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ABC Exporters in Pathogenesis: Role of Synthetic Anti-Microbial Peptides

Ritika Kabra[1] · **Shailza Singh**[1]

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Abstract
ABC exporters are involved in diverse cellular processes including lipid trafficking, drug resistance, pathogenesis etc. The greatest thrust has been in the area of drug resistance that explains the underlying well-crafted canonical architecture of its structure. Interestingly, ranging from structural organisation to subsequent design and delivery aspects lays the niche of antimicrobial peptides. One of the major highlight of this paper is the role of synthetic antimicrobial peptides in current scenario.

Keywords ABC · Synthetic AMPs · Antibacterial resistance

Abbreviations

ABC	ATP binding cassette
ALD	Adrenoleukodystrophy
AMPs	Antimicrobial peptides
CFTR	Cystic fibrosis transmembrane conductance regulator
MATE	Multidrug and toxin compound extrusion
MDR	Multi drug resistance
MFS	Major facilitator superfamily
MMP7	Matrix metalloproteinase 7
NBD	Nucleotide binding domain
OABP	Organic anion binding protein
PACE	Proteobacterial antimicrobial compound efflux
PXE	Pseudoxanthoma elasticum
RND	Resistance nodulation division
SMR	Small multidrug resistance
SUR	Sulfonylurea receptor
TAP	Transporters associated with antigen processing
TMD	Transmembrane domain
TMH	Transmembrane helices

1 Introduction

An emerging crisis all over the world is a large number of antimicrobial drugs becoming ineffective against most microbes due to emergence of resistance. It has been observed that most of the microbes are exhibiting insensitivity for more than one drug, a condition termed as Multi Drug Resistance (MDR). Studies have concluded that occurrence of resistance, mainly in bacteria, is mostly because of one or combination of the two mechanisms. First, expression of multiple genes responsible for resistance to a single or multiple drugs in a cell. Second, increased activity or overexpression of efflux proteins (or exporters) [1–3]. The efflux proteins are classified into six major classes namely small multidrug resistance (SMR), proteobacterial antimicrobial compound efflux (PACE), major facilitator superfamily (MFS), multidrug and toxin compound extrusion (MATE), resistance nodulation division (RND) and ATP binding cassette (ABC) superfamily. Of these, ABC superfamily is the largest [4]. In order to solve the resistance issues, ABC exporters have always been the desired target protein. Great efforts have been taken in either inhibiting or modulating them. But the major obstacles in their success was their high level of conservedness resulting in toxicity and gradual ineffectiveness of inhibitors due to rapid rate of mutations [5, 6]. With the failure of first and second line of drugs against Multi drug resistant microbes, due to either emergence of resistance or adverse side effects, there has been a constant search for new therapeutics. Antimicrobial peptides (AMPs) provide a ray of hope as an alternative strategy. Being specific in nature, AMPs withhold advantage

✉ Shailza Singh
shailza_iitd@yahoo.com; singhs@nccs.res.in

[1] National Centre for Cell Science, NCCS Complex, Ganeshkhind, Pune 411007, India

over small molecules by minimizing toxicity and increased specificity [7]. Herein, we have focused on ABC superfamily of efflux proteins with potent role of antimicrobial peptides for targeting them in combating with MDR microbes.

2 ABC Exporters

ABC Exporters are a wide variety of ATP dependent proteins that confer translocation from cytoplasmic leaflet to exoplasmic leaflet of cell membrane, thus, also known as Floppases [8, 9]. They are found in all living organisms and play key roles in many biological processes. They utilize energy obtained from ATP hydrolysis to efflux a diverse range of molecules like amino acids, sugars, ions, small organic or inorganic compounds, peptides or proteins, hydrophobic drugs and a variety of toxins. The ABC exporters are often associated with MDR and several human diseases. The structural composition of ABC exporters includes 2 transmembrane domains (TMDs) each having six transmembrane helices and 2 nucleotide binding domains (NBDs). ABC transporter class is a highly conserved class of proteins categorised further in 7 subfamilies (ABCA to ABCG). In humans, 51 ABC genes are known while in *L. major* 40 members have been reported till date [10, 11]. The subfamilies of Human ABC transporters as classified by HUGO Gene Nomenclature Committee (HGNC) are mentioned below [12].

2.1 Classification

2.1.1 ABCA

The subfamily A of ABC family also known as ABC1 constitutes of few of the largest proteins amongst ABC transporters. It consists of 14 genes which are further sub grouped into 7 and 5 genes. The first sub group is ABCA1 like genes consisting of 50 exons. These sub groups includes ABCA1, ABCA2, ABCA3, ABCA4, ABCA7, ABCA12 and ABCA13. Mutations in these genes are associated with various genetic diseases. For instance, mutations in ABCA1 cause Tangier's disease. Mutations in ABCA4 may lead to Stargardt disease, age-related macular degeneration and retinitis pigmentosa (Table 1). The other sub group consists of ABCA5, ABCA6, ABCA8, ABCA9 and ABCA10 genes consisting of 37–38 exons [13, 14].

2.1.2 ABCB

The subfamily B is the only human subfamily of ABC Transporters having half transporters as well as full transporters. If all the domains of an ABC exporter (i.e. 2TMDs and 2 NBDs) are present as a single polypeptide chain, then they are referred as full transporters. But if 2TMDs and 2NBDs are represented by more than one polypeptide chains, they are referred as half transporters. 1TMD and 1NBD comprises in a single polypeptide and such two polypeptides dimerize to form a complete functional unit. The dimers may either be homo- or hetero- in nature [11, 18]. The schematic arrangement of full transporters and half transporters are represented in Fig. 1. It constitutes of 11 unique genes of which, 4 are full transporters while the rest 7 are half transporters. Many members of this subfamily extrudes multiple drugs and imparts multi drug resistance, hence ABCB subfamily is aliased as MDR. ABCB1, commonly known as P-gp or P-glycoprotein or MDR1, is one of the major exporters responsible for MDR. P-gp is the first Human ABC to be cloned and also one of the best characterized and studied protein. Apart from MDR, mutations in ABCB genes are associated with diseases like spondylitis, diabetes type 2, coeliac disease, X-linked sideroblastic anaemia, and several cholestatic liver diseases (Table 1) [13, 14]. The ABCB2 and ABCB3 are half transporters and form a heterodimer to transport peptides into ER. These two transporters are associated with antigen processing (TAP) protein complex, hence, also named as TAP1 and TAP2 respectively [19].

2.1.3 ABCC

The subfamily C of ABC family of exporters constitutes of 13 gene members of which nine are involved in multidrug resistance. This family is aliased as Multidrug Resistant Proteins, MRPs. The members of this subfamily are involved in diverse functional activities ranging from extrusion of small molecules, drugs and toxins to ions and nucleoside transport as well as acting as receptors. The ABCC7 (CFTR, Cystic fibrosis transmembrane conductance regulator) functions as chloride ion channel and mutations in this gene results in cystic fibrosis, a genetic autosomal recessive disease. The sulfonylurea receptor (SUR) genes, ABCC8 and ABCC9, are associated with hypo- and hyper-glycaemia, dilated cardiomyopathy, familial atrial fibrillation and hypertrichotic osteochondrodysplasia. The other diseases associated with mutations in genes of ABCC subfamily are Dubin-Johnson Syndrome, Pseudoxanthoma Elasticum (PXE), diabetes mellitus 2, Borna disease, congenital bilateral aplasia, etc. (Table 1) [14, 19].

