Global Scientific JOURNALS
GSJ: Volume 12, Issue 3, March 2024, Online: ISSN 2320-9186
www.globalscientificjournal.com

# An Investigation into the Mysteries of Antibiotic Resistance: Bacterial Porins as a Critical Player and New Approaches to Overcoming Antibiotic Resistance

Syed Yawar Saeed[1], Shahzar Khan, Inam UR Rehman Zafar[1], Asim Gamaryani[3], , Afaq Ahmad[1], Laiba Raees[1], Humaira Nawaz[1] , Jawad Ali[1] , Nimrah shah[1], Asma Mukhtiar[1]

[1]Department of Microbiology, Faculty of Biological sciences, Quaid-I-Azam University Islamabad ,452320, Pakistan.

[2]Department of Microbiology, Abdul Wali Khan University Mardan Pakistan

[3]*School of Health and society, University of Wollongong Australia*

Corresponding Author; Shahzar khan, Department of Microbiology, Quaid-I-Azam University Islamabad Pakistan

**Email:** shahzarkhankmu@gmail.com



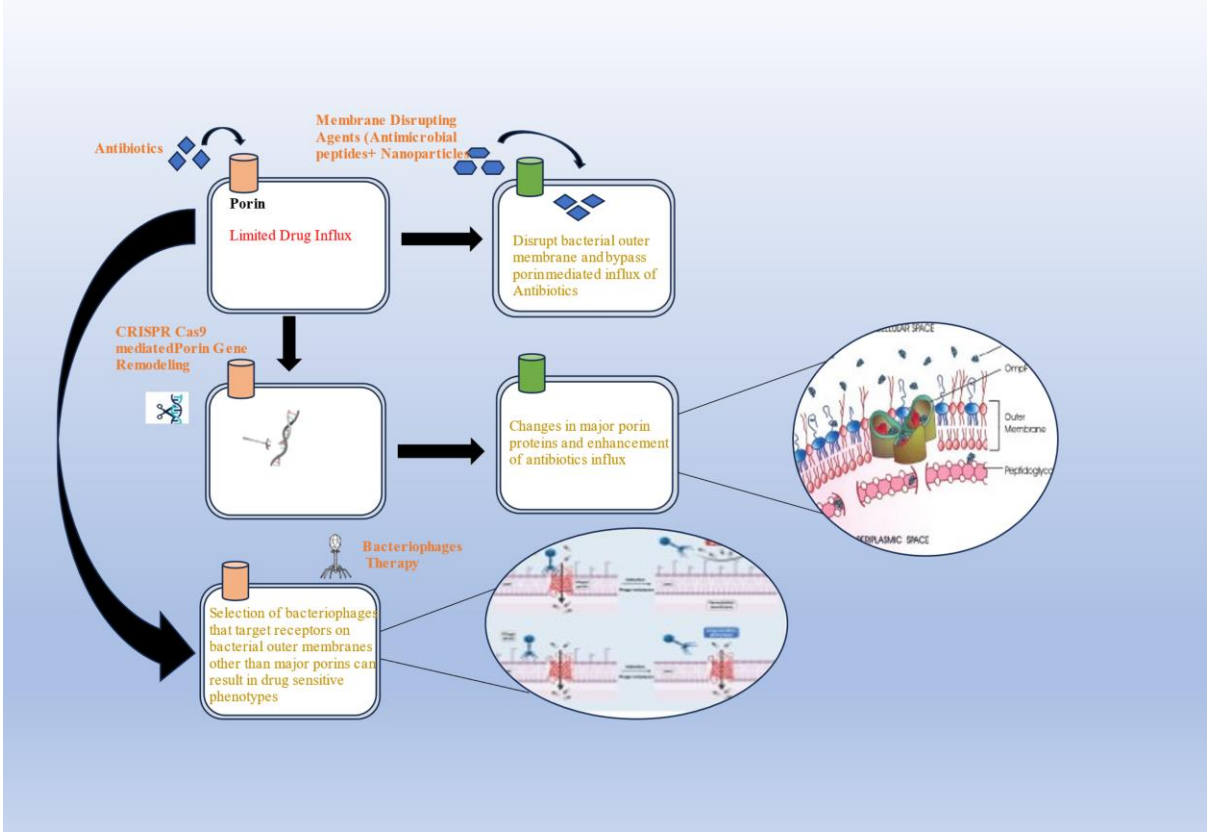
**Funding**

This study received no funding from any source.

**Abstract**

A multimodal strategy is required to effectively address bacterial infections because of the worldwide health challenge of antibiotic resistance. The modification of vital outer membrane proteins underlies porin-mediated resistance in Gram-negative bacteria, which presents a significant obstacle in successful antimicrobial therapy. To combat porin-mediated resistance, this paper examines an integrated approach that involves the use of bacteriophages, membrane-disrupting agents, and CRISPR-Cas9 gene editing. Destabilizing the bacterial outer membrane is possible with the use of membrane-disrupting substances such antimicrobial peptides and nanoparticles. This disturbance directly affects the integrity of porins, decreasing their ability to perform their protective role, as well as increasing bacterial sensitivity to bacteriophages and other natural predators. To defeat resistant strains, bacteriophages must get beyond porin-mediated barriers due to their high host-specificity for bacteria. Additionally, the precise change of the genes in charge of porin production is made possible by CRISPR-Cas9-mediated gene editing. This method can improve bacterial permeability to drugs by either restoring or reconfiguring porin function. A viable path to overcome porin-mediated resistance in Gram-

negative bacteria is shown by the synergistic combination of various tactics. The all-encompassing strategy described in this paper gives a fresh and holistic viewpoint on addressing porin-mediated resistance. We can pave the way for the revival of antibiotic therapy and the reduction of antibiotic resistance in Gram-negative bacteria by utilizing the advantages of membrane-disrupting drugs, bacteriophages, and gene editing.




