

# The role of bacterial transport systems in the removal of host antimicrobial peptides in Gram-negative bacteria

Jessica M.A. Blair<sup>1</sup>, Kornelius Zeth<sup>2</sup>, Vassilij N. Bavro<sup>3</sup>, Enea Sancho-Vaello<sup>1,\*</sup>

<sup>1</sup>College of Medical and Dental Sciences, Institute of Microbiology and Infection, University of Birmingham, Edgbaston, Birmingham, B15 2TT, United Kingdom

<sup>2</sup>Department of Science and Environment, Roskilde University, Universitetsvej 1, 4000 Roskilde, Denmark

<sup>3</sup>School of Life Sciences, University of Essex, Colchester, CO4 3SQ, United Kingdom

\*Corresponding author. College of Medical and Dental Sciences, Institute of Microbiology and Infection, University of Birmingham, Edgbaston, Birmingham, B15 2TT, United Kingdom. E-mail: [E.Sancho-Vaello.1@bham.ac.uk](mailto:E.Sancho-Vaello.1@bham.ac.uk)

Editor: Miguel Camara.

## Abstract

Antibiotic resistance is a global issue that threatens our progress in healthcare and life expectancy. In recent years, antimicrobial peptides (AMPs) have been considered as promising alternatives to the classic antibiotics. AMPs are potentially superior due to their lower rate of resistance development, since they primarily target the bacterial membrane ('Achilles' heel' of the bacteria). However, bacteria have developed mechanisms of AMP resistance, including the removal of AMPs to the extracellular space by efflux pumps such as the MtrCDE or AcrAB–TolC systems, and the internalization of AMPs to the cytoplasm by the Sap transporter, followed by proteolytic digestion. In this review, we focus on AMP transport as a resistance mechanism compiling all the experimental evidence for the involvement of efflux in AMP resistance in Gram-negative bacteria and combine this information with the analysis of the structures of the efflux systems involved. Finally, we expose some open questions with the aim of arousing the interest of the scientific community towards the AMPs—efflux pumps interactions. All the collected information broadens our understanding of AMP removal by efflux pumps and gives some clues to assist the rational design of AMP-derivatives as inhibitors of the efflux pumps.

**Keywords:** efflux pumps, antimicrobial peptides, antimicrobial resistance mechanisms, MtrCDE, AcrAB–TolC, Sap system

## Introduction to antimicrobial peptides

Antibiotic resistance is a major global challenge that threatens the progress in healthcare and life expectancy. Although there are several antibiotics in preclinical and clinical trials, it is urgent to find new and more effective candidates to deal with this health emergency situation (Theuretzbacher et al. 2019, Butler and Paterson 2020).

Over the last decades, natural weapons such as bacteriocin proteins and peptides, endolysins, and antibodies have received substantial attention as potential clinical antimicrobials and as possible immune-modulating agents (Rios et al. 2016, Soltani et al. 2021). In particular, antimicrobial peptides (AMPs) are being considered as an alternative to the classical antibiotic approach. AMPs are a diverse group of peptides produced by multicellular organisms as a part of their first-line defence mechanism against pathogen invasion (Zasloff 2002, Wang and Wang 2004, Wang et al. 2022). AMPs can inhibit proinflammatory responses induced by lipopolysaccharides (LPS), act as adjuvants, modulate cytokine production, exert direct chemotactic action on neutrophils, macrophages, immature dendritic cells, mast cells, monocytes, and T-lymphocytes, or even activate endothelial cells to proliferate and form vessel-like structures in wound repair (Diamond et al. 2009).

These small peptides (10–50 amino acids) are amphipathic molecules, mostly cationic (with a charge of +2 to +11), although anionic AMPs have also been reported (Schitteck et al. 2001, Lai et al. 2007, Harris et al. 2009, Mahlapuu et al. 2016). Structurally,

AMPs can be divided into linear  $\alpha$ -helical,  $\beta$ -sheet, mixed, and linear extended/unfolded molecules (Table 1; Koehbach and Craik 2019). The linear  $\alpha$ -helical group members typically contain one  $\alpha$ -helix (e.g. LL-37, magainin, cecropin, and melittin). The  $\beta$ -sheet members contain at least two  $\beta$ -strands in their structure stabilized by two to four disulfide bridges (e.g. HD-5 and protegrin-1). The mixed AMPs contain both  $\alpha$ - and  $\beta$ - structural elements (e.g. HBD-1). The linear extended structures do not exhibit any clear structural arrangement (e.g. indolicidin). In addition, AMPs can exhibit a huge variability in conformation and oligomerization, as seen by the different 3D structures/oligomers that the same AMP (e.g. LL-37) can adopt (Zeth and Sancho-Vaello 2021). Some of the AMPs show oligomeric structures such as LL-37 with dimers and tetramers or dermcidin as a hexameric channel (Fig. 1; Song et al. 2013, Sancho-Vaello et al. 2017, 2020).