2.1.4 ABCD

ABCD subfamily consists of 4 genes, ABCD1-4. They encode half transporters and are expressed only in peroxisomes. They may function as either homodimer or heterodimer. Mutations in genes ABCD1 and ABCD2 are associated with X-linked adrenoleukodystrophy (ALD). Thus, this subfamily is also called as ALD. The other diseases associated

Table 1 Human ABC Transporter genes, functions, associated diseases and their tissue location

Gene	Aliases	Function	Disease associated	Tissue specificity
ABCA (ABC1)				
ABCA1	TGD	Cholesterol efflux and phospholipid transport	Tangier disease T1, familial hypoapoproteinemia	Placental cells
ABCA2	–	Drug resistance	–	Brain, monocytes
ABCA3	ABC-C	Multidrug resistance	Pulmonary surfactant metabolism dysfunction type 3 (SMDP3)	Brain, lung, colon, liver, kidney, lymph node and testis
ABCA4	STGD, FFM	Rod photoreceptor, retinoid transport, N-retinylidene-phosphatidylethanolamine (PE) efflux	Stargardt disease, retinitis pigmentosa, Cone-rod dystrophy and age-related macular degeneration	Photoreceptor cells in retina
ABCA5	–	Urinary diagnostic marker for prostatic intraepithelial neoplasia	Hypertrichosis, congenital generalized, with or without gingival hyperplasia and lysosomal disease	Cytoplasmic expression in most tissues
ABCA6	–	Multidrug Resistance	Ichtyosis, congenital, autosomal recessive 4b, autosomal recessive congenital ichtyosis	Liver[#]
ABCA7	ABCX	Cholesterol efflux	Alzheimer's disease	Bone marrow and immune cells
ABCA8	–	Transports certain lipophilic drugs	Ichtyosis, congenital, autosomal Recessive 4B, autosomal recessive congenital ichtyosis	Abundant in stromal cells
ABCA9	–	Monocyte differentiation and macrophage lipid homeostasis	–	Adipose tissue[#]
ABCA10	–	Cholesterol-responsive gene	–	Most tissues
ABCA11P*	–	–	–	–
ABCA12	–	Prenatal diagnosis	Autosomal recessive congenital ichthyosis type 4B	Skin and tongue[#]
ABCA13	–	Inherited disorder affecting the pancreas	Schizophrenia and stargardt disease	Bone marrow, lung and lymphoid tissue
ABCA17P*	–	–	–	–
ABCB (MDR)				
ABCB1	P-gp, MDR1 Bacterial homologs: Sav1866, MsbA, LmrA, McjD, BmrC/D	Drug resistance	Colchicine resistance and Inflammatory bowel disease 13	Intestine, liver, kidney, placenta and blood–brain barrier
ABCB2	TAP1 Bacterial homologs: Sav1866, Tm287/88, LmrCD	Peptide transport	Immune deficiency	Most tissues
ABCB3	TAP2 Bacterial Homologs: Sav1866, Tm287/88, LmrCD	Peptide transport	Immune deficiency	Kidney, urinary bladder, lymphoid tissues
ABCB4	MDR2	Bile—acid transport, phosphatidylcholine (PC) transport	Progressive familial intrahepatic cholestasis 3, Intrahepatic cholestasis of pregnancy, Gall-bladder disease	Liver

Table 1 (continued)

Gene	Aliases	Function	Disease associated	Tissue specificity
ABCB5	–	Melanogenesis	Borna disease and melanoma	Epididymis, retina[#]
ABCB6	MTABC3, PRP, UMAT	Iron transport	Colobomatous microphtalmia 7, dyschromatosis Universalis Hereditaria 3	Most tissues
ABCB7	ABC7, ASAT	Iron transport, Fe/S Cluster transport	X-linked sideroblastosis and anemia	Lung, endothelial cells and muscle tissues
ABCB8	M-ABC1, MITOSUR	Intracellular peptide trafficking across membranes	Anemia, Sideroblastic and Spinocerebellar Ataxia and Intestinal Tuberculosis	Most tissues
ABCB9	TAPL, HABC9	Located in lysosomes	Nemaline myopathy 4	Cytoplasmic expression in most tissues
ABCB10	M-ABC2, MTABC2	Export of peptides derived from proteolysis of inner-membrane proteins	Developmental coordination disorder and stereotypic movement disorder	Cytoplasmic expression in most tissues
ABCB11	BSEP, PFIC2, SPGP, ABC16	Bile—acid transport	Progressive familial intrahepatic cholestasis 2 and Benign recurrent intrahepatic cholestasis 2	Liver
ABCC (MRP)				
ABCC1	MRP1, GS-X, ABC29	Drug resistance	Dubin-Johnson syndrome and pseudoxanthoma elasticum	All tissues
ABCC2	MRP2, CMOAT, CMRP, ABC30	Bile—acid transport, Organic anion efflux	Dubin–Johnson syndrome and bilirubin metabolic disorder	Liver, kidney, intestine
ABCC3	MRP3, MLP2, MOAT-D, ABC31	Drug resistance	Dubin-Johnson syndrome and extrahepatic cholestasis	Pancreas, kidney, intestine, liver, adrenal glands
ABCC4	MRP4, MOATB	Nuceloside transport	Biliary tract disease and Dubin-Johnson syndrome	Prostate, testis, ovary, intestine, pancreas, lung
ABCC5	MRP5, SMRP, MOAT-C, ABC33	Nuceloside transport	Primary angle-closure glaucoma and choroid plexus meningioma	Most tissues
ABCC6	MRP6, MLP1, PXE, ABC34	Expressed primarily in liver and kidney	Pseudoxanthoma elasticum (PXE)	Liver, kidney
ABCC7	CFTR, ABC35, MRP7 Bacterial Homologs: Tm287/88	Chloride ion channel	Cystic fibrosis	Pancreas, gall-bladder
ABCC8	MRP8, ABC36, SUR1, HHF1, PHHI, TNDM2	Sulfonylurea receptor	Hypoglycemias and hyperglycemias	Brain, pancreas, pituitary gland
ABCC9	SUR2, CMD10	Encodes the regulatory SUR2A subunit of the cardiac Kþ(ATP) channel	Dilated cardiomyopathy 10, familial atrial fibrillation and hypertrichotic osteochondrodysplasia	Liver, gall-bladder, muscle tissues[#]
ABCC10	MRP7, SIMRP7	Multidrug resistance	Borna disease	Liver, heart, kidney
ABCC11	MRP8, EWWD	Drug resistance in breast cancer	Apocrine gland secretion and lateral sinus thrombosis	Cytoplasmic expression in most tissues
ABCC12	MRP9	Multidrug resistance	Familial cold autoinflammatory syndrome 1 and episodic kinesigenic dyskinesia 1	Brain, testis[#]
ABCC13*	ABCC13P, PRED6	Encodes a polypeptide of unknown function	–	–

Table 1 (continued)

Gene	Aliases	Function	Disease associated	Tissue specificity
ABCD (ALD)				
ABCD1	ALD, ALDP, AMN	Very long chain fatty acids transport	X-linked adrenoleukodystrophy (ALD) and hypoadrenocorticism	Intestine[#]
ABCD2	ALD1, ALDR, ALDRP	Major modifier locus for clinical diversity in X-linked ALD	X-linked adrenoleukodystrophy (ALD) and Zellweger Syndrome	Adipose tissue, brain[#]
ABCD3	PXMP1, PMP70, ZWS2,ABC43	Involved in import of fatty acids and/or fatty acyl-coenzyme As into the peroxisome	Bile acid synthesis defect, congenital and Zellweger syndrome	General cytoplasmic expression with a granular pattern
ABCD4	PXMP1L, PMP69, P70R, ABC41, MAHCJ	May modify the ALD phenotype	Methylmalonic aciduria and homocystinuria, cblj type (MAHCJ) and disorders of intracellular cobalamin metabolism	General cytoplasmic expression in several tissues, including membranous expression in fallopian tube
ABCE (OABP)				
ABCE1	OABP, RLI, RLI1, ABC38	Oligoadenylate-binding protein	Noonan syndrome	General cytoplasmic expression
ABCF (GCN20)				
ABCF1	ABC50, ABC27	Susceptibility to autoimmune pancreatitis	Autoimmune pancreatitis	General cytoplasmic expression
ABCF2	ABC28, M-ABC1, HUSSY18	Drug resistance, tumor suppression at metastatic sites and in endocrine pathway for breast cancer	Cystic fibrosis and intestinal tuberculosis	General cytoplasmic expression
ABCF3	–	Displays an antiviral effect against flaviviruses such as west Nile virus (WNV) in the presence of OAS1B	Hemophagocytic lymphohistiocytosis	General cytoplasmic expression
ABCG (white)				
ABCG1	ABC8, WHITE1, WHT1	Cholesterol transport	Cardiometabolic disease	Placenta, intestine, breast, liver, macrophage
ABCG2	MXR, BCRP, ABC-P, CD338, GOUT1	Drug resistance, toxicant efflux	Gout disease	Breast, liver, testis, brain
ABCG4	WHITE2	Macrophage lipid homeostasis	Sitosterolemia and hypolipoproteinemia	Macrophage, eye, brain and spleen
ABCG5	STSL	Sterol transport	Sitosterolemia	Intestine, liver[#]
ABCG8	GBD4	Sterol transport	Gall-bladder disease 4 and sitosterolemia	Intestine, liver[#]

The data is listed as per HGNC database, Reactome Pathway Knowledgebase, The Human Protein Atlas and GeneCards, The Human Gene Database [12, 15–17]