## 1. Introduction

Protein plays a significant role in every aspect of life. They are found in different forms like enzymes, and cell membrane channels and play vital roles in the functioning of cells [1]. It is the amino acid sequence of the protein that determines the activity of the protein. If any change is made in this amino acid sequence, it may disrupt the proper biological activity and proper functioning of the protein [2]. One of the proteins which we are targeting in this review is the Porin channels present in the outer membrane of gram-negative bacteria shown in **Figure 1**.

The gram-negative outer membrane contains porins which act as a barrier during the transport of materials across the cell membrane. An important functional feature of porin is its opening and closing which facilitate its transporting property [3]. Nutrients and other solutes diffuse into the cells through these porin channels which are made of β- barrel strands. β- barrel strands give rise to water-filled channels which act as diffusion barriers and give the porins a sieve-

like appearance. The name porins are given to it because of its pore shape and the term ‘porin’ was exclusively affected by large non-specific outer membrane β-barrel channels such as OmpF or OmpC [4] .

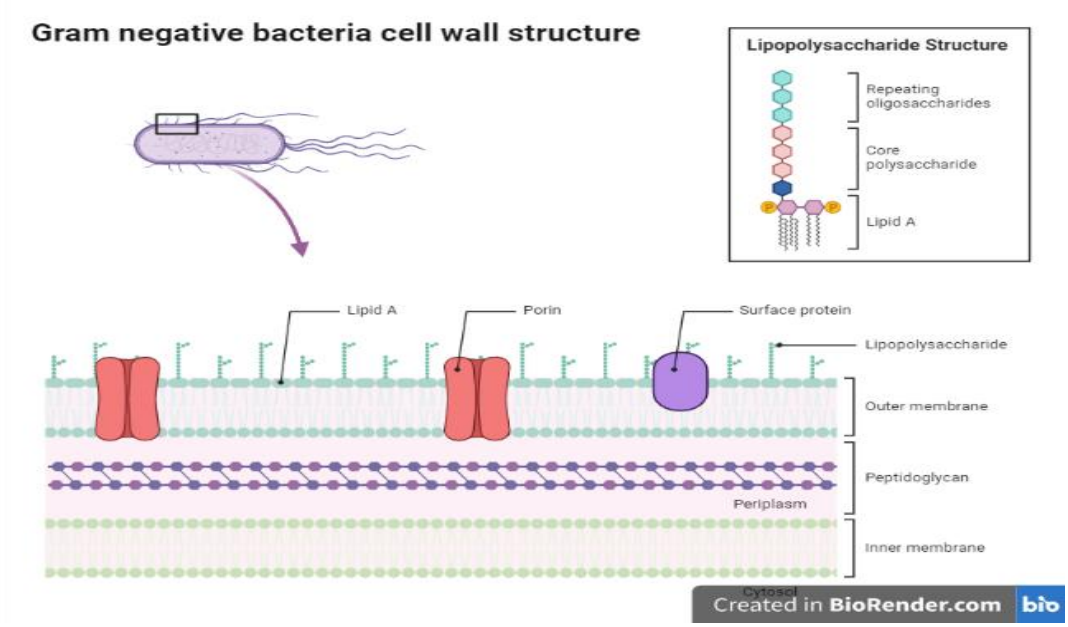



*Figure 1Showing Outer membrane of gram negative bacteria containing Porins*



Porins consist of β-barrel strands having a trimeric structure and consist of different loops that join these β-strands [5]. Among these multiple loops, the most important loop is L3 which folds back into the lumen of the β-barrel strand and here it forms a constriction zone at the mid of the porin that plays a vital role in narrowing the porin and increasing the selectivity of the porin [6]. This L3 consists of clusters of tyrosine and also possesses some other residues. These residues are termed Y and B faces. As are familiar, porin molecules undergo conformational changes and this conformation is an active process and is driven by utilizing energy. OprP and OmpF are involved in specific and nonspecific channels through which the bacteria uptake nutrients. Porins account for 70% mass of the total outer membrane [6]. Porins are highly involved in antibiotic resistance as bacteria always seek to change their porin confirmation in response to environmental stress. Strategies like editing porin genes through CRISPER-cas9, use of membrane destabilizer, bacteriophages can help to bypass porin mediated resistance and make bacteria susceptible to already present antibiotics [7].

## 2. Types of Porins

There are two types of porins involved in the transport of materials across the cell. These include specific and non-specific porins.

### Non-specific Porins

This type of porin has less affinity to binds with permeable solutes. They are large size porins with pore sizes greater than 10 Å. They can transport molecules up to 600 KD in size. There are several dozen non-specific porins identified in different microorganisms [8]. OmpF and PhoE are some of the examples of non-specific porins. The function of non-specific porin is to provide a primary route to several classes of antibiotics. Thus, understanding structural modifications adopted by these pores leading to antibiotic resistance is of prime importance [9].

### Specific Porins

Another type of porins is specific porins which can be called substrate-specific porins. These porins have a strong affinity because of certain binding sites for different substrates. These affinities are measured in micromolar. Because of these binding sites, they make protein channels (porins) that are very efficient even at the low levels of molecules [10]. The determination of specific proteins reveals that bacteria exhibit diversity in several β-strands, oligomeric states, pore diameters, and amino acid distributions. The main difference between the general porins and specific porins is that specific porins have more than one extracellular loop, i.e., usually two to four, folds inward, lowering the pore diameter and significantly reducing the size exclusion limit e.g. ~200 Da or lower [11].

