. For example, some bacteria having tendency to decrease the expression of porins, alter the structure of LPS, or produce efflux pumps that expel antibiotics from the cell (Wasfi et al, 2016).

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Gram-negative bacterial multidrug resistance is a challenging health issue for the landscape of health care system. Antibiotic resistance frequently reported in clinical Gram-negative bacteria, and severely limits the available therapeutic options in health care system to treat infections. Consequently, due to the shortage of novel antibiotics, there is an immense need to understand the molecular mechanisms of antibiotic resistance, especially toward key Gram-negative clinical pathogens, such as Klebsiella, Enterobacter, Pseudomonas, Campylobacter, Acinetobacter, and Salmonella species.

The current innovative method to improving the antibiotics potential, is to efficiently introduce them into the bacterial cell and further prevent them from degradation by bacterial enzymes before they reach their targets. This is the strategy, which can counter the problem of antibiotic resistance. The main mechanisms employed by Gram-negative bacteria against available antibiotic therapy include the enzymatic barrier, which primarily destroys the antibiotics; the membrane barrier, which limits the entrance of antibiotics within the cell; and antibiotic target modification, resulting in the overall failure of antibiotic therapy (Durães & Sousa, 2019).

LPS form a permeation barrier and stop the entrance of antibiotics inside the cell, which leads to drug resistance. To improve the efficacy of antibiotics, it is necessary to understand the methods that improve the diffusion of antibiotics and bypass the bacterial membrane barrier, which is responsible for the antibiotic resistance of Gram-negative bacteria. Permeabilizers are compounds that weaken the outer membrane of Gram-negative bacteria, enhance the permeability of bacterial cells to exogenous products, including antimicrobial agents. They are potentiate the antibacterial activity of antibiotics that interact with intracellular targets mainly due to the perturbation of the lipid fraction of the cell membrane as they disintegrate the LPS layer (Ghai & Ghai, 2018).

OUTER MEMBRANE PERMEABILITY ENHANCERS

Outer membrane permeability enhancers are compounds designed to disrupt the structural integrity of the outer membrane, thereby enhancing the permeability and allowing antibiotics to bypass the mebrain barrier. These enhancers operate through various mechanisms, including interactions with lipopolysaccharides or porins, the protein channels responsible for controlling molecule passage (Lin et al, 2009).

They can overcome the intrinsic resistance of these bacteria, caused by the low permeability of their outer membrane and the presence of beta-lactamase enzymes that degrade some antibiotics. Some examples of natural outer membrane permeabilizers are thymol, Gallic acid, quercetin, and epigallocatechin gallate. These

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phytochemicals can also act as natural beta-lactamase inhibitors and synergize with antibiotics to kill resistant bacteria.

Outer membrane permeability and beta-lactamase activity are key factors mediating the resistance of bacteria to antibiotics. Entrance of antibiotics inside the bacterial cell is an important factor that affect bactericidal activity, and effective permeabilization of the outer membrane may overcome intrinsic resistance pathways. Therefore, understanding the molecular mechanisms of antibiotic entry via outer membrane proteins (OMPs) and lipid-mediated pathways is essential for developing new strategies to combat Gram-negative bacterial infections. Some methods that can improve the diffusion of antibiotics and bypass the bacterial membrane barrier are gamma irradiation, which can alter the permeability barrier, beta lactamase activity, and outer membrane protein profiles of the tested isolates1, and control of antibiotic permeability by modifying the structure or expression of Omps and LPS. These approaches can enhance the efficacy of antibiotics and reduce the emergence of resistance (Farrag et al, 2019).

One of the case studies of outer membrane permeability enhancers in drug resistance is the use of natural phytochemicals to boost the antibiotic action against irradiated resistant bacteria. This study search for develop new strategies avoid the resistance of pathogenic Gram-negative bacilli by a combination of conventional antibiotics, potent permeabilizers and natural beta lactamase inhibitors enhancing the activity of various antibiotics.