*Pseudogenes; [#]Gene expression specificity at RNA level

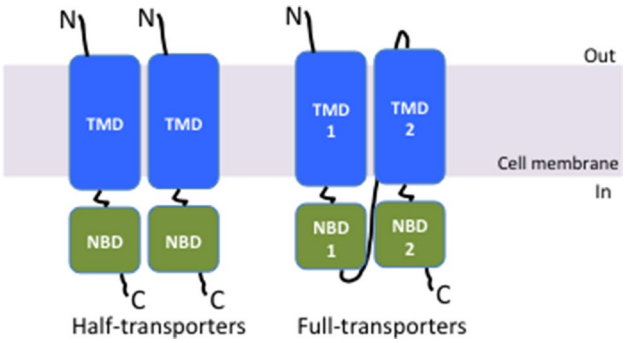

Fig. 1 The schematic arrangement of transmembrane domain (TMD) and nucleotide binding domain (NBD) in ABC exporters. In full transporters, 2TMDs and 2NBDs are expressed as single polypeptide. While in half transporters, more than one polypeptide are present either in homodimer or in heterodimer to form functional unit. The arrangement of domains is in head to tail fashion, i.e., C-terminal of first domain connects with N-terminal of the second

are Bile Acid Synthesis Defect, Congenital and Zellweger syndrome, Methyl malonic Aciduria and Homocystinuria, cblj type (MAHCJ) and Disorders Of Intracellular Cobalamin Metabolism (Table 1) [14, 19].

2.1.5 ABCE

ABCE subfamily has only one member, ABCE1, also known as organic anion binding protein (OABP). ABCE1 is an ABC transporter protein with ATP-binding domain but no transmembrane domain and recognizes oligoadenylate for binding and help in promoting interferon activity during viral infections. Any defects in this gene results in Noonam Syndrome (Table 1) [14, 19].

2.1.6 ABCF

ABCF subfamily is also known as GCN20 after the most characterized GCN20 gene of *S. cerevisiae*. Similar to ABCE subfamily, it also has only ATP-binding domain and lacks transmembrane domains. It consists of 3 members, ABCF1-3. These genes play role in inflammatory processes as they were found to be upregulated by tumour necrosis factor-α. Autoimmune Pancreatitis, Hemophagocytic Lymphohistiocytosis and Intestinal Tuberculosis are the diseases associated with ABCF subfamily (Table 1) [14, 19].

2.1.7 ABCG

ABCG subfamily or White consists of 5 genes that encode half transporters. The domain orientation is opposite to other ABC transporters with ATP-binding site at N' and transmembrane domain at C' end, and hence is also known as 'reverse half transporters'. ABCG1 is involved in cholesterol transport and any defects are linked with cardio-metabolic diseases. ABCG5 and ABCG8 helps in sterol transport and are linked with sitosterolemia disease. ABCG2 is the most studied member and its function is efflux of toxins and drugs thereby causing multi drug resistance (Table 1) [14, 19].

2.2 General Architecture

ABC Transporters are one of the largest families of known proteins that are highly conserved and share common architecture which includes 2 TMDs and 2 NBDs arranged in a head to tail fashion (Fig. 1). Each TMD consist of 6 helices and are capable of recognizing a wide variety of substrates. TMDs are arranged in the form of two wings such that the helices TM1 and TM2 of a subunit and TM3-6 of other domain form one wing in a domain swapped arrangement. ABC transporter proteins transport a wide variety of substrates through TMDs and hence compared to NBDs, TMDs are much more diverse. Being diverse in nature is supported by varying number of trans-membrane helices in different subfamilies. The other domains of ABC proteins, i.e. NBDs, are more conserved in nature. NBDs consist of a larger and a smaller domain. Larger domain, also known as catalytic sub domain, consist of 2 β sheets and 6 α helices. The Walker A motif (also called P-loop; GXXGXGKS/T) and Walker B motif (ΦΦΦΦD) are part of this domain. The Φ in Walker B motif represents any hydrophobic residue. The smaller helical domain has 3–4 helices and the conserved ABC signature motif or C-motif (LSGGQ). Apart from this, ABC domain has a glutamine residue also called Q-loop and is thought to be connecting TMD and NBD. Another conserved region known as H motif or switch region having highly conserved histidine residue play an important role in the interaction of ATP with ABC domain. The Walker A motif of NBD1 and signature motif of NBD2 interacts with ϒ-Phosphate of ATP and vice versa, thereby sandwiching it between two NBDs [11, 20, 21]. The interaction of ATP with NBDs in ABCB1 are shown in Fig. 2.

2.3 Mechanism of Action

The transport of substrate through ABC transporter proteins requires binding of two ATP molecules followed by their hydrolysis. Since, an ATP molecule interacts with both NBDs, thus binding of ATP brings both NBDs closer to each other in a dimeric form. This is known as closed-dimer conformation. The 12 transmembrane helices (TMH) are arranged in a manner to form a large cavity which can either be in open-inwards conformation (open towards cytosol) or in open-outwards conformation. The rearrangement between these two conformations is guided by ATP hydrolysis. The open-inward conformation has a high affinity binding site for substrate. Upon ATP binding, the NBDs dimerizes and

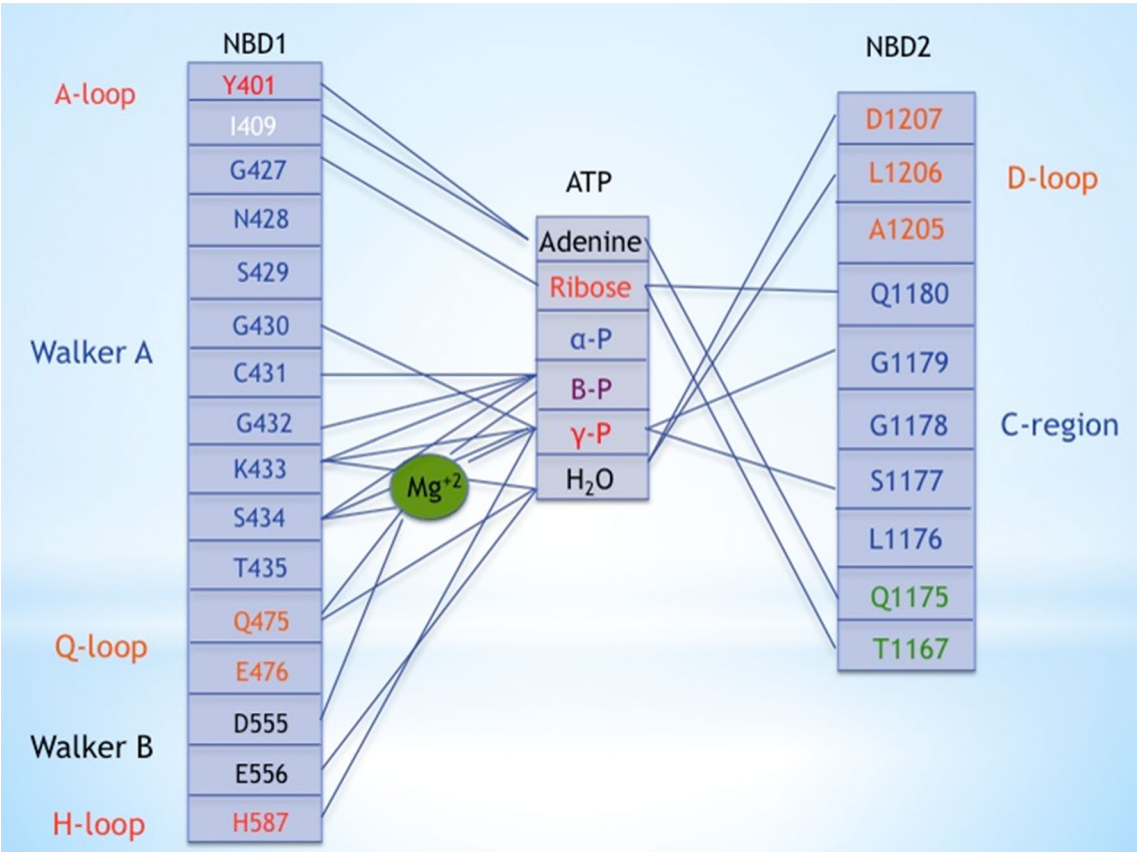

Fig. 2 The interacting partners of ATP with NBD of ABC Transporter Protein ABCB1

results in rearrangement of membrane cavity to form open-outward conformation. This conformation has low affinity for substrate. Thus, ATP hydrolysis results in the release of substrate and conformational change back to open-inward form. In this manner, they alternate between these two conformations, hence called alternating access model. Apart from this, two models have been proposed explaining the mechanism of catalytic cycle of ABC exporters: (i) The ATP Switch Model and (ii) The Constant Contact Model.