### Biogenesis of β-Barrel Outer Membrane Proteins

The outer membrane in gram-negative consists of some integral membrane proteins that possess the β-barrel fold present in the porin molecules. These β-barrel folds contain 8-26 β-barrel strands that are in an antiparallel manner to each other. These β-barrel strands have the role of anchoring the porins with the outer membrane of the bacteria. In previous literature, it is reported that the synthesis of porins involves specific machinery along with a general pathway known as BAM (β-barrel assembly machinery [12]**.** The synthesis of porins occurs in the cytoplasm and it occurs in the form of small precursors having N-terminal peptide. These precursors along with its N-terminal direct the Sec translocon to be transported into the inner membrane. Molecular chaperones like SurA and Skp in the periplasm escort the mature OMPs during their transit to the outer membrane [12].

**Structural Aspects**

The hydrophobic surfaces of the barrels and extracellular loop L2, which latches into a groove of a neighboring monomer and creates a multitude of polar interactions, provide inter-monomer connections for porins, which are frequently organized as trimers, the structure of porin molecule is given in **Figure 2**. The trimer entity appears to have no clear function because there isn't strong evidence to support cooperativity within the trimer, meaning that the porin monomers probably carry out autonomous functions. The trimeric configuration might just add more stability [13].

The porins from Enterobacteriaceae, such as OmpF from E. coli, have undergone considerable structural and biochemical characterization. Only one of the eight loops present on the extracellular side of the homotrimer is formed by the independent monomers of the OmpF channel and the majority of other generic porins fold back into the barrel to minimize the pore size [14]. The term "constriction" region is frequently used to describe this constrictive area of the channel. At the channel's center, each OmpF monomer creates a cavity with a diameter of 6.5–7, creating a tunnel with an hourglass shape along the channel axis [15]. 72 Additionally, the eyelet region's asymmetrical distribution of charged amino acids includes basic residues on one side of loop L3 and acidic residues on loop L3. There are some basic residues like charged amino acids that are present on the opposite side of the barrel wall of porins and they generate a transverse electric field which results in enhancing the permeability of porins [16].



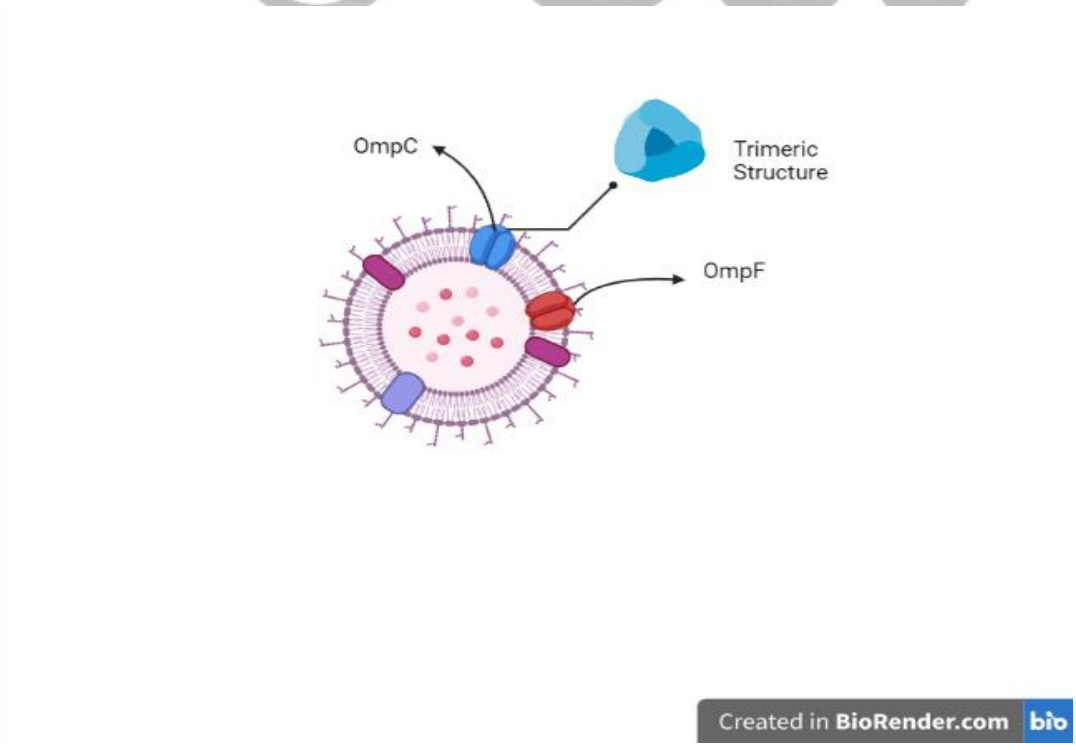



*Figure 2 Shows the structure of outer membrane of bacteria having porins*

**Genomic expression and regulation aspect**

Most of the genes present in porins are transcribed as mRNA. If we talk about the number so it will be 16 out of the 26 genes that are transcribed as mRNA. The two important regulators of porin gene expression are; The first one is a two component system that can detect a signal and is dependent on phosphorylation [4]. The second one is extra cytoplasmic function (ECF) sigma factors which are activated during stress and are expelled out from the cytoplasm and involved in the regulation of multiple genes [17].

