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REVIEW

Understanding antibiotic resistance via outer membrane permeability

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Abstract: Collective antibiotic drug resistance is a global threat, especially with respect to Gram-negative bacteria. The low permeability of the bacterial outer cell wall has been identified as a challenging barrier that prevents a sufficient antibiotic effect to be attained at low doses of the antibiotic. The Gram-negative bacterial cell envelope comprises an outer membrane that delimits the periplasm from the exterior milieu. The crucial mechanisms of antibiotic entry via outer membrane includes general diffusion porins (Omps) responsible for hydrophilic antibiotics and lipid-mediated pathway for hydrophobic antibiotics. The protein and lipid arrangements of the outer membrane have had a strong impact on the understanding of bacteria and their resistance to many types of antibiotics. Thus, one of the current challenges is effective interpretation at the molecular basis of the outer membrane permeability. This review attempts to develop a state of knowledge pertinent to Omps and their effective role in solute influx. Moreover, it aims toward further understanding and exploration of prospects to improve our knowledge of physicochemical limitations that direct the translocation of antibiotics via bacterial outer membrane.

Keywords: antibiotics, Gram-negative bacteria, drug-resistance, outer membrane proteins, porins, membrane permeability, influx

Introduction

At the end of the 20th century, the attention of the scientific as well as the pharmaceutical community regarding the threat of antibiotic resistance was mainly focused on multiresistant Gram-positive bacteria.[1,2] This significantly contributed towards the development of new compounds with the specific activity against this particular group of microorganisms.[1] Regrettably, the introduction of antibiotics for Gram-negative bacteria has not developed at a similar pace.[1] Gram-negative bacterial multidrug resistance is a worrying health issue. Antibiotic resistance is frequently reported in clinical Gram-negative bacteria, and severely limits the available therapeutic options in hospital acquired infections.[2,3] Consequently, due to the shortage of novel active antibacterials, there is an immense need to interpret the molecular mechanisms of antibiotic resistance, especially toward key Gram-negative clinical pathogens, such as *Klebsiella, Enterobacter, Pseudomonas, Campylobacter, Acinetobacter*, and *Salmonella* species.[4–8]

The current innovative mode of improving the potential of antibiotics is to efficiently introduce them into the bacteria and further prevent them from degradation by bacterial enzymes before they reach their targets.[7,8] This is, however, an extreme method for countering the problem of antibiotic resistance.[9,10] The main mechanisms employed by Gram-negative bacteria against available antibiotic therapy include the enzymatic barrier, which primarily destroys the antibiotics; the membrane barrier,

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which limits the intracellular access of antibiotics; and antibiotic target modification, resulting in the overall failure of antibiotic therapy.[7] Significantly, these mechanisms can work together in clinical isolates, thus creating an elevated level of antibiotic resistance.[4,6,8] Of these mechanisms, antibiotic infusion across the bacterial membranes[11] is one of the crucial mechanisms that needs to be studied thoroughly.[5–9] Passing over toward the outer membrane barricade to scope the inhibitory concentration inside the bacterial cell is a key step for antibiotic molecules to work effectively,[11] thus, understanding the mechanism of transport across the outer membrane will give a crucial insight towards designing futuristic "smart" antibiotics.[7,8,10] The outer membrane of Gram-negative bacteria performs the crucial role of providing an extra layer of protection to the organism without conceding the exchange of material required for sustaining life. In this dual capacity, this barrier appears to be an extremely sophisticated macromolecular assemblage, the complexity of which has been explored only in recent years.[5,8,12–15] By combining a highly hydrophobic lipid bilayer containing pore-forming proteins (Omps) (Tables 1 and 2) of specific size-exclusion properties, the outer membrane acts as a selective barricade.[7,8] The permeability properties of this barrier, therefore, have a major impact on the susceptibility of the microorganism to antibiotics. Small hydrophilic drugs, such as β-lactams, use the pore-forming porins to gain access to the cell interior, while macrolides and other hydrophobic drugs diffuse across the lipid bilayer.[4,12,13] The existence of drug-resistant strains in many bacterial species due to modifications in the lipid or protein composition of the outer membrane indeed highlights the importance of the outer membrane barrier in antibiotic sensitivity. For instance, any structural changes in the available outer membrane proteins can significantly account for antibiotic resistance.[5] Further, the situation becomes serious when the permeability barrier synchronizes with the β-lactamases in the periplasmic space, potentially leading to third-generation cephalosporin resistance.[4–7] In Gram-negative bacteria, the outer membrane is an asymmetric bilayer of phospholipid and lipopolysaccharides (LPS), with the latter exclusively found in the outer leaflet.[4,5] A typical LPS molecule consists of three parts, together with a relatively short core oligosaccharide, lipid A, a glucosamine-based phospholipid, and a distal polysaccharide O-antigen.[12] Since part of the core oligosaccharide and the O-antigen are not required for the growth of *Escherichia coli*, strains can exhibit varying lengths of these structures.[4,5,12,13] The phospholipid composition of the inner leaflet of the outer membrane contains approximately 15% phosphatidylglycerol, 80% phosphatidylethanolamine, and 5% cardiolipin, like that of the cytoplasmic membrane.[12] Many different types of proteins reside in the outer membrane (Table 1). Some of them are extremely abundant. Different outer membrane proteins have been characterized in Gram-negative bacteria (Table 2) and are distinguished according to their substrate specificities, functional structure (monomeric or trimeric), and their regulation and expression.[4–6,12,13]