As per ATP Switch Model, the two NBDs fully dissociate from each other at the end of catalytic cycle. The binding of ATPs followed by dimerization of NBDs is the power stroke driving the conformational changes from open-inwards to open-outwards form. Due to this rearrangement, the high affinity binding site converts into low affinity resulting in the release of substrate in the extracellular space. Finally, hydrolysis of either or both ATPs occurs which disrupts the closed NBD dimer and results the conformation back to open-inwards form. This is the most accepted model for the catalytic cycle mechanism. This model is being supported by various structures obtained in inward-facing form like ABCB1, ABCC7, Sav1866, MsbA, MetNI, $MalFGK_2$, ModBC, etc. [22–24].

According to the Constant Contact Model, the two NBDs do not dissociate from each other during catalysis, rather each ATP hydrolyses and binding site open alternately. This means that in every cycle, one NBD will hydrolyse a bound ATP and another NBD will release ADP and P*i* hydrolysed in the previous cycle. Thus, the NBDs always exists in a dimer form. Example of this model is explained by heterodimeric ABC transporter TM287/TM288 from *T. maritima* [25]. Similar activity was also observed in ABCC7 as well as ABCB1 suggesting that there may be more than one mechanism for transport of substrates in a single transporter or a transporter might transport different substrates with different mechanisms [11, 23, 24, 26].

3 Antimicrobial Peptides

Antimicrobial Peptides (AMPs) are small peptides ranging from 12 to 100 amino acid residue length and can be linear or combination of alpha helices or beta sheets or loops or mixed structures. AMPs have a broad spectrum of target organisms ranging from viruses to bacteria to parasites and have been fighting against various microbes including

antibiotic-resistant microbes for the last two decades. A database, linking AMPs (LAMP2; https://biotechlab.fudan.edu.cn/database/lamp/index.php), currently have 23,253 sequences of which 7824 are natural while 15,429 are synthetic AMPs [27, 28]. Apart from this, other databases of AMPs are dbAMP (https://140.138.77.240/~dbamp/), DRAMP (https://dramp.cpu-bioinfor.org/), SATPdb (https://crdd.osdd.net/raghava/satpdb/), DBAASP (https://dbaasp.org/), CAMP (https://www.camp3.bicnirrh.res.in/index.php), APD (https://aps.unmc.edu/AP/main.php), etc. The first discovery of AMP dates back to 1939, where a mixture of AMPs, gramicidins, was obtained from soil *Bacillus brevis* [29–31]. Most of the natural AMPs are transcribed and expresses as pro-peptides and later are post translationally modified in their active form at their respective target cells. For instance, a human cathelicidin gene of 18Kda weight, known as hCAP18, is processed at C-terminus end to release a 37 amino acid residue peptide beginning with two leucines, thus, named LL37. This processing occurs in neutrophils. In case of skin, LL37 is further cleaved by serine proteases to produce diffeerent variants with varied degree of activity (like RK-31, KS-30, K20). The LL37 peptide was later found to exhibit potent and broad spectrum antimicrobial activity [32–34]. Similarly, defensins are also stored as unprocessed propeptides form in the paneth cells of small intestine. They subsequently undergo proteolytic cleavage resulting in the activation of mature peptides. This proteolytic cleavage is done by trypsin in humans and matrix metalloproteinase 7 (MMP7) in mouse [35]. AMPs have been discovered in both prokaryotes and eukaryotes. In higher order animals, they are mostly found in tissues and organs that are more susceptible for airborne pathogens and hence act as first line of innate immune defence [36, 37].

3.1 Mechanism of Action

AMPs target their cell either by disrupting membrane integrity or by penetrating inside cell and targeting intracellular proteins, DNA or RNA synthesis. Thus, on the basis of their mechanism of action, AMPs can be classified as either Membrane—Active or Intracellularly—Active. The membrane active AMPs initially interact with the membrane and then either aggregate or form pores in the membrane thereby permeablising it. Since the AMPs that interact with the membrane, are mostly amphiphilic in nature (having both ionic and hydrophobic residues). The mechanism of action of membrane actives can further be classified as Carpet like, Membrane thinning, aggregate formation, Barrel stave or Toroidal pore formation as represented in Fig. 3. In the barrel stave model, the AMPs insert into the membrane bilayer perpendicularly by forming pores. The hydrophobic and hydrophilic associations of phospholipid chains line the lumen of this pore thereby allowing AMP to penetrate and disrupt the membrane bilayer. In the toroidal pore model, the AMPs enter perpendicular to the membrane with inward folding of the membrane lipids resulting in formation of continuous channels between inner and outer leaflet of bilayer membrane. The pore is lined by both peptides and lipids head group. The basic differecne between toroidal pore and barrel-stave pore is that in the former peptides are always in contact with phospholipid head even when they enter the lipid bilayer. Less regular pore structures and peptides oriented parallel to membrane are characteristic features of

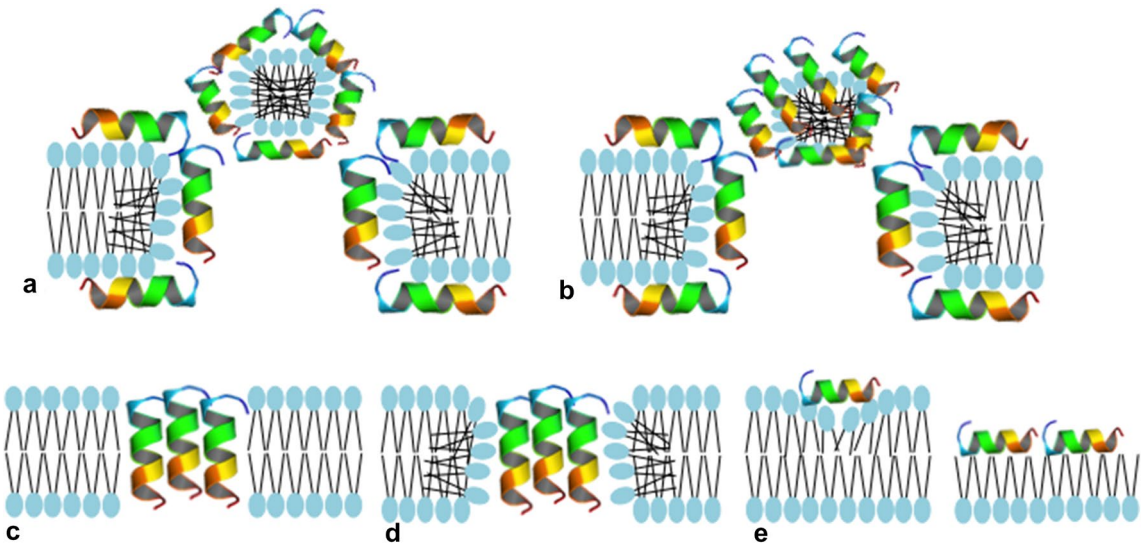

Fig. 3 Mechanism of action of AMPs. **a** Carpet model; **b** aggregate formation; **c** barrel-stave pore formation; **d** toroidal pore formation; **e** membrane thinning

disordered toroidal pore model [38, 39]. As the name suggests, in the carpet mechanism, the AMPs bind parallel to the surface of membrane and upon reaching above a threshold concentration, they permeate and disperse the membrane in a detergent-like manner without pore formation [39, 40]. The aggregate formation mechanism is similar to carpet formation, the difference being that in carpet model peptides interact only with phospholipid head while in aggregate form they may interact with complete lipid or rather no fixed pattern is followed [41]. Sometimes AMPs insert themselves only in one leaflet of membrane bilayer causing gaps in the membrane. To fill it, neighbouring lipid molecules are pulled in and gradually may also lead to replacement of complete monolayer with AMP. Such phenomenon is known as membrane thinning [42, 43]. As for intracellular active AMPs, they can either directly penetrate inside cell or through endocytosis. The AMPs falling in this category like PR-39, indocilin, tPMP-1, aHNP-1, etc. targets and inhibit DNA and protein synthesis. They may also inhibit enzymatic activities or may mediate immunomodulatory effects in hosts [7, 44–46].

3.2 Nature of AMPs

The widely explored and most studied AMPs are against bacteria. The majority of these AMPs are either cationic or amphipathic and target bacterial cell membrane. The examples of antibacterial peptides are Magainin, Cecropin A, Mellitin, Buforin II, Protegrin, Polyphemusin, Indolicidin, PR-39, LL-37, α/β Defensins, Drosocin,etc. An AMP, Nisin, was found to be effective against methicillin resistant *Staphylococcus aureus* thus opening a new door for fighting against resistant microbes [45, 47].