**Porins in Outer Membrane**

**a. OprF:** As there on E. coli OmpA is outer membrane protein similarly in P. aeruginosa OprF is present both belong to OmpA protein family. OprF consists of three domains; the first domain is in the outer membrane of the cell and is referred to as a crystallized N-terminal. It has 8 stranded β-barrel. Another domain is known as a cysteine-rich linker which may be partially exposed at the surface. The third domain which is last domain is the C-terminal part which consists of both α-helixes and/or β-strands [4]. The function of OprF being a non-specific water channel allows the transport of ions and low molecular mass sugars. It also allows the passage of toluene. OprF is also responsible for the diffusion of non-cognate iron-chelating siderophores. If the Oprf level is increased in anaerobically grown bacteria. The structural role of Oprf is to maintain cell shape under low osmotic conditions [18]. The C-terminal part of Oprf contains a peptidoglycan binding domain that tethers the outer membrane of bacteria to the peptidoglycan layer. The C-terminal of OprF is required for a stable assembly of porins with peptidoglycan. If there any mutation occurs in the OprF it will be sensitive to low osmolarity and the length of the cell will be reduced [19].

**b. OmpF and OmpC porins:**

E. coli has two main proteins which include OmpC and OmpF. These both play their role in the passive transport of materials into the cell. These are passive diffusion channels responsible for the transport of various materials like small size molecules, some nutrients, and antibiotics. The OmpF porin is in the outer membrane having different antigenic epitopes. These epitopes are located on different loops [20]. OmpF plays an important role in the pathogenesis of bacteria. In a study distribution of ompF and ompC was examined in the APEC collection, and the results showed that the distribution rate of these porin proteins was prominently different and was y higher in group B2 (70.3%) and D (62.7%), suggesting that OmpF and OmpC were mainly encoded in virulent isolates of groups B2 and D, thus made a contribution to APEC pathogenicity [21]. Previous observations of APEC adhesion to and invasion of mouse brain microvascular endothelial cells (bEnd.3) show a relation between the value of these cells as a

model for research underlying the mechanism by which APEC adhesion and invasion occur. OmpF and OmpC were analyzed for their effect on APEC adhesion and invasion using a model based on bEnd.3 cells. It is reported that mutant strains having these two porins have reduced capacity for adhesion and invasion as compared to the wild-type strain. Additionally, genetic complementation was able to partially restore the adhesion and invasion capabilities, proving that ompF and ompC were essential for bEnd.3 cells' capacity to adhere to and invade APEC [21].

## 3. Porins and antibiotic susceptibility

The alteration in OmpF and OmpC porin expression and its response to antibiotics have been discussed in different research. According to recent literature, chemicals from the beta-lactam class, the association between porin susceptibility and porin channel features (OmpC type versus OmpF type) appears to be related to one another [22]. The loss of both major porins results in the highest level of resistance with complete impermeability to β -lactams, which correlates with the resistance phenotype seen in different Enterobacteriaceae isolates. Study of the isolates that are susceptible express both major porins, low level/intermediate resistant isolates exhibit one truncated porin, and high level/extremely resistant isolates exhibit both major porins [23]. Additionally, a larger spectrum of resistance was reported to be produced by the expression of a shortened OmpK36 after carbapenem therapy. According to reports, further changes in ompK35 and ompK36 are the most likely cause of ceftazidime-avibactam resistance in several K. pneumoniae strains [24]. In the OmpK36 internal loop, mutations can also alter the activity of combinations such as imipenem-relebactam or meropenem-varbobactam by directly affecting the expression of the porin [25].

### 3.1 Antibiotic resistance in gram negative bacteria

Resistance to antibiotics in gram-negative bacteria, which represent a danger to the health of the public, must be combated through antimicrobial intervention. Porins, which are proteins in the outer membrane of bacteria that regulate bacterial cell entrance, are crucial to this resistance, underscoring the need of comprehending these resistance mechanisms in order to successfully target and treat bacteria that are resistant to antibiotics [26].

### 3.2 Efflux Pumps

Antibiotics are rendered ineffective by bacteria's efflux pumps, a defensive mechanism that stops antibiotics from entering their target areas. Higher pumping activity brought on by overexpression or mutation of these pumps can result in antibiotic resistance in a variety of gram-negative bacteria, adding to the worldwide issue of antibiotic resistance [27]**.**

### 3.2 Beta-Lactamase Production

Beta-lactamase enzymes are a frequent form of resistance, rendering beta-lactam medicines useless. Since treating bacterial infections in particular offers a substantial issue for medical care, new methods for combating resistance to antibiotics are being developed [28].

### 3.4 Target Site Modifications

By changing the target locations of antibiotics, gram-negative microbes can potentially acquire resistance. For instance, modifications to Penicillin-binding proteins (PBPs) might lower beta-lactam antibiotics' affinity for their targets, reducing the effectiveness of the medication [29].

### 3.5 Adaptations in Porins to Resist Antibiotics

Porins, which are essential parts of the bacterial outer membrane, are crucial for antibiotic absorption. But bacteria have developed ways to modify their porins, which makes antibiotics less effective. Modifications to the porin architecture or levels of expression may be a part of these adaptations [30].



### 3.6 Structural Modifications

Porin proteins' structures may be changed by bacteria to decrease the penetration of antibiotics. These alterations may involve modifications that reduce the total pore size or constrict the porin channel [31].

### 3.7 Downregulation of Porin Expression:

In response to antibiotics exposure, certain bacteria decrease the production of porin proteins. Regulatory mechanisms that decrease porin production may cause this downregulation. As a result, there are fewer entry points for antibiotics, which makes it harder for medications to enter cells and reach their intended objectives [32]**.**

### 3.8 Porin-Specific Antibiotic Exclusion

Some bacteria have evolved defenses that prevent some antibiotics from invading cells through porins. These methods can stop drugs from passing while permitting the flow of vital nutrients. They may entail interactions among antibiotics and certain porin residues [22].