The study used thymol and Gallic acid as the most potent permeabilizers and quercetin and epigallocatechin gallate as natural beta lactamase inhibitors. The combination of these phytochemicals with antibiotics induced greater susceptibility of resistant isolates compared to antibiotic treatment with beta lactamase inhibitors alone. The study also showed that gamma irradiation altered the permeability barrier, beta lactamase activity, and outer membrane protein profiles of the tested isolates, which enhanced the efficacy of antibiotics (Farrag et al, 2019).

The study concluded that the synergistic effects of the natural phytochemicals and antibiotics led to new clinical choices via outer membrane destabilization (permeabilizers) and/or inactivation of the beta lactamase enzyme, which enabled the use of older, more cost-effective antibiotics against resistant strains.

Ethylenediamine tetra-acetic acid (EDTA): Ethylenediamine, often abbreviated as “EDA,” is a chemical compound with the formula $C_2H_8N_2$. It is a colorless, clear liquid with a strong ammonia-like odour. Ethylenediamine is widely used in various industrial applications, including as a precursor in the production of polymers, adhesives, and coatings. It also serves as an outer membrane permeability enhancer (Raad et al, 2008).

EDTA increased outer membrane permeability to many compounds in a wide range of gram-negative bacteria, act as an outer membrane permeability enhancer.

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The mechanism of action of EDTA is its strong divalent cation-chelating function. Although it should be stressed that, with the exception of P. aeruginosa. An organic cation and mild alkaline pH are also required. The actual mechanism of outer membrane permeabilization is usually unknown. EDT A apparently causes the loss of substantial amounts of LPS [between 30% and 67% of the total in *E. coli* (Raad et al, 2008).

Polymyxins: Polymyxins are a class of antibiotics that can act as outer membrane permeability enhancers for Gram-negative bacteria. They can bind to the lipopolysaccharides (LPS) on the bacterial surface and disrupt the integrity of the outer membrane, allowing other antibiotics to enter and kill the bacteria. Polymyxins used as a last-line treatment against infections caused by multidrug-resistant (MDR) Gram-negative bacteria.

However, polymyxins have some drawbacks, such as nephrotoxicity and limited spectrum of activity. Therefore, researchers have tried to modify the structure of polymyxins to reduce their toxicity and increase their potency. One of the modifications is to replace the amino groups in the polymyxin backbone with guanidine groups, resulting in guanidinylated polymyxins. Guanidinylated polymyxins have lower antibacterial activity than polymyxins, but they can still act as outer membrane permeabilizers and potentiate the activity of other antibiotics, such as rifampicin, erythromycin, ceftazidime and aztreonam. Guanidinylated polymyxins can also synergize with avibactam, a β-lactamase inhibitor, to overcome β-lactam resistance in some Gram-negative bacteria (Ramirez et al, 2022).

Aminoglycosides: Aminoglycosides are a group of antibiotics that can kill Gram-negative bacteria by binding to their ribosomes and interfering with protein synthesis. However, aminoglycosides have to cross the outer membrane of the bacteria to reach their target, which is a major obstacle for their effectiveness. The outer membrane is composed of lipopolysaccharides (LPS) and phospholipids, and it contains protein channels called porins that allow the passage of small hydrophilic molecules.

One way to enhance the uptake of aminoglycosides is to use outer membrane permeabilizers, which are compounds that can weaken or disrupt the outer membrane and increase its permeability to antibiotics. Some examples of natural outer membrane permeabilizers are thymol and Gallic acid, which can bind to LPS and cause its release, making the bacteria more sensitive to aminoglycosides and other antibiotics1. Another example is EDTA, which can chelate divalent cations that stabilize the LPS layer and facilitate the entry of aminoglycosides through the lipid bilayer (Ramirez et al, 2022).

Another way to enhance the uptake of aminoglycosides is to exploit the existing porins in the outer membrane. Different porins have different sizes and selectivity for various molecules. For example, OmpF and OmpC are general porins that can allow the passage of aminoglycosides such as kanamycin, gentamicin and

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amikacin, while LamB is a sugar-specific porins that has no significant permeation for these antibiotics. Therefore, by modulating the expression or activity of specific porins, it may be possible to increase or decrease the susceptibility of bacteria to aminoglycosides (Ude et al, 2021).