Antifungal peptides are also mostly amphipathic in nature and kill by targeting cell wall or binding to chitin or targeting intracellular components. D-V13K, P18, Indolicin, Defensins are a few examples of AMPs possessing antifungal property [4, 7]. As for the antiviral activity of AMPs, they either integrate into the envelope of both DNA/RNA viruses and cause membrane instability or disruption or by preventing viral particles from entering host cell by blocking their receptors on cell membrane or preventing cell to cell spread of viral particles. Magainin, Cecropin, Mellitin, LL-37, Defensin, Brevinin-1, Dermaseptin, Tachyplesin, Protegrin, Polyphemusin, Lactoferricin, Indolicidin, etc. are a few antiviral AMPs targeting a wide spectra of viruses like HIV, Herpes Simplex Virus (HSV), Influenza A Virus (IAV), Jumin Virus, Adenovirus, Human Papillomavirus (HPV), Human Cytomegalovirus (HCMV), Vesicular Stomatitis Virus (VSV), etc.[7, 45]. The AMPs with antiparasitic properties form a small group when compared with those AMPs having antibacterial, antifungal and antiviral properties. The first AMP presenting antiparasitic activity was Magainin 2 which can kill *Paramecium caudautum* by swelling and eventual bursting of cells. Analogues of Defensins (synthetic peptides) were found to be effective against *Leishmania major* and *Trypanosoma brucei.* Apart from antiprotozoal, antinematodal peptides have also been reported against *Caenorhabditis elegans* like PMAP-23, cathelicidin, etc. An acylated synthetic AMP, Oct-CA(1–7)-M(2–9), was found to be effective against canine leishmaniasis. Dermaseptin S1-S5 AMPs were found to kill *Leishmania mexicana* by disturbing their lipid membrane composition. Cecropin A and their derivatives were more effective on the intracellular amastigote form compared to the extracellular promastigote form as tested on *Leishmania aethiopica*. Another AMP, Tachyplesin, is effective on *Leishmania braziliensis* as well as *Trypanosoma cruzi*. Similar to multidrug chemotherapy, combinatorial effect of AMPs have also been studied. For example, a combination of Mellitin and Cecropin A derivative shows greater effectiveness against *Leishmania donovani* than administered alone [7, 45, 48, 49].

The various studies conducted by diverse research groups imply that AMPs possessing antiprotozoal activity might have different/multiple mechanistic actions which depend on certain peptide motifs and these differ from those having anti-bacterial/fungal/viral activity [45]. The emerging resistance in microbes renders a constant pressure on the need of new drugs to fight against antibiotic resistance and multi-drug resistance (MDR). Nagarajan D. and group have tried to fight against MDR bacteria's by using machine learning technique to design a library of synthetic AMPs. Of these, two AMPs were quite successful against MDR clinical isolates of *Escherichia coli, Acinetobacter baumannii, Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus,* and coagulase-negative *staphylococci* (CoNS) strains [50]. Their work has upheld the importance of computationally designed antimicrobial peptides which is the need of the hour. Other limitations of natural occurring AMPs are their long translating sequences resulting in high production cost, limited availability and low potency, their susceptibility to enzymatic degradation and cytotoxicities. With these limitations and avaialble information withheld by the sequence and structures, natural AMPs have led to the design and development of a new generation of synthetic AMPs with more potency, less toxicity apparently being more useful for therapeutics [7, 51].

In the initial era of AMPs discovery, it was believed that AMPs only target membranes and by disrupting them causes antimicobial effect. Thus, the stereospecific orientation of amino-acids in an AMP doesn't affect their activity. Although later it was revealed that AMPs do have intracellular targets with specific stereochemical orientation like proteins, DNA or RNA and the presence of D- or L-amino acids in an AMP makes a difference. The AMPs with intracellular targets do not permeabilize membrane but penetrates

them and causes antimicrobial activity [52]. Some examples of AMPs having stereochemical targets are buforin II, human α defensin 5, human α defensin 1, human β defensin 4, indolicidin, PR-39, etc. [53–57]. The effect of stereospecificity is not only limited to amino acids. The D- or L-enantiomeric changes in sugar (post-translational modification of AMPs) have drastically affected the antimicrobial activity of Drosocin [58]. The stereochemical isomers of MP196, a hexapeptide, showed improved activity against penicillin-binding protein of *E. coli* [59].

3.3 Designing of New Synthetic AMPs

While designing synthetic AMPs, few properties that need to be considered includes length of peptide, net charge, helicity, hydrophobicity, amphipathicity and last but not the least, their solubility in aqueous solution. Depending on the design and structure activity relationship, designing of synthetic AMPs can be categorised in three different classes, i.e., by modification of sequences of naturally occurring antimicrobial peptides, de novo designing of peptides and using computational assistance to design combinatorial libraries to help in identification of new synthetic AMPs.

3.3.1 Modification of Existing AMPs

This strategy starts with choosing an appropriate natural occurring active AMP as a starting step. A large number of databases containing details of all the naturally occurring AMPs from diverse species like plants, insects, mammals, etc., serve as grand reservoir for selecting the initial template sequence for designing new AMP. This template sequence can further be used for sequence truncation, amino acid substitution, cyclization which may result in improvising its structural activity, reducing size and hence improving antimicrobial activity with reduced toxicity [7, 60].

As most natural AMPs are longer in length, thus, truncating them to short 20–25 residues length not only decreases the cost facilitating large scale production, but also increases stability, decreases the chances of proteolytic cleavage hence making them more active. Removal of few residues may result in increase in the activity as well as specificity while decreasing the toxicity. Zasloff et al. have demonstrated through their study that truncating N-terminus of Magainin 2 by three residues have increased its antimicrobial activity drastically [61]. Similarly, truncating C-terminus of human cationic antimicrobial peptide (hCAP-18) has resulted in shorter and more functional α-helical peptide [62]. Many cationic host defense peptides have been modified using this strategy, for e.g., α-helical cecropins, lactoferrin, cathelicidins and magainins and β-sheet or β-hairpin like AMPs Defensins, gramicidins, protegrins, etc.[60].

Another strategy to increase stability against protease cleavage is by cyclization of AMPs. The cyclization can be done either by amide linkage between backbone in head-to-tail fashion, or disulphide bridging or native chemical ligation. The disulphide linkage cyclization of an indolicidin analogue CP-11 has led to a more rigid backbone as being demonstrated by Hancock and group [63–65]. One more way of modification of an existing AMP is the creation of hybrid peptides from truncated fragments of multiple naturally occurring peptides with antimicrobial activity. In such a hybrid peptide, usually one component is having more antimicrobial activity with higher toxicity and the other component is less toxic but less active too. Such a combination leads to a novel chimeric peptide having higher activity and less toxicity. The examples of hybrid peptides are a series of combinations of cecropin A (CA) and melittin; protegrin-1 (PG-1) with CA or PG-LB (bovine lactoferricin) [60, 66, 67].

The most widely used strategy is modifying an AMP by changing its amino acid content. Numerous studies have been reported where researchers have tried varied number of permutations and combinations to study the effect of different amino acids on their antimicrobial activity. It has been observed that higher proline content reduces the ability of peptide to penetrate the cell membrane. It was also professed that swapping neutral amino acids like Asn and Gln with positively charged residues have resulted in reduction of cytotoxic effects on eukaryotic cells. Introduction of D-amino acids in a peptide sequence have shown more stability against proteases [68, 69]. Addition of an amide group at the end of sequence has also shown to increase the efficiency of a peptide. For instance, amidation at C-terminal end of PMAP-23 increases the uptake by 10 folds, better and faster interaction with cell membrane of gram negative bacteria, better membrane permeabilisation, etc.[70]. The use of an unnatural AMP like β-didehydrophenylalanine have shown higher stability against proteolytic cleavages and hence been widely used in medicinal chemistry to alter a native bioactive AMPs [71]. Another classic example of modifying natural amino acids by substitution is represented by Jiang et al. group. They have used 2 natural AMPs, Piscidin 1 (fish origin) and dermaseptin S4 (frog origin), and substituted hydrophobic positions with one or two Lys residues. This subsitution not only improved antimicrobial activity against *A. baumannii* and *P. aeruginosa* but also decreased hemolysis of human red blood cells. I9K substitution in Piscidin 1 imrpoved activity by 55-fold and 32-fold respectively, against *A. baumannii* and *P. aeruginosa.* While L7K and A14K substitutions in sequence of dermaseptin S4 increased activity by 730-fold and 980-fold respectively, against *A. baumannii* and *P. aeruginosa* [72]. Apart from these different post-translational modifications can be incorporated

like phosphorylation, methylation, glycosylation, amidation, formation of disulphide linkage, etc.[7].