In this present review, we discuss and tabulate different attributes to understand various outer membrane proteins mainly responsible for solute influx in Gram-negative bacteria.[4,10] This active knowledge can be used towards under-

Table 1 Crucial Omps studied in different bacteria

Protein	Pathogens
OmpX,[14] OmpA,[15–17] OmpT,[18] Tsx,[19] FadL,[20] OmpF,[7,8,21,22] OmpC,[23–31] PhoE,[32] LamB,[33,34] BtuB,[35] FepA,[36] FhuA,[37,38] TolC	*Escherichia coli*
Omp36,[3,31,39–41] Omp35[8,31,39,40,42]	*Enterobacter aerogenes*
OmpE36,[43] OmpE35[8]	*Enterobacter cloacae*
OmpK36,[30,31,44] OmpK35[8,30,31,44]	*Klebsiella pneumoniae*
MOMP,[45–49] Omp50[49,50]	*Campylobacter jejuni*
(OccAB1-OccAB5),[51] rOprD,[52] CarO,[53,54] Omp25[55]	*Acinetobacter baumannii*
NspA,[56] OpcA,[57] NalP[58]	*Neisseria meningitidis*
Hia[59]	*Haemophilus influenzae*
CymA[60,61]	*Klebsiella oxytoca*
α-hemolysin[62,63]	*Staphylococcus aureus*
MspA[64]	*Mycobacterium smegmatis*
ScrY[65]	*Salmonella typhimurium*
OmpPst1,[66,67] OmpPst2[67]	*Providencia stuartii*
(OccD1 (OprD), OccD2 (OpdC), OccD3 (OpdP), OccD4 (OpdT), OccD5 (OpdI), OccD6 (OprQ), OccD7 (OpdB), OccD8 (OpdJ))[8,68–76]	*Pseudomonas aeruginosa*
(OccK1 (OpdK), OccK2 (OpdF), OccK3 (OpdO), OccK4 (OpdL), OccK5 (OpdH), OccK6 (OpdQ), OccK7 (OpdD), OccK8 (OprE))[8,73,74,77–84]	
OprP,[75,85–88] OprO[87]	

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Table 2 Conclusive investigations with different Omps studied in pathogens