In the interest of brevity, this review is focussed predominantly on the eukaryotic AMPs, however, the medical importance of prokaryotic bactericidal peptides is well-established. The ribosomally synthesized bacteriocin peptides often have high antimicro-

Received: December 16, 2021. Revised: May 23, 2022. Accepted: June 22, 2022

© The Author(s) 2022. Published by Oxford University Press on behalf of FEMS. This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted reuse, distribution, and reproduction in any medium, provided the original work is properly cited.

**Table 1.** Structural properties and classification of the AMPs mentioned in this review.

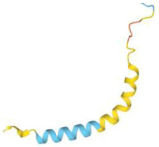
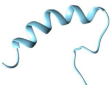
AMP	Organism	Known biophysical properties and mode of action	Ribosomal AMPs	PDB	Amino acids	Charge	References
LL-37	The 18-KD protein hCAP-18 is the only cathelicidin found in humans so far. It consists of a cathelin-like domain and a C-terminal peptide called LL-37, which is released after a proteolytic event.	Disordered in water but helical structure in presence of ions, salts, detergents, or bacterial membranes. It is a very flexible peptide exhibiting multiple conformations and quaternary structures.	Ribosomal AMPs	See different LL-37 3D structures and the corresponding PDBs in Fig. 1(A)–(D).	37	+6	Sørensen et al. (2001), Porcelli et al. (2008), Wang (2008), Morizane et al. (2010), Vandamme et al. (2012), Shahmiri et al. (2016), Sancho-Vaello et al. (2017, 2020), Engelberg and Landau (2020).
CRAMP	Cathelin-Related Antimicrobial Peptide is the mouse analogue of human LL-37 peptide. It is released from the cathelicidin proform after a proteolytic event.	$\alpha$ -helical structure as other cathelicidins. CRAMP-38 seems to be equivalent to CRAMP-2. In the AMP database, CRAMP is composed of 34 amino acids.	Linear $\alpha$ -helical		33 (CRAMP-1) 38 (CRAMP-2)	+6	Gallo et al. (1999), Kovach et al. (2012).
Cecropin	Cecropin was the first insect AMP isolated from the bacteria-challenged <i>Hyalophora cecropia</i> pupa.	Unstructured in aqueous solution but forming a high percentage of $\alpha$ -helical structure in the presence of LPS vesicles, or liposomes.		 PDB: 2N92	35 (Cecr.B) 31(Cecr.P1)	+7	Steiner et al. (1981), Hultmark et al. (1982), Wang et al. (1998), Bland et al. (2001).

Table 1. Continued


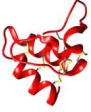

AMP	Organism	Known biophysical properties and mode of action	PDB	Amino acids	Charge	References
<b>Ribosomal AMPs</b>						
<b>Linear <math>\alpha</math>-helical</b>						
Melittin	Melittin constitutes 50% of the dry weight of the honey bee venom.	Random coil configuration at low ionic concentration and neutral pH. It is a very flexible peptide exhibiting multiple conformations and quaternary structures (from monomer to tetramer).	 PDB: 6DST	26	+5	Altenbach and Hubbell (1988), Goto and Hagiwara (1992), Son et al. (2007).
Snakin-1	Snakin-1 is a peptide isolated from potato tubers.	Mostly $\alpha$ -helical with six disulfide bonds between its 12 cysteines.	 PDB: 5E5Q	63	+8	Segura et al. (1999)
<b><math>\beta</math>-sheet</b>						
$\alpha$ -defensins	Human neutrophil peptides HNP-1, -2, -3, -4, and human enteric HD-5, -6. Defensins are synthesized as inactive precursors being activated through posterior proteolytic removal of the inhibitory propeptide.	Able to dimerize and form barrel-stave pores that span anionic membranes. A concentration-dependent equilibrium between monomers and higher oligomers has also been proposed. The structure of the membrane-bound HNP-1 showed a similar conformation to the water-soluble state, except for the turn connecting the $\beta 2$ and $\beta 3$ strands, supporting in this way a 'dimer pore' topology. They have three disulfide bonds.	 PDB: 1ZMM	30 (HNP-1) 29 (HNP-2) 30 (HNP-3) 33 (HNP-4) 32 (HD-5,-6)	+3 (HNPs) +4 (HD-5) +2 (HD-6)	Hill et al. (1991), Liu and Ganz (1995), Valore et al. (1996), Zhang et al. (2010), Shafee et al. (2017).

Table 1. Continued

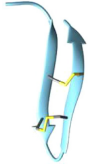

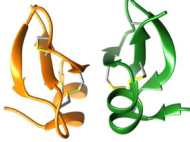
AMP	Organism	Known biophysical properties and mode of action	PDB	Amino acids	Charge	References
			<b>Ribosomal AMPs</b>			
			<b>Linear <math>\alpha</math>-helical</b>