3.3.2 De novo Design

This method is based on the intrinsic characteristics features of the naturally occurring antimicrobial peptides. These features include cationicity, amphipathicity, residue length, or structural features like formation of α-helical or β-sheet conformations or having mixed extended secondary structures. These features are responsible for stability as well as strong microbial activity. This method combines a few basic residues with non-polar residues. The basic residues like Arg, Lys or His assists in initial electrostatic interactions with microbial membrane surfaces which is negatively charged. The non-polar residues like Ala, Val, Leu, Phe, Tyr and Trp mediates the peptide's insertion into microbial membrane lipid bilayers. This stratagem is widely used in drug development area as it leads to the identification of motifs which are smaller in size and can be further used to develop pharmacophore essential for antimicrobial effects. This, in turn, provides an opportunity to increase the ease of optimization through systematic modifications to the short peptide sequence. It also reduces the production cost as well as toxicities and immunogenicity's which are the demerits associated with peptides with longer length of amino acid residues. As mentioned earlier, the combination of Arg and Lys residues have been found to possess strong antimicrobial activity. Similarly, combinations of Arg with a hydrophobic residue Trp also have strong effect on microbes and hence have been investigated vigorously by researchers. A handful of studies have revealed that combination of Arg and Trp along with a critical length of peptides (5–6 residue) not only balances amphipathicity but also accomplishes minimal requirement for showing antibacterial activities [73–75].

Along with maintaining amphipathic nature, the design of synthetic AMPs in terms of secondary structure should also be considered. Synthetic AMPs which can self-assemble into stabilized α-helical, β-sheet and/or hairpin structures are favoured. Due to charge repulsion between cationic residues, AMPs remain unstructured in an aqueous solution. This unstructured form helps them in interacting with cell membrane components of microbes like lipopolysaccharides, lipoteichoic acids and phospholipid head groups which neutralizes the charges on peptides leading to their self-assembly in a specified secondary structure via hydrogen bonding and hydrophobic interactions with the phospholipid bilayer causing membrane damage and perturbation. α-helix is the most studied class closely followed by β-sheet and hairpin structures. Decades of study have revealed that the degree of helicity is correlated with the length of peptide. Increase in the length of the peptide up to a certain extent increases the antimicrobial activity of the peptide. If the length is increased beyond a certain threshold then it results in loss of activity, selectivity or even stability. Synthetic α-helical AMPs like $(FFRR)_3$, $(LLRR)_3$ and $(LLKK)_3$ displays the most optimal balance between α-helicity, antimicrobial activities and hemolytic potentials. Another important factor is the arrangement of hydrophobic and hydrophilic residues. Interestingly, arranging these hydrophobic and hydrophilic residues facing opposite to each other in the helical wheel projection has found to achieve ideal amphiphilicity. Yet another study have focused on the positioning of Trp residues at the hydrophobic–hydrophilic interface in helical wheel projection to enhance the stability as well as membrane penetration ability of the AMP [60, 76–78].

Another advantage of using de novo design is the liberty to use amino acids of D-conformation. Mant et. al. have successfully designed de novo new AMPs with selective activity against gram-negative bacteria while having no toxicity against human red blood cells under the most stringent conditions (18 h at 37 °C and up to 1000 μg/ml or > 350 μM of AMP). They have also shown that the unusual use of all D-conformation amino acids in peptides, diaminopropionic acid (Dap) and diaminobutyric acid (Dab), on the polar surface of amphipathic alpha-helix AMP have resulted in increased specificity for prokaryotes (bacteria) over eukaryotic cells. Aditionally, when tesetd on over 27 strains of *A. baumannii* resistant against atleast 20 antibiotics, the AMPs showed identical antimicrobial activity with MIC in range of 0.5 to 1.2 μM. Thus introduction of D-amino acids have significant advantage on antimicrobial activity with minimized hemolytic effect on red blood cells [79].

A new wave in direction of designing synthetic peptides is the use of machine learning approach to study sequences and nature of natural occurring peptides and on that basis predicting a whole new library of primary sequences. The structure of these newly designed peptides and then their function is predicted with computer assistance. Various databases like LAMP (linking antimicrobial peptides) have been created to store and compare various AMPs.

4 Discussion

ABC transporters being the largest family of efflux transport proteins and playing key role in MDR have always been an attractive target protein. But their highly conserved nature resulted in targeting them to be quite challenging. Most of the ABC inhibitors available currently leads to toxicity. Even the presence of modulators have helped in minimizing their toxic effects to some extent but failed to improve their effectiveness. Antimicrobial peptides with specificity as their characteristic feature have given a ray of hope in fighting against resistant microbes. Both natural and synthetic AMPs

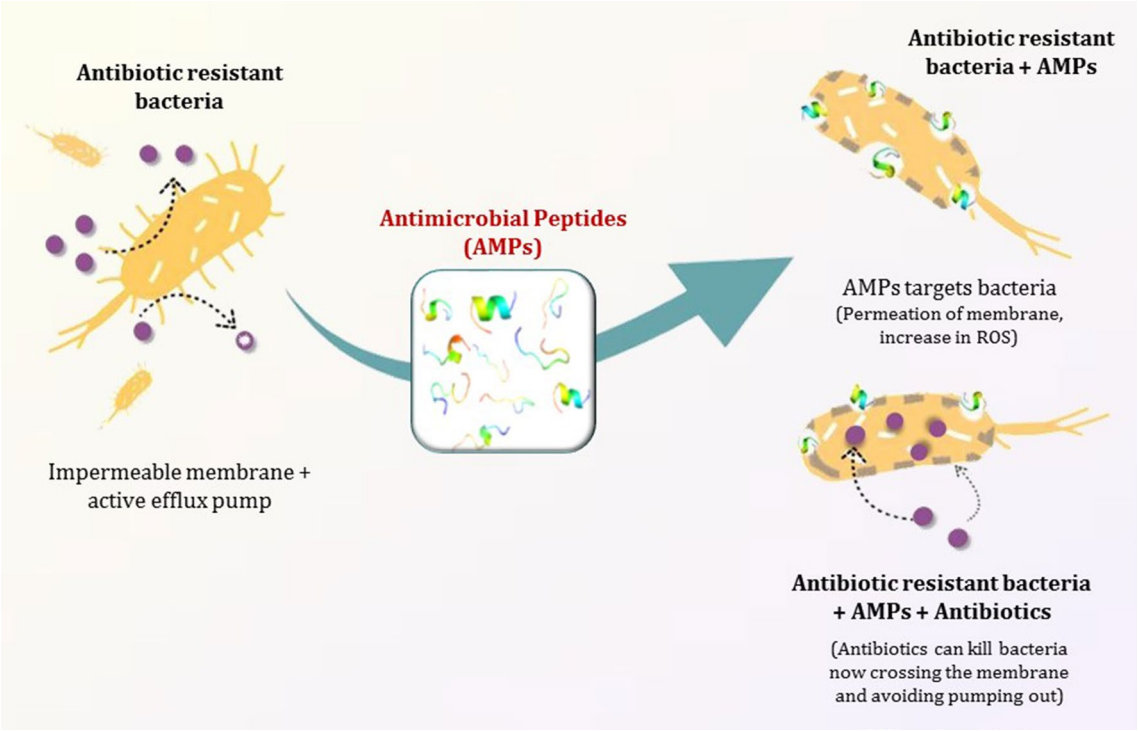

Fig. 4 Schematic representation of the role of AMPs when administered alone or in combination with existing antibiotics

have shown great potential with their antimicrobial activity. For instance, LTX-109, a synthetic AMP, and their stereoisomers have proven quite effective against *S. aureus* as well as methicillin resistant *S. aureus* (MRSA) infections [80, 81]. In our recent work we have demonstrated that small peptides can either be used to modulate activity of transporter proteins leading to reversal of Miltefosine resistance in *Leishmania major* or can directly act as AMPs wildtype *L. major* strains [82]. In nutshell, AMPs provide an effective solution against resistant microbes and can be used either directly or in combination with existing antibiotics to maximize the antimicrobial effect (Fig. 4).