Decisive investigation	Omp	Pathogens
Studied interaction of β-lactam molecule meropenem using ETP.[89]	*OmpF*	*Escherichia coli*
Studied interaction of ampicillin, penicilloic-acid, and benzylpenicillin with Omp using ETP.[7,123]	*OmpF*	*E. coli*
Studied and showed effect of access resistance in Omp using ETP.[22]	*OmpF*	*E. coli*
Studied transport of divalent metal ions and their effect on conductance and selectivity of Omp.[90]	*OmpF*	*E. coli*
Studied the effect of salts of divalent cations on the Omp conductance, particularly the role of the electrolyte and the counterion accumulation induced by the Omp charges, and other effects not found in salts of monovalent cations using ETP.[91]	*OmpF*	*E. coli*
Studied effect of divalent cations toward pH sensitivity of Omp via inducing the pKa shift of key acidic residues using ETP.[92]	*OmpF*	*E. coli*
Studied mechanism of selectivity inversion in the Omp using ETP.[93]	*OmpF*	*E. coli*
Studied ciprofloxacin permeation pathways across Omp using MS.[94]	*OmpC*	*E. coli*
Studied recombinant form of the Omp and demonstrated the monomeric nature of Omp using ETP.[95]	*OmpG*	*E. coli*
Determined the X-ray crystal structure of the Omp.[96]	*OmpG*	*E. coli*
Determined the crystal structure of the Omp in two dimensions.[97]	*OmpG*	*E. coli*
Studied mechanism of folding of Omp in detergent solution.[98]	*OmpG*	*E. coli*
Studied structural configuration of different Omps and measured penetration rates of different β-lactams using LSA.[99]	OmpA	*E. coli*
Studied binding regions of Omp using site-directed fluorescence study.[17]	OmpA	*E. coli*
Studied function of Omp in stress survival using microbiological assay.[16]	OmpA	*E. coli*
Studied crystal structure of Omp and further explained possible mechanisms of virulence.[14]	OmpX	*E. coli*
Studied the Omp behavior and described the effect of expanded channel protein using ETP.[100]	*FhuA*	*E. coli*
Studied transfer of DNA via Omp using LSA.[101]	*FhuA*	*E. coli*
Studied structural parameters of Omp using size exclusion chromatography, sedimentation equilibrium, and velocity experiments.[102]	*FhuA*	*E. coli*
Studied structures and the interaction of proteins and protein subdomains, and also demonstrated the role of the Omp in outer membrane permeability.[103]	FhuA,	*E. coli*
Demonstrated Fe^{3+} as ferrichrome complex transport through the outer membrane.[104]	FhuA	*E. coli*
Studied interaction of β-lactam molecules ertapenem, cefepime, and cefoxitin, using ETP and MIC assay.[67]	OmpPst1 and OmpPst2	*Providencia stuartii*
Studied Omp structure, including function of surface-exposed loops and Omp interaction with membrane components (e.g., LPS) using conventional ETP and MS.[66]	OmpPst1 and OmpPst2	*P. stuartii*
Studied role of Omp in carbapenem transport across outer membrane using ETP and LSA.[105]	OmpPst1	*P. stuartii*
Described and explained biophysical properties of the Omp.[45]	MOMP	*Campylobacter jejuni*
Studied and confirmed conformational analyses showing the presence of a native trimeric state generated by association of the three folded monomers, and further compared the stability with that of *Escherichia coli* porins.[46]	MOMP	*C. jejuni*
Studied translocation of short poly-arginines across Omp using ETP.[41]	MOMP	*C. jejuni*
Studied the three-dimensional structure of Omp and elucidated the underlying molecular mechanisms using X-ray diffraction.[47]	MOMP	*C. jejuni*
Studied sequence polymorphism and showed secondary structures, and surface-exposed conformational epitopes of the Omp.[106]	MOMP	*C. jejuni*
Studied channel-forming properties of Omp as trimer and monomer using ETP, and transition of trimer to monomer using light scattering; further examined the secondary structures of these two molecular states by infra-red spectroscopy.[48]	MOMP	*C. jejuni*
Studied different environmental regulation factors controlling Omp expression in *Escherichia coli* using fluorescent spectroscopy.[49]	MOMP and Omp50	*C. jejuni*
Studied pore-forming ability of the Omp and performed biophysical characterization using conventional ETP.[50]	Omp50	*C. jejuni*
Studied key residues in the channel constriction and their effect on substrate specificity of the Omp using ETP and MS.[107]	OprP and OprO	*Pseudomonas aeruginosa*
Studied transport of fosfomycin via Omp using ETP.[108]	OprP and OprO	*P. aeruginosa*
Showed decreased Omp production to be one of the contributing factors for carbapenem heteroresistance.[109]	OprD	*P. aeruginosa*
Studied role of Omp in increasing MICs of carbapenems in clinical isolate.[110]	OprD	*P. aeruginosa*

(*Continued*)