### 3.9 Preserving Protein Function: A Key Consideration

When porin proteins are being considered as prospective targets for antimicrobial intervention, maintaining their structural integrity and functionality is crucial. Any change in the porins' amino acid composition can have a significant impact on their functioning and, as a result, on their capacity to promote nutrition absorption and antibiotic penetration [33].

**4. The Importance of Maintaining Porin Structural Integrity for bacterial survival**

Porins are essential membrane proteins that help Gram-negative bacteria create holes or channels in their outer membrane. These channels act as gatekeepers, letting vital molecules like nutrients and ions pass while preventing the entry of potentially hazardous chemicals [34]. Porins depend on a precise three-dimensional structure and a clearly defined arrangement of amino acids to retain their essential activities. Disruptions to this structural integrity can compromise the selective permeability of porins. A change in porin structure may prevent nutrients from being absorbed, depriving the bacteria of important supplies needed for growth and survival. Bacteria may become more susceptible to external stresses including osmotic shock and changes in temperature or pH as a result of porin structural alterations [35].

**4. Consequences of Amino Acid Sequence Alterations**

Porins' amino acid sequence can vary as a result of genetic mutations or adaptive responses to environmental stresses, such as antibiotic exposure due to which the porin channel's dimensions and shape may change as a result of mutations, which may have an impact on the molecules that may flow through [36]. The equilibrium between nutrient intake and antibiotic exclusion may be thrown off by this. Porin channels can lose their selectivity due to amino acid sequence changes, allowing a larger spectrum of compounds, including antibiotics, to enter the bacterial cell. Changes in the porin channel's internal charge distribution may have an influence on the channel's affinity for certain molecules, which might have an effect on both nutrition absorption and antibiotic penetration [37].

**5. Novel Antimicrobial Approaches Targeting Porins**

Innovative porin-targeting techniques have come to light in the search for efficient antimicrobial therapies as antibiotic resistance continues to represent a serious threat to global health. These strategies provide intriguing ways to deal with drug-resistant bacteria since they attempt to modify porin activity without affecting their structural integrity [38]. The approaches to combat antibiotic resistance are listed in Table 1

**Table 1; list of approaches to combat antibiotic resistance.**

**6. Inhibitors of Porin Channel Function**

A cutting-edge method to selectively alter the activity of these important outer membrane

| Approach | Mechanism | Target Porin(s) | Advantages | Challenges |
|---|---|---|---|---|
| Phage Therapy | Utilizing bacteriophages that bind specific porins | Various | Highly specific, reduces antibiotic resistance | Phage-host specificity, regulatory approval |
| Porin-blocking Peptides | Peptides that block porin channels | OmpF, OmpC, etc. | Directly disrupts bacterial nutrient uptake | Developing effective and stable peptides |
| Porin-based Vaccines | Immunization against specific porins | OmpA, OmpC, etc. | Prevents infection by inducing immune response | Identifying immunogenic and safe porin targets |
| Small Molecule Inhibitors | Molecules that inhibit porin function | LamB, FhuA, etc. | Can be designed for broad or narrow specificity | Potential off-target effects, resistance |
| Antibody Therapeutics | Monoclonal antibodies targeting porins | Selected porins | High specificity, can enhance immune clearance | Cost of development, delivery challenges |
| Synthetic Biology Approaches | Engineering synthetic organisms or systems to target porins | Customizable | Precision targeting, programmable functions | Complexity of design, biocontainment |
| Nanoparticle Delivery Systems | Using nanoparticles to deliver antimicrobials through porins | Non-specific/targeted | Enhanced delivery and penetration, controlled release | Safety, potential toxicity, manufacturing challenges |
| Enzymatic Degradation | Enzymes that degrade outer membrane components including porins | Non-specific | Bypasses traditional resistance mechanisms | Stability of enzymes, delivery to infection site |
| Structural Modification Inhibitors | Compounds that alter porin structure or expression | Specific porins | Prevents correct porin folding/function | Resistance development, specificity |
| CRISPR-Cas Systems | Targeting bacterial DNA to disrupt porin gene expression | Gene-specific | Highly specific gene editing, irreversible effects | Delivery mechanisms, off-target effects |

proteins is to use inhibitors of porin channel function. In order to prevent the entry of hazardous

chemicals like antibiotics while maintaining the structural integrity of the porin protein, porin channels must be blocked or controlled. Small compounds that bind with certain porin areas and change their shape to lessen their permeability to antibiotics have been discovered recently. The possibility of causing harmful changes in the porin protein is reduced by this tailored strategy, which also decreases antibiotic resistance [39].

## 7. Strategies for bypassing Antibiotic influx through porin

### 7.1 Outer Membrane Permeabilizers

Compounds called outer membrane permeabilizers are made to increase how permeable the outer membrane of bacteria is antibiotics that previously had trouble entering the cell can more easily reach their intracellular targets by reducing the membrane barrier. The bilayer of lipids of the outer membrane is disrupted by outer membrane permeabilizers, making the membrane more vulnerable to the action of conventional antibiotics [40]. Antibiotics are able to more efficiently reach their targets because to this synergistic strategy, which also circumvents resistance mechanisms involving decreased porin permeability [41].

### 7.2 Bacterial Membrane-Disrupting Agents

Bacterial membrane disrupting agents are a family of substances that can compromise the integrity of bacterial membranes without requiring antibiotic penetration through porins. For instance, antimicrobial peptides and nanoparticles have shown that they may damage the bacterial outer membrane, causing cell lysis and death [42].

The potential of membrane-disrupting drugs as alternatives to conventional antibiotics is highlighted by research by Li et al. (2020). These substances directly attack the bacterial membrane, making it challenging for bacteria to produce porin-based resistance mechanisms [43].