Tris or tris (hydroxymethyl) aminomethane: Tris (hydroxymethyl) aminomethane, often referred to as "Tris," is a chemical compound with the molecular formula $C_4H_{11}NO_3$. It is a white, crystalline solid used in various laboratory and biochemical applications. Tris have buffering properties, making it valuable in maintaining stable pH levels in solutions. . It also serves as an outer membrane permeability enhancer (Farrag et al, 2019).

Tris, or tris (hydroxymethyl) aminomethane, is a chemical compound that act as a buffer for biological and chemical reactions. It can also increase the permeability of the outer membrane of Gram-negative bacteria, which is a barrier for many antibiotics. Tris can bind to the lipopolysaccharides (LPS) on the bacterial surface and weaken their structure, allowing other molecules to enter the cell more easily. Tris can also chelate metal ions that stabilize the LPS layer and disrupt the integrity of the outer membrane (Farrag et al, 2019).

Tris used as a component of some COVID-19 vaccines, such as the Moderna and Pfizer-BioNTech vaccines, to enhance their stability and delivery. Tris used in combination with other antibiotics to overcome bacterial resistance by facilitating their penetration through the outer membrane. For example, Tris can enhance the activity of aminoglycosides, which are antibiotics that target the ribosomes of bacteria and inhibit protein synthesis. Tris can also potentiate the effect of beta-lactam antibiotics, which are antibiotics that interfere with the cell wall synthesis of bacteria.

Serum and complement: Serum and complement are components of the immune system that can help fight against infections caused by Gram-negative bacteria. These bacteria have a complex cell envelope that consists of an inner membrane, a cell wall and an outer membrane. The outer membrane is composed of lipopolysaccharides (LPS) and phospholipids, and it acts as a barrier for many antibiotics.

One way that serum and complement can enhance the permeability of the outer membrane is by forming the membrane attack complex (MAC), which is a pore-like structure that inserts into the bacterial surface and disrupts its integrity. The MAC formed by the activation of the complement cascade, which is a series of reactions involving different proteins in the serum. The MAC can allow other antimicrobial proteins in the serum, such as lysozyme and phospholipase A2, to enter the cell and degrade the cell wall and the inner membrane of the bacteria.

Another way that serum and complement can enhance the permeability of the outer membrane is by modulating the expression or activity of specific porins, which are protein channels that allow the entrance of small molecules through the outer membrane. Some porins are permeable for certain antibiotics, and their expression

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influenced by environmental factors, such as temperature, pH and osmolarity. Serum and complement can also affect the activity of porins by binding to them or altering their conformation (Heesterbeek et al, 2021).

MECHANISMS OF OUTER MEMBRANE PERMEABILITY ENHANCERS

Outer membrane permeability enhancers operate through a variety of mechanisms, each targeting specific components of the outer membrane. Some compounds interact with lipopolysaccharides, which are integral to the outer membrane's stability. By binding to these molecules, outer membrane permeability enhancers induce structural changes that compromise the integrity of the membrane, allowing antibiotics to breach its defences more effectively. Other enhancers focus on porins, which are protein channels responsible for regulating the entry of molecules into the bacterial cell. By altering the conformation of these channels, outer membrane permeability enhancers can facilitate the passage of antibiotics, effectively nullifying one of the primary resistance mechanisms employed by Gram-negative bacteria.

The mechanisms of outer membrane permeability enhancers in drug resistance, related to how these compounds can weaken the outer membrane of Gram-negative bacteria and increase the uptake of antibiotics or other antimicrobial agents. Some of the possible mechanisms are:

Inducing the number of porins: The number of porins in the outer membrane of Gram-negative bacteria can affect their permeability to various molecules, including antibiotics. Porins are protein channels that allow the passage of small hydrophilic compounds, such as nutrients and some antibiotics, through the outer membrane. However, porins can also be a target for antibiotic resistance, as bacteria can modulate their expression or activity in response to environmental stress, such as antibiotic exposure.