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References

1. Nikaido H (2009) Multidrug resistance in bacteria. Annu Rev Biochem 78:119
2. Piddock LJV (2006) Multidrug-resistance efflux pumps—not just for resistance. Nat Rev Microbiol. https://doi.org/10.1038/nrmicro1464
3. Tanwar J, Das S, Fatima Z et al (2014) Multidrug resistance: an emerging crisis, multidrug resistance: an emerging crisis. Interdiscipl Perspect Infect Dis Interdiscipl Perspect Infect Dis 2014:e541340. https://doi.org/10.1155/2014/541340
4. Kabra R, Chauhan N, Kumar A et al (2019) Efflux pumps and antimicrobial resistance: paradoxical components in systems genomics. Prog Biophys Mol Biol 141:15–24. https://doi.org/10.1016/j.pbiomolbio.2018.07.008
5. Choi Y, Yu A-M (2014) ABC transporters in multidrug resistance and pharmacokinetics, and strategies for drug development. Curr Pharm Des. https://doi.org/10.2174/1381612820666140214165212
6. Palmeira A, Sousa E, Vasconcelos MH, Pinto M (2012) Three decades of P-gp inhibitors: skimming through several generations and scaffolds. Curr Med Chem 19:1946–2025. https://doi.org/10.2174/092986712800167392
7. Bahar AA, Ren D (2013) Antimicrobial peptides. Pharmaceuticals 6:1543–1575. https://doi.org/10.3390/ph6121543
8. Daleke DL (2003) Regulation of transbilayer plasma membrane phospholipid asymmetry. J Lipid Res 44:233
9. Contreras FX, Sánchez-Magraner L, Alonso A, Goñi FM (2010) Transbilayer (flip-flop) lipid motion and lipid scrambling in membranes. FEBS Lett 584:1779
10. Locher KP (2016) Mechanistic diversity in ATP-binding cassette (ABC) transporters. Nat Struct Mol Biol 23:487–493. https://doi.org/10.1038/nsmb.3216
11. Seeger MA, van Veen HW (2009) Molecular basis of multidrug transport by ABC transporters. Biochim Biophys Acta Proteins Proteomics 1794:725–737. https://doi.org/10.1016/j.bbapap.2008.12.004
12. Yates B, Braschi B, Gray KA et al (2017) Genenames.org: the HGNC and VGNC resources in 2017. Nucleic Acids Res. https://doi.org/10.1093/nar/gkw1033
13. Dean M, Hamon Y, Chimini G (2001) The human ATP-binding cassette (ABC) transporter superfamily. J Lipid Res 42:1007

14. Vasiliou V, Vasiliou K, Nebert DW (2009) Human ATP-binding cassette (ABC) transporter family. Hum Genomics 3:281–290
15. Jassal B, Matthews L, Viteri G et al (2020) The reactome pathway knowledgebase. Nucleic Acids Res 48:D498–D503. https://doi.org/10.1093/nar/gkz1031
16. Uhlen M, Fagerberg L, Hallstrom BM et al (2015) Tissue-based map of the human proteome. Science 347:1260419–1260419. https://doi.org/10.1126/science.1260419
17. Stelzer G, Rosen N, Plaschkes I et al (2016) The GeneCards suite: From gene data mining to disease genome sequence analyses. Curr Protoc Bioinform. https://doi.org/10.1002/cpbi.5
18. Van Veen HW, Margolles A, Müller M et al (2000) The homodimeric ATP-binding cassette transporter LmrA mediates multidrug transport by an alternating two-site (two-cylinder engine) mechanism. EMBO J. https://doi.org/10.1093/emboj/19.11.2503
19. Štefková J, Poledne R, Ek JAHČ (2004) ATP-binding cassette (ABC) transporters in human metabolism and diseases. Physiol Res 53:235–243
20. Rees DC, Johnson E, Lewinson O (2009) ABC transporters: the power to change. Nat Rev Mol Cell Biol 10:218
21. Oswald C, Holland IB, Schmitt L (2006) The motor domains of ABC-transporters: what can structures tell us? Naunyn Schmiedebergs Arch Pharmacol 372:385
22. Higgins CF, Linton KJ (2004) The ATP switch model for ABC transporters. Nat Struct Mol Biol 11:918
23. Zoghbi ME, Krishnan S, Altenberg GA (2012) Dissociation of ATP-binding cassette nucleotide-binding domain dimers into monomers during the hydrolysis cycle. J Biol Chem. https://doi.org/10.1074/jbc.M112.340281
24. López-Marqués RL, Poulsen LR, Bailly A et al (2015) Structure and mechanism of ATP-dependent phospholipid transporters. Biochim Biophys Acta Gen Subj 1850:461–475. https://doi.org/10.1016/j.bbagen.2014.04.008
25. Hohl M, Briand C, Grütter MG, Seeger MA (2012) Crystal structure of a heterodimeric ABC transporter in its inward-facing conformation. Nat Struct Mol Biol. https://doi.org/10.1038/nsmb.2267
26. George AM, Jones PM (2012) Perspectives on the structure-function of ABC transporters: the switch and constant contact models. Prog Biophys Mol Biol 109:95–107. https://doi.org/10.1016/j.pbiomolbio.2012.06.003
27. Zhao X, Wu H, Lu H et al (2013) LAMP: a database linking antimicrobial peptides. PLoS ONE. https://doi.org/10.1371/journal.pone.0066557
28. Ye G, Wu H, Huang J et al (2020) LAMP2: a major update of the database linking antimicrobial peptides. Database (Oxford). https://doi.org/10.1093/database/baaa061
29. Dubos RJ (1939a) Studies on a bactericidal agent extracted from a soil bacillus: I. Preparation of the agent. Its activity in vitro. J Exp Med. https://doi.org/10.1084/jem.70.1.1
30. Dubos RJ (1939b) Studies on a bactericidal agent extracted from a soil bacillus: II. Protective effect of the bactericidal agent against experimental pieuococcus infections in mice. J Exp Med. https://doi.org/10.1084/jem.70.1.11
31. Hotchkiss RD, Dubos RJ (1940) Fractionation of the bactericidal agent from cultures of a soil bacillus. J Biol Chem 132:791
32. Zanetti M (2005) The role of cathelicidins in the innate host defenses of mammals. Cur Issues Mo Biol 7:179
33. Zanetti M (2004) Cathelicidins, multifunctional peptides of the innate immunity. J Leukoc Biol. https://doi.org/10.1189/jlb.0403147
34. Murakami M, Lopez-Garcia B, Braff M et al (2004) Postsecretory processing generates multiple cathelicidins for enhanced topical antimicrobial defense. J Immunol. https://doi.org/10.4049/jimmunol.172.5.3070
35. Zhang LJ, Gallo RL (2016) Antimicrobial peptides. Curr Biol 26:R14
36. Lai Y, Gallo RL (2009) AMPed up immunity: how antimicrobial peptides have multiple roles in immune defense. Trends Immunol 30:131–141
37. Radek K, Gallo R (2007) Antimicrobial peptides: natural effectors of the innate immune system. Semin Immunopathol 29:27–43
38. Yang L, Harroun TA, Weiss TM et al (2001) Barrel-stave model or toroidal model? A case study on melittin pores. Biophys J. https://doi.org/10.1016/S0006-3495(01)75802-X
39. Brogden KA (2005) Antimicrobial peptides: pore formers or metabolic inhibitors in bacteria? Nat Rev Microbiol 3:238
40. Ladokhin AS, White SH (2001) "Detergent-like" permeabilization of anionic lipid vesicles by melittin. Biochim Biophys Acta Biomembr. https://doi.org/10.1016/S0005-2736(01)00382-0
41. Xiao H, Shao F, Wu M et al (2015) The application of antimicrobial peptides as growth and health promoters for swine. J Anim Sci Biotechnol 6:1–6
42. Ludtke S, He K, Huang H (1995) Membrane thinning caused by magainin 2+. Biochemistry. https://doi.org/10.1021/bi00051a026
43. Mecke A, Lee DK, Ramamoorthy A et al (2005) Membrane thinning due to antimicrobial peptide binding: an atomic force microscopy study of MSI-78 in lipid bilayers. Biophys J. https://doi.org/10.1529/biophysj.105.062596
44. Madani F, Lindberg S, Langel U et al (2011) Mechanisms of cellular uptake of cell-penetrating peptides. J Biophys 2011:414729
45. Jenssen H, Hamill P, Hancock REW (2006) Peptide antimicrobial agents. Clin Microbiol Rev 19:491–511
46. Melo MN, Ferre R, Castanho MARB (2009) Antimicrobial peptides: linking partition, activity and high membrane-bound concentrations. Nat Rev Microbiol. https://doi.org/10.1038/nrmicro2095
47. Brumfitt W (2002) Nisin, alone and combined with peptidoglycan-modulating antibiotics: activity against methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant enterococci. J Antimicrob Chemother 50:731–734. https://doi.org/10.1093/jac/dkf190
48. Alberola J, Rodríguez A, Francino O et al (2004) Safety and efficacy of antimicrobial peptides against naturally acquired leishmaniasis safety and efficacy of antimicrobial peptides against naturally acquired leishmaniasis. Antimicrob Agents Chemother 48:2–5. https://doi.org/10.1128/AAC.48.2.641
49. Pérez-Cordero JJ, Lozano JM, Cortés J, Delgado G (2011) Leishmanicidal activity of synthetic antimicrobial peptides in an infection model with human dendritic cells. Peptides 32:683–690. https://doi.org/10.1016/j.peptides.2011.01.011
50. Nagarajan D, Nagarajan T, Roy N et al (2018) Computational antimicrobial peptide design and evaluation against multidrug-resistant clinical isolates of bacteria. J Biol Chem 293:3492–3509. https://doi.org/10.1074/jbc.M117.805499
51. Brogden NK, Brogden KA (2011) Will new generations of modified antimicrobial peptides improve their potential as pharmaceuticals? Int J Antimicrob Agents 38:217
52. Kumar P, Kizhakkedathu JN, Straus SK (2018) Antimicrobial peptides: diversity, mechanism of action and strategies to improve the activity and biocompatibility in vivo. Biomolecules 8:4
53. Park CB, Kim HS, Kim SC (1998) Mechanism of action of the antimicrobial peptide buforin II: Buforin II kills microorganisms by penetrating the cell membrane and inhibiting cellular functions. Biochem Biophys Res Commun. https://doi.org/10.1006/bbrc.1998.8159
54. Subbalakshmi C, Sitaram N (1998) Mechanism of antimicrobial action of indolicidin. FEMS Microbiol Lett. https://doi.org/10.1016/S0378-1097(98)00008-1