### 7.3 Bacteriophages

Bacteriophages—viruses that infect and reproduce inside of bacteria—have drawn interest as a possible tool for selectively focusing on and modifying bacterial porin channels. These bacterial natural predators can provide a highly targeted and precise strategy to tackle antibiotic-resistant strains [44].

#### 7.3.1 Bacteriophages as Porin-Targeting Agents its advanatages and drawbacks

Porins are frequently among the receptors on the surface of bacteria that bacteriophages may identify and interact with. Phages can efficiently target different bacterial species and strains depending on their porin profiles by taking advantage of this selectivity as shown in table 2.

Bacteriophages with the capacity to bind to the outer membrane porins have been found in recent investigations (Smith et al., 2023) and are thought to facilitate entrance into bacterial cells as shown in table 3 .

**Table 2; shows bacteriophages and their family along with their target bacteria.**

| Bacteriophage | Target Bacteria | Phage Family | Type | Application Note |
|---|---|---|---|---|
| T4 | Escherichia coli | Myoviridae | Lytic | Research, potential therapeutic uses |
| φX174 | Escherichia coli | Microviridae | Lytic | Genetic research, vaccine development |
| P1 | Escherichia coli | Myoviridae | Lysogenic | Gene mapping, DNA packaging studies |
| λ (Lambda) | Escherichia coli | Siphoviridae | Lysogenic | Molecular biology research, genetic engineering |
| M13 | Escherichia coli | Inoviridae | Filamentous | Phage display technology, antibody development |
| φ6 | Pseudomonas aeruginosa | Cystoviridae | Lytic | Study of viral replication, potential therapeutic uses |
| T7 | Escherichia coli | Podoviridae | Lytic | High-efficiency gene expression systems |
| Felix O1 | Salmonella Typhimurium | Myoviridae | Lytic | Phage typing, therapeutic uses against Salmonella |
| Pf1 | Pseudomonas aeruginosa | Inoviridae | Filamentous | Structural studies, understanding filamentous phage biology |
| N4 | Escherichia coli | Podoviridae | Lytic | Study of lytic cycle, RNA polymerase interactions |
| F116 | Pseudomonas aeruginosa | Inoviridae | Lytic | Potential use in phage therapy |
| LUZ19 | Pseudomonas aeruginosa | Myoviridae | Lytic | Investigated for treating P. aeruginosa infections |
| PEV2 | Pseudomonas aeruginosa | Podoviridae | Lytic | Potential therapeutic application in cystic fibrosis |

| | | | | |
|---|---|---|---|---|
| AP22 | Acinetobacter baumannii | Myoviridae | Lytic | Studied for its antibacterial activity against A. baumannii |
| BS46 | Burkholderia cepacia | Not specified | Lytic | Potential for treating infections in cystic fibrosis |
| PYO2 | Pseudomonas aeruginosa | Myoviridae | Lytic | Phage therapy research |
| LKD16 | Klebsiella pneumoniae | Not specified | Lytic | Studied for effectiveness against K. pneumoniae |
| phiCTX | Pseudomonas aeruginosa | Podoviridae | Lytic | Phage therapy for multidrug-resistant strains |
| KPP10 | Klebsiella pneumoniae | Not specified | Lytic | Investigated for phage therapy applications |
| Sb-1 | Shigella boydii | Myoviridae | Lytic | Studied for its application in combating shigellosis |

Once inside, the phages can interfere with porin function in a number of ways, such as:

**7.3.2 Blocking Porin Channels:** Porin channels may be physically blocked by phage proteins, which lowers the flow of nutrients as well as ions necessary for bacterial viability.

**7.3.3 Inducing Structural Changes:** Certain phage-encoded proteins can cause porins to shift in shape, which alters their selectivity and prevents the entry of certain compounds, including antibiotics.

**7.3.4 Lysing Bacterial Cells:** By destroying the peptidoglycan covering of the cell wall, several bacteriophages have developed the ability to lyse bacterial cells. Porins and other outer membrane proteins are directly disrupted by this method [45].

**Table 3; shows interaction of phages with porin**

| **Bacteriophage** | **Bacterial Target** | **Porin Targeted** | **Interaction** |
|---|---|---|---|
| T4 | Escherichia coli | OmpC, OmpF | T4 phage uses OmpC and OmpF as receptors for initial attachment. |
| φX174 | Escherichia coli | LamB (Maltoporin) | φX174 recognizes LamB for entry into the bacterial cell. |
| Lambda (λ) | Escherichia coli | LamB (Maltoporin) | Lambda phage binds to LamB, initiating infection process. |

| | | | |
|---|---|---|---|
| P22 | Salmonella enterica | OmpC | P22 targets OmpC porin in Salmonella for bacterial invasion. |
| FhuA | Escherichia coli | FhuA | Utilizes FhuA receptor, typically involved in iron uptake. |
| T5 | Escherichia coli | FhuA | T5 phage targets FhuA porin to inject its DNA. |
| M13 | Escherichia coli | F-pilus | While not a porin, M13 targets F-pilus related to porin function in conjugation. |

### 7.3.5 Advantages of Bacteriophages for Porin Targeting

Utilizing bacteriophages to target porin channels offers several advantages:

**Precision:** As a result of bacteriophages' great host-specificity, porin profiles of certain bacteria can be used to target them with extreme accuracy.

**Lack of Resistance:** Because phage-host interactions are specialized and may evolve quickly in response to bacterial alterations, bacteria are less likely to acquire resistance to phages.