One way to induce the number of porins is to alter the environmental conditions that regulate their expression. For example, temperature, pH, osmolarity, nutrient availability and oxygen levels influence some porins. By changing these factors, it may be possible to increase or decrease the number of porins in the bacterial outer membrane. However, this may also affect other aspects of bacterial physiology and metabolism, and may not be feasible in clinical settings (Ude et al, 2021).

Another way to induce the number of porins is to use genetic engineering or chemical agents that can directly affect the genes or proteins that code for porins. For example, some researchers have used CRISPR-Cas9 technology to knock out or overexpress specific porins in *Escherichia coli* and Pseudomonas aeruginosa and studied their effects on antibiotic susceptibility. Alternatively, some compounds,

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such as salicylate and indole, shown to modulate the expression or activity of porins by interacting with their regulatory systems or altering their conformation (Choi & Lee, 2019).

Inducing the number of porins can have different consequences for antibiotic resistance, depending on the type and mechanism of action of the antibiotic. For some antibiotics, such as β-lactams and aminoglycosides, increasing the number of porins can enhance their entry into the bacterial cell and reduce resistance. For other antibiotics, such as carbapenems and fluoroquinolones, decreasing the number of porins can limit their access to their targets and increase resistance. Therefore, inducing the number of porins may be a potential strategy to modulate antibiotic resistance in Gram-negative bacteria, but it requires careful consideration of the specific porin-antibiotic interactions and the possible trade-offs for bacterial fitness (Zhou et al, 2023).

Reducing drug efflux pumps: Drug efflux pumps (EPs) are proteins that can transport various substances, including antibiotics, out of the bacterial cell, reducing their intracellular concentration and effectiveness. EPs are one of the main mechanisms of antibiotic resistance in Gram-negative bacteria, which have a complex outer membrane that acts as a barrier for many drugs. Reducing the number or activity of EPs can enhance the permeability of the outer membrane and increase the susceptibility of bacteria to antibiotics (Shriram et al, 2018).

One way to reduce the number of EPs is to inhibit their gene expression or translation. This can be achieved by using antisense RNA, ribozymes, or CRISPR-Cas9 technology to target the specific genes or mRNA that code for EPs. However, this approach may have some limitations, such as specificity, stability, delivery, and safety issues.

Another way to reduce the activity of EPs is to use efflux pump inhibitors (EPIs), which are compounds that can block or interfere with the function of EPs. EPIs can be synthetic or natural molecules, such as phytochemicals, that can bind to the EPs and prevent them from pumping out antibiotics. EPIs can also modulate the regulatory systems or signaling pathways that control the expression or activity of EPs. EPIs can gives in combination with antibiotics to enhance their efficacy and overcome resistance (Huang et al, 2022).

Efflux Pump Inhibitors (EPIs): Efflux pump inhibitors are compounds designed to block the activity of drug efflux pumps. By inhibiting these pumps, EPIs prevent bacteria from expelling antibiotics, allowing the drugs to accumulate within the cell and exert their intended effects.

Combination Therapy: Combining efflux pump inhibitors with antibiotics creates a synergistic effect. While the inhibitors prevent the pumps from expelling antibiotics, the drugs can more effectively target and eliminate bacterial pathogens.

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Causing LPS release: One method to cause LPS release is to use natural or synthetic compounds that can bind to LPS and weaken their structure, allowing other molecules to enter the cell more easily. Some examples of natural compounds that can act, as outer membrane permeabilizers are thymol and Gallic acid, which are phytochemicals found in plants. These compounds can sensitize bacteria to SDS and Triton X-100, which are detergents that can disrupt the outer membrane2. They can also enhance the activity of various antibiotics, such as aminoglycosides, beta-lactams, and quinolones, by facilitating their penetration through the outer membrane (Farrag et al, 2019).