55. Sharma H, Nagaraj R (2015) Human β-defensin 4 with non-native disulfide bridges exhibit antimicrobial activity. PLoS ONE. https://doi.org/10.1371/journal.pone.0119525
56. Lehrer RI, Barton A, Daher KA et al (1989) Interaction of human defensins with *Escherichia coli*. Mechanism of bactericidal activity. J Clin Invest. https://doi.org/10.1172/JCI114198
57. Boman HG, Agerberth B, Boman A (1993) Mechanisms of action on *Escherichia coli* of cecropin P1 and PR-39, two antibacterial peptides from pig intestine. Infect Immun. https://doi.org/10.1128/iai.61.7.2978-2984.1993
58. Lele DS, Talat S, Kumari S et al (2015) Understanding the importance of glycosylated threonine and stereospecific action of Drosocin, a Proline rich antimicrobial peptide. Eur J Med Chem. https://doi.org/10.1016/j.ejmech.2015.01.032
59. Kaur K, Kaur P, Mittal A et al (2017) Design and molecular docking studies of novel antimicrobial peptides using autodock molecular docking software. Asian J Pharm Clin Res 10:28. https://doi.org/10.22159/ajpcr.2017.v10s4.21332
60. Ong ZY, Wiradharma N, Yang YY (2014) Strategies employed in the design and optimization of synthetic antimicrobial peptide amphiphiles with enhanced therapeutic potentials ☆. Adv Drug Deliv Rev 78:28–45. https://doi.org/10.1016/j.addr.2014.10.013
61. Zasloff M, Martin B, Chen HC (1988) Antimicrobial activity of synthetic magainin peptides and several analogues. Proc Natl Acad Sci USA. https://doi.org/10.1073/pnas.85.3.910
62. Oren Z, Lerman JC, Gudmundsson GH et al (1999) Structure and organization of the human antimicrobial peptide LL-37 in phospholipid membranes: relevance to the molecular basis for its non-cell-selective activity. Biochem J. https://doi.org/10.1042/0264-6021:3410501
63. Dathe M, Nikolenko H, Klose J, Bienert M (2004) Cyclization increases the antimicrobial activity and selectivity of arginine- and tryptophan-containing hexapeptides. Biochemistry. https://doi.org/10.1021/bi035948v
64. Molhoek EM, Van Dijk A, Veldhuizen EJA et al (2011) Improved proteolytic stability of chicken cathelicidin-2 derived peptides by d-amino acid substitutions and cyclization. Peptides. https://doi.org/10.1016/j.peptides.2011.02.017
65. Chan LY, Zhang VM, Huang YH et al (2013) Cyclization of the antimicrobial peptide gomesin with native chemical ligation: influences on stability and bioactivity. ChemBioChem. https://doi.org/10.1002/cbic.201300034
66. Boman HG, Wade D, Boman IA et al (1989) Antibacterial and antimalarial properties of peptides that are cecropin-melittin hybrids. FEBS Lett 259:103–106. https://doi.org/10.1016/0014-5793(89)81505-4
67. Liu YF, Xia X, Xu L, Wang YZ (2013) Design of hybrid β-hairpin peptides with enhanced cell specificity and potent anti-inflammatory activity. Biomaterials. https://doi.org/10.1016/j.biomaterials.2012.09.032
68. Giuliani A, Rinaldi AC (2011) Beyond natural antimicrobial peptides: multimeric peptides and other peptidomimetic approaches. Cell Mol Life Sci 68:2255
69. Zhang L, Benz R, Hancock REW (1999) Influence of proline residues on the antibacterial and synergistic activities of α-helical peptides. Biochemistry. https://doi.org/10.1021/bi9904104
70. Kim JY, Park SC, Yoon MY et al (2011) C-terminal amidation of PMAP-23: translocation to the inner membrane of Gram-negative bacteria. Amino Acids. https://doi.org/10.1007/s00726-010-0632-1
71. Gupta M, Chauhan VS (2011) De novo design of α, β-didehydrophenylalanine containing peptides: from models to applications. Biopolymers. https://doi.org/10.1002/bip.21561
72. Jiang Z, Vasil A, Vasil M, Hodges R (2014) "Specificity determinants" improve therapeutic indices of two antimicrobial peptides piscidin 1 and dermaseptin S4 against the gram-negative pathogens *Acinetobacter baumannii* and *Pseudomonas aeruginosa*. Pharmaceuticals 7:366–391. https://doi.org/10.3390/ph7040366
73. Liu Z, Brady A, Young A et al (2007) Length effects in antimicrobial peptides of the (RW)n series. Antimicrob Agents Chemother. https://doi.org/10.1128/AAC.00828-06
74. Strøm MB, Rekdal Ø, Svendsen JS (2002) Antimicrobial activity of short arginine- and trytophan-rich peptides. J Pept Sci. https://doi.org/10.1002/psc.398
75. Strøm MB, Haug BE, Skar ML et al (2003) The pharmacophore of short cationic antibacterial peptides. J Med Chem. https://doi.org/10.1021/jm0340039
76. Wiradharma N, Sng MYS, Khan M et al (2013) Rationally designed α-helical broad-spectrum antimicrobial peptides with idealized facial amphiphilicity. Macromol Rapid Commun. https://doi.org/10.1002/marc.201200534
77. Deslouches B, Phadke SM, Lazarevic V et al (2005) De novo generation of cationic antimicrobial peptides: Influence of length and tryptophan substitution on antimicrobial activity. Antimicrob Agents Chemother. https://doi.org/10.1128/AAC.49.1.316-322.2005
78. Rekdal Ø, Haug BE, Kalaaji M et al (2012) Relative spatial positions of tryptophan and cationic residues in helical membrane-active peptides determine their cytotoxicity. J Biol Chem. https://doi.org/10.1074/jbc.M111.279281
79. Mant CT, Jiang Z, Gera L et al (2019) De Novo designed amphipathic α-helical antimicrobial peptides incorporating dab and dap residues on the polar face to treat the gram-negative pathogen, *Acinetobacter baumannii*. J Med Chem. https://doi.org/10.1021/acs.jmedchem.8b01785
80. Nilsson AC, Janson H, Wold H et al (2015) LTX-109 is a novel agent for nasal decolonization of methicillin-resistant and -sensitive *Staphylococcus aureus*. Antimicrob Agents Chemother 59:145–151. https://doi.org/10.1128/AAC.03513-14
81. Isaksson J, Brandsdal BO, Engqvist M et al (2011) A Synthetic antimicrobial peptidomimetic (LTX 109): stereochemical impact on membrane disruption. J Med Chem 54:5786–5795. https://doi.org/10.1021/jm200450h
82. Kabra R, Ingale P, Singh S (2020) Computationally designed synthetic peptides for transporter proteins imparts allostericity in Miltefosine resistant L. major. Biochem J 477:2007–2026. https://doi.org/10.1042/BCJ20200176