**Synergy with Antibiotics:** Phages and antibiotics can complement one other's effects. Phages can increase the ability to penetrate antibiotics into bacterial cells by focusing on porins, which increases their potency against resistant strains.



## 8. Challenges and Considerations

While bacteriophages offer exciting potential for porin targeting, several challenges must be addressed, including:

**8.1 Phage Host Range:** Phages have a limited ability to infect a variety of bacterial species because of their narrow host ranges.

**8.2 Phage Delivery:** It might be difficult to ensure effective phage delivery to the infection site, particularly for systemic diseases.

**8.3 Resistance Evolution:** Bacteria can acquire phage resistance over time, thus continual research is required to create new phage strains.

## 9. CRISPR-Cas9 editing

The targeted porin genes within the genome of bacterial cells can be altered after CRISPR-Cas9 editing to increase the sensitivity of bacteria to antimicrobials particular changes can be induced by precise gene editing, such as lowering the permeability of the porin to particular nutrients while selectively enabling the passage of antimicrobial drugs [46]. Since the bacterium's capacity to limit the delivery of these therapeutic drugs is disrupted, this modification may make the bacteria more susceptible to antimicrobials. Edited bacteria are more vulnerable to antimicrobial interventions, which increases their efficiency and presents a viable approach to the fight against antimicrobial resistance [47].

## Conclusion

In conclusion, innovative antimicrobial methods that specifically target porins provide promising means of preventing antibiotic resistance. Porin channel function inhibitors, membrane permeabilizers, and drugs that disrupt bacterial membranes provide creative approaches to boost the effectiveness of current antibiotics while maintaining the structural integrity of porins. These strategies mark a critical development in the field of antimicrobial treatment and a response to the rising problem of drug-resistant bacteria.



## 4. References

1. Hall-Stoodley, L., J.W. Costerton, and P. Stoodley, *Bacterial biofilms: from the natural environment to infectious diseases.* Nature reviews microbiology, 2004. **2**(2): p. 95-108.
2. Costerton, J.W., P.S. Stewart, and E.P. Greenberg, *Bacterial biofilms: a common cause of persistent infections.* science, 1999. **284**(5418): p. 1318-1322.
3. Liu, Y.-Q., Y. Liu, and J.-H. Tay, *The effects of extracellular polymeric substances on the formation and stability of biogranules.* Applied Microbiology and Biotechnology, 2004. **65**: p. 143-148.
4. Chevalier, S., et al., *Structure, function and regulation of Pseudomonas aeruginosa porins.* FEMS microbiology reviews, 2017. **41**(5): p. 698-722.
5. Arnold, T., et al., *Gene duplication of the eight-stranded β-barrel OmpX produces a functional pore: a scenario for the evolution of transmembrane β-barrels.* Journal of molecular biology, 2007. **366**(4): p. 1174-1184.
6. Prajapati, J.D., U. Kleinekathöfer, and M. Winterhalter, *How to enter a bacterium: bacterial porins and the permeation of antibiotics.* Chemical reviews, 2021. **121**(9): p. 5158-5192.

7. Raza, S., et al., *Resistance and adaptation of bacteria to non-antibiotic antibacterial agents: Physical stressors, nanoparticles, and bacteriophages.* Antibiotics, 2021. **10**(4): p. 435.
8. Ženíšková, K., *Effect of various stress factors on mitochondrial processes of pathogenic protists.* 2023.
9. King, D.T., S. Sobhanifar, and N.C. Strynadka, *One ring to rule them all: Current trends in combating bacterial resistance to the β-lactams.* Protein Science, 2016. **25**(4): p. 787-803.
10. Zgurskaya, H.I. and V.V. Rybenkov, *Permeability barriers of Gram-negative pathogens.* Annals of the New York Academy of Sciences, 2020. **1459**(1): p. 5-18.
11. Vollan, H.S., et al., *In silico structure and sequence analysis of bacterial porins and specific diffusion channels for hydrophilic molecules: conservation, multimericity and multifunctionality.* International journal of molecular sciences, 2016. **17**(4): p. 599.
12. Masi, M., M. Winterhalter, and J.-M. Pagès, *Outer membrane porins.* Bacterial cell walls and membranes, 2019: p. 79-123.
13. Kumar, A., A. Bhandari, and S. Krishnaswamy, *Sequence and structural perspectives of bacterial β-stranded porins.* Protein and Peptide Letters, 2015. **22**(1): p. 8-22.
14. D'Agostino, T., *Enhanced sampling methods and their application in the study of molecular permeation in gram-negative bacteria.* 2016.
15. Bajaj, H., et al., *Bacterial outer membrane porins as electrostatic nanosieves: exploring transport rules of small polar molecules.* ACS nano, 2017. **11**(6): p. 5465-5473.
16. Vergalli, J., et al., *Porins and small-molecule translocation across the outer membrane of Gram-negative bacteria.* Nature Reviews Microbiology, 2020. **18**(3): p. 164-176.
17. Paget, M.S., *Bacterial sigma factors and anti-sigma factors: structure, function and distribution.* Biomolecules, 2015. **5**(3): p. 1245-1265.
18. Nikaido, H., *Molecular basis of bacterial outer membrane permeability revisited.* Microbiology and molecular biology reviews, 2003. **67**(4): p. 593-656.
19. Ude, J., et al., *Outer membrane permeability: Antimicrobials and diverse nutrients bypass porins in Pseudomonas aeruginosa.* Proceedings of the National Academy of Sciences, 2021. **118**(31): p. e2107644118.
20. Patel, D.S., et al., *Dynamics and interactions of OmpF and LPS: influence on pore accessibility and ion permeability.* Biophysical journal, 2016. **110**(4): p. 930-938.
21. Hejair, H.M., et al., *Functional role of ompF and ompC porins in pathogenesis of avian pathogenic Escherichia coli.* Microbial pathogenesis, 2017. **107**: p. 29-37.