Table 2 (*Continued*)

Decisive investigation	Omp	Pathogens
Studied Omp levels in carbapenem-resistant isolates using real-time polymerase chain reaction.[111]	OprD	*P. aeruginosa*
Studied and characterized discrepant carbapenem susceptibility profile including alterations in outer membrane permeability.[112]	OprD	*P. aeruginosa*
Studied in vitro activity of ceftazidime-avibactam and ceftolozane-tazobactam against meropenem-resistant isolates using MIC.[113]	OprD	*P. aeruginosa*
Studied and identified unique in-frame deletions in Omp among clinical isolates.[114]	OprD	*P. aeruginosa*
Studied variations of Omp dominating in imipenem-resistant isolates.[115]	OprD	*P. aeruginosa*
Developed whole-cell-based assay, system to characterize the structure of Omp and its role in permeation for a set of novel carbapenem analogs.[116]	OprD	*P. aeruginosa*
Studied effect of Omp polymorphisms, particularly the amino acid substitution at codon 170 toward carbapenem resistance.[117]	OprD	*P. aeruginosa*
Studied the impact of single amino acid substitutions in Omp on carbapenem resistant strains.[118]	OprD	*P. aeruginosa*
Studied and showed incapacitating mutation and decreased expression of Omp to be one of the factors contributing toward imipenem and meropenem resistance.[119]	OprD	*P. aeruginosa*
Studied and showed the role of Omp in 70 different carbapenem-resistant clinical isolates.[120]	OprD	*P. aeruginosa*
Studied channel-forming properties and other physicochemical properties of Omp using ETP and mass spectrometry.[55]	CarO and Omp25	*Acinetobacter baumannii*
Studied L-ornithine uptake via Omp, also showed L-ornithine's effect over pathogen sensitivity to imipenem.[121]	CarO	*A. baumannii*

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Abbreviations: LSA, liposome swelling assay; LPS, lipopolysaccharides; MS, molecular simulations; ETP, electrophysiology.

standing the effect of outer membrane influx in antibiotic resistance in Gram-negative bacteria which can be further used for future antibiotic drug development.

Conclusion

In this review, we continued to explore different outer membrane proteins by extending and recapitulating the progressive systematic evidence elucidating the role of Omps in solute membrane permeability in Gram-negative bacteria.[7,8] Bacterial membrane transport is a multifaceted process that is strongly controlled by a complicated network of activities that sense and respond to external stress.[8] Significantly, bacteria make use of these cultured controlled cascades that perceive and distinguish different toxic compounds and respond by triggering various resistance mechanisms, including modification of specific Omps.[4–6,13,122] Membrane penetrability, which further, along with added resistance mechanisms, including drug inactivation or target modification, has become one of the major problems in effective antibiotic therapy. Effective information regarding the role of effective Omps in substrate uptake and further explaining their structural relationship toward the uptake, highlights the capability of the scientific community in the direction of understanding the bacterial resistance machinery generated mainly via modification of membrane permeability.[4–8,13,122] Understanding translocation via Omps can be regarded as a first step toward defining a pathway of an antibiotic specific to its target. Consequently, interpretation of antibiotic translocation through Omps is crucial for understanding the connection between influx and activities in bacteria. The function of the general diffusion Omp has been well studied based on Omp characteristics, alteration, and mutations. We also tried to combine data from different studies concerning the Omps. Our understanding of the structure of the pore-forming complex has been extremely improved over the last decade with emergence of the computational approach, crystallographic data from X-rays, electron microscopy, mass spectrometry, and electrophysiology. However, significant key knowledge regarding the transformation of outer membrane pores' transportation mechanism is still required to further elaborate their conditional role in antibiotic/antimicrobial transport. The molecular basis of antibiotic transport via specific porins is presently open to interpretation, and additional rigorous studies are required to give insight into the structural–activity relationship between Omp geometry and antibiotic transport. Collectively, the current and previous[8] data can be employed in an effort to explain substrates, especially antibiotic uptake pathways, and may provide insights into molecular mechanisms that could enable rational drug design to enhance permeation and provide novel strategies to solve the "impermeability" issue of antibiotic resistance.

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Disclosure

The authors report no conflicts of interest in this work.

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