Another method to cause LPS release is to use agents that can chelate metal ions that stabilize the LPS layer and disrupt the integrity of the outer membrane. One example of such an agent is EDTA, which is a synthetic compound that can bind to divalent cations, such as calcium and magnesium. EDTA can increase the permeability of the outer membrane and potentiate the effect of antibiotics, such as polymyxins, aminoglycosides, and beta-lactams (Langendonk et al, 2021).

Causing LPS release offers several potential benefits:

1. Enhanced Antibiotic Penetration: By disrupting the outer membrane barrier, antibiotics can enter bacterial cells more effectively, increasing their potency.
2. Combating Resistance: This strategy can help combat antibiotic resistance by bypassing the protective mechanisms that bacteria develop.

Sensitizing bacteria to detergents: Detergents are substances that can solubilize and remove lipids and proteins from the outer membrane of Gram-negative bacteria, making them more permeable to antibiotics and other molecules. However, detergents are usually toxic to both bacteria and host cells, and therefore it is not used as therapeutic agents. Sensitizing bacteria to detergents is a strategy to enhance the efficacy of antibiotics by weakening the outer membrane barrier and facilitating the entry of drugs into the bacterial cell (Farrag et al, 2019).

One way to sensitize bacteria to detergents is to use natural or synthetic compounds that can bind to lipopolysaccharides (LPS), which are the main components of the outer membrane. These compounds can cause LPS release, which reduces the stability and integrity of the outer membrane. For example, thymol and Gallic acid, which are phytochemicals found in plants, can act as outer membrane permeabilizers and sensitize bacteria to SDS and Triton X-100, which are common detergents. These compounds can also enhance the activity of various antibiotics, such as aminoglycosides, beta-lactams, and quinolones, by facilitating their penetration through the outer membrane.

Gallic acid is a natural chemical compound that has numbers of health benefits. It is comes in category of phenolic acid found in many plants, especially fruits,

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nuts, wine, and tea. It has antioxidant properties that can protect your cells from damage caused by free radicals. It may also have antimicrobial properties that can help fight infections and prevent food spoilage. Some studies suggest that Gallic acid may also help with weight management, cancer prevention, and brain health, but more research needed to confirm these effects in humans.

Thymol is a natural compound that has many uses and health benefits. It is a type of essential oil extracted from plants such as thyme, bee balms, and wildflowers. It has strong antiseptic and antimicrobial properties that can help fight infections, pests, and spoilage. It may also have anti-inflammatory, antioxidant, and analgesic effects that can support your health and wellness.

Another way to sensitize bacteria to detergents is to use agents that can activate the complement system, which is a part of the innate immune system that can target and kill bacteria. The complement system can form the membrane attack complex (MAC), which is a pore-like structure that inserts into the bacterial surface and disrupts its integrity. The MAC can increase the permeability of the outer membrane and potentiate the effect of antimicrobial proteins in serum and phagocytes, such as lysozyme and phospholipase A2. The MAC can also sensitize bacteria to detergents, such as SDS and Triton X-100, by allowing them to enter the cell more easily (Heesterbeek et al, 2021).

Modifying OMPs and LPS: OMPs, or outer membrane proteins, and LPS, or lipopolysaccharides, are two major components of the outer membrane of Gram-negative bacteria. They play important roles in maintaining the structure and function of the outer membrane, which acts as a barrier for many antibiotics. Modifying OMPs and LPS can affect the permeability of the outer membrane and influence the susceptibility of bacteria to antibiotics.

One way to modify OMPs and LPS is to use natural or synthetic compounds that can bind to them and weaken their structure, allowing other molecules to enter the cell more easily. For example, thymol and Gallic acid, which are phytochemicals, found in plants, could act as outer membrane permeabilizers and cause LPS release, which reduces the stability and integrity of the outer membrane. These compounds can also enhance the activity of various antibiotics, such as aminoglycosides, beta-lactams, and quinolones, by facilitating their penetration through the outer membrane (Wang et al, 2021).

Another way to modify OMPs and LPS is to use genetic engineering or chemical agents that can directly affect the genes or proteins that code for them. For example, some researchers have used CRISPR-Cas9 technology to knock out or overexpress specific OMPs in *Escherichia coli* and Pseudomonas aeruginosa and studied their effects on antibiotic susceptibility. Alternatively, some compounds, such as salicylate and indole, show to modulate the expression or activity of OMPs by interacting with their regulatory systems or altering their conformation (Sperandeo et al, 2009).

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Modifying OMPs and LPS can have different consequences for antibiotic resistance, depending on the type and mechanism of action of the antibiotic. For some antibiotics, such as β-lactams and aminoglycosides, decreasing the number or activity of OMPs can limit their access to their targets and increase resistance. For other antibiotics, such as carbapenems and fluoroquinolones, increasing the number or activity of OMPs can enhance their entry into the bacterial cell and reduce resistance. Therefore, modifying OMPs and LPS may be a potential strategy to modulate antibiotic resistance in Gram-negative bacteria, but it requires careful consideration of the specific OMP-LPS-antibiotic interactions and the possible trade-offs for bacterial fitness (Ghai & Ghai, 2018).

Revitalizing Antibiotics, Applications and Potential: Outer membrane permeability enhancers can be natural or synthetic molecules, such as phytochemicals, detergents, chelators, or complement components. They can act by binding to lipopolysaccharides (LPS), which are the main components of the outer membrane, or by modulating the expression or activity of outer membrane proteins (OMPs), which are protein channels that allow the passage of small molecules. Revitalizing antibiotics by using outer membrane permeability enhancers is a promising strategy to combat the growing problem of antibiotic resistance in Gram-negative bacteria. This strategy can enhance the efficacy of existing antibiotics, such as beta-lactams, aminoglycosides, quinolones, and polymyxins, by increasing their access to their targets inside the bacterial cell. This strategy can also enable the use of older, more cost-effective antibiotics that are resist by resistance mechanisms. Moreover, this strategy can reduce the toxicity and side effects of some antibiotics by lowering their required doses. Antibiotics that are ineffective against Gram-negative bacteria due to their inability to penetrate the outer membrane, re-evaluated with the assistance of these enhancers. This offers a unique opportunity to expand the utility of antibiotics that had lost their efficacy, potentially restoring them to the forefront of infection treatment (Farrag et al, 2019).

Moreover, outer membrane permeability enhancers hold promise in the fight against drug-resistant bacterial strains. The rise of such strains has underscored the urgent need for novel approaches to combat resistance. By disrupting the outer membrane barrier, outer membrane permeability enhancers offer a new avenue to target bacteria that have developed intricate resistance mechanisms against traditional antibiotics. This could potentially extend the lifespan of existing antibiotics while reducing the reliance on developing entirely new drug classes (Farrag et al, 2019).

The revitalization of antibiotics using outer membrane permeability enhancers represents a groundbreaking strategy in the fight against antibiotic resistance. By breaking down the barriers that resistant bacteria have erected, this approach offers a pathway to rekindling the effectiveness of antibiotics. As research advances, outer membrane permeability enhancers may play a pivotal role in preserving our

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antibiotic arsenal, providing renewed hope for patients and healthcare providers grappling with drug-resistant infections.

While the concept of outer membrane permeability enhancers is promising, there are several challenges and considerations to keep in mind. First, ensuring that these enhancers only affect bacterial membranes without harming human cells is crucial. Research is ongoing to develop selective enhancers with minimal side effects. Additionally, there is a risk that bacteria may develop resistance to outer membrane permeability enhancers themselves. Continuous monitoring and adaptation will be essential to stay one-step ahead of evolving bacterial defenses. Finally, bringing new treatments to the market involves rigorous testing and regulatory approval. Outer membrane permeability enhancers will need to meet strict safety and efficacy standards.

In the battle against antibiotic resistance, innovation is our greatest ally. Outer membrane permeability enhancers offer a promising avenue for revitalizing antibiotics and improving their effectiveness against drug-resistant bacteria. As research in this field continues, we can hope for a future where antibiotics remain a potent tool in our fight against bacterial infections (Ghai, 2023).

BALANCING EFFICACY AND SAFETY

While the concept of OMPEs holds immense potential, it also introduces critical considerations. Disrupting the outer membrane, while facilitating antibiotic entry, could render the bacterial cell more vulnerable to other threats, such as the host's immune response. Striking the delicate balance between enhancing antibiotic efficacy and ensuring the overall safety of the host remains a key challenge in the development of OMPE-based therapies. Comprehensive studies are essential to elucidate the potential consequences and address any safety concerns that may arise.

Balancing efficacy and safety is a key challenge in developing and using outer membrane permeability enhancers for Gram-negative bacteria. These compounds can enhance the effectiveness of antibiotics by weakening or disrupting the outer membrane barrier, which is a major obstacle for many drugs. However, these compounds can also have adverse effects on the host cells and tissues, such as toxicity, inflammation, and immunogenicity.

One factor that affects the balance between efficacy and safety is the specificity of the outer membrane permeability enhancers. Some compounds, such as detergents and chelators, can act on both bacterial and mammalian membranes, causing damage to both cells. Other compounds, such as phytochemicals and complement components, can selectively target bacterial membranes, sparing the host cells.

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Therefore, designing and screening outer membrane permeability enhancers with high specificity and low toxicity is a crucial step to improve their safety profile.

Another factor that affects the balance between efficacy and safety is the dosage and delivery of the outer membrane permeability enhancers. Some compounds, such as polymyxins and EDTA, can have dose-dependent toxicity, meaning that higher doses can cause more harm than lower doses. Other compounds, such as thymol and Gallic acid, can have dose-independent toxicity, meaning that any dose can cause damage regardless of the concentration. Therefore, optimizing and controlling the dosage and delivery of outer membrane permeability enhancers is an important step to minimize their adverse effects (Bos et al, 2004).

FUTURE PROSPECTS AND CONCLUDING REMARKS

The emergence of outer membrane permeability enhancers marks a paradigm shift in the field of antibiotics. Rather than exclusively seeking novel compounds to combat bacterial infections, scientists now have the opportunity to enhance the activity of existing antibiotics. This approach not only offers a potential solution to bacterial resistance but also provides a strategy to maximize the utility of antibiotics that are resist by bacteria.

Outer membrane permeability enhancers are a promising strategy to revitalize antibiotics and overcome the problem of antibiotic resistance in Gram-negative bacteria. By increasing the permeability of the outer membrane, these compounds can facilitate the entry of antibiotics into the bacterial cell and enhance their efficacy. Outer membrane permeability enhancers can be natural or synthetic molecules, such as phytochemicals, detergents, chelators, or complement components. They can act by binding to lipopolysaccharides (LPS), which are the main components of the outer membrane, or by modulating the expression or activity of outer membrane proteins (OMPs), which are protein channels that allow the passage of small molecules.

However, there are still many challenges and limitations in developing and using outer membrane permeability enhancers. One of them is to balance efficacy and safety, as these compounds also have adverse effects on the host cells and tissues, such as toxicity, inflammation, and immunogenicity. Therefore, it is important to design and screen outer membrane permeability enhancers with high specificity and low toxicity, and to optimize and control their dosage and delivery. Another challenge is to understand the complex interactions and trade-offs between outer membrane permeability enhancers, antibiotics, bacteria, and host cells. For example, modifying OMPs and LPS can have different consequences for antibiotic resistance, depending on the type and mechanism of action of the antibiotic. Therefore, it is

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necessary to study the specific OMP-LPS-antibiotic interactions and the possible impacts on bacterial fitness.

Outer membrane permeability enhancers are a potential strategy to revitalize antibiotics and combat antibiotic resistance in Gram-negative bacteria. However, more research and development needed to improve their efficacy and safety, and to explore their applications and potential in different settings. Outer membrane permeability enhancers may not be a magic bullet for antibiotic resistance, but they may be a valuable tool to complement other strategies and extend the lifespan of existing antibiotics.

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