22. Masi, M. and J.-M. Pagès, *Suppl 1: Structure, function and regulation of outer membrane proteins involved in drug transport in Enterobactericeae: the OmpF/C–TolC Case.* The open microbiology journal, 2013. **7**: p. 22.
23. Ionescu, M.I., et al., *Carbapenem resistance in non-fermentative bacterial species and in Enterobacteriaceae isolates from hospitalized patients in different health-care settings.* Clujul Medical, 2014. **87**(4): p. 235.
24. Nelson, K., et al., *Resistance to ceftazidime-avibactam is due to transposition of KPC in a porin-deficient strain of Klebsiella pneumoniae with increased efflux activity.* Antimicrobial agents and chemotherapy, 2017. **61**(10): p. 10.1128/aac. 00989-17.
25. Vrancianu, C.O., et al., *Present and future perspectives on therapeutic options for carbapenemase-producing Enterobacterales infections.* Microorganisms, 2021. **9**(4): p. 730.
26. Gootz, T.D., *The global problem of antibiotic resistance.* Critical Reviews™ in Immunology, 2010. **30**(1).
27. Blair, J.M., G.E. Richmond, and L.J. Piddock, *Multidrug efflux pumps in Gram-negative bacteria and their role in antibiotic resistance.* Future microbiology, 2014. **9**(10): p. 1165-1177.
28. Ghafourian, S., et al., *Extended spectrum beta-lactamases: definition, classification and epidemiology.* Current issues in molecular biology, 2015. **17**(1): p. 11-22.
29. Breijyeh, Z., B. Jubeh, and R. Karaman, *Resistance of gram-negative bacteria to current antibacterial agents and approaches to resolve it.* Molecules, 2020. **25**(6): p. 1340.
30. Wani, A.K., et al., *Microbial adaptation to different environmental conditions: molecular perspective of evolved genetic and cellular systems.* Archives of Microbiology, 2022. **204**(2): p. 144.
31. Galdiero, S., et al., *Microbe-host interactions: structure and role of Gram-negative bacterial porins.* Current Protein and Peptide Science, 2012. **13**(8): p. 843-854.
32. Lin, X.-M., et al., *A novel negative regulation mechanism of bacterial outer membrane proteins in response to antibiotic resistance.* Journal of proteome research, 2010. **9**(11): p. 5952-5959.
33. Nikoo, M., J.M. Regenstein, and H. Ahmadi Gavlighi, *Antioxidant and antimicrobial activities of (-)-epigallocatechin-3-gallate (EGCG) and its potential to preserve the quality and safety of foods.* Comprehensive Reviews in Food Science and Food Safety, 2018. **17**(3): p. 732-753.

34. Avakh, A., et al., *The Art of War with Pseudomonas aeruginosa: Targeting Mex Efflux Pumps Directly to Strategically Enhance Antipseudomonal Drug Efficacy.* Antibiotics, 2023. **12**(8): p. 1304.
35. Zeidler, S. and V. Müller, *Coping with low water activities and osmotic stress in Acinetobacter baumannii: significance, current status and perspectives.* Environmental microbiology, 2019. **21**(7): p. 2212-2230.
36. Chen, A. and H.S. Seifert, *Saturating mutagenesis of an essential gene: a majority of the Neisseria gonorrhoeae major outer membrane porin (PorB) is mutable.* Journal of bacteriology, 2014. **196**(3): p. 540-547.
37. Lou, H., et al., *Altered antibiotic transport in OmpC mutants isolated from a series of clinical strains of multi-drug resistant E. coli.* PloS one, 2011. **6**(10): p. e25825.
38. Kim, S., et al., *Matching amino acids membrane preference profile to improve activity of antimicrobial peptides.* Communications Biology, 2022. **5**(1): p. 1199.
39. Vranakis, I., et al., *Proteome studies of bacterial antibiotic resistance mechanisms.* Journal of proteomics, 2014. **97**: p. 88-99.
40. Farrag, H.A., et al., *Natural outer membrane permeabilizers boost antibiotic action against irradiated resistant bacteria.* Journal of Biomedical Science, 2019. **26**(1): p. 1-14.
41. Impey, R.E., et al., *Overcoming intrinsic and acquired resistance mechanisms associated with the cell wall of gram-negative bacteria.* Antibiotics, 2020. **9**(9): p. 623.
42. Zhou, G., et al., *Outer membrane porins contribute to antimicrobial resistance in Gram-negative bacteria.* Microorganisms, 2023. **11**(7): p. 1690.
43. Li, J., et al., *Metal-organic framework membranes for wastewater treatment and water regeneration.* Coordination Chemistry Reviews, 2020. **404**: p. 213116.
44. Selvaraj, C. and S.K. Singh, *Phage protein interactions in the inhibition mechanism of bacterial cell.* Biocommunication of phages, 2020: p. 121-142.
45. Smith, L.M. and P.C. Fineran, *Phage capsid recognition triggers activation of a bacterial toxin-antitoxin defense system.* Molecular Cell, 2023. **83**(2): p. 165-166.
46. Li, X., et al., *A combination therapy of Phages and Antibiotics: Two is better than one.* International Journal of Biological Sciences, 2021. **17**(13): p. 3573.
47. Wan, F., et al., *Novel strategy to combat antibiotic resistance: a sight into the combination of CRISPR/Cas9 and nanoparticles.* Pharmaceutics, 2021. **13**(3): p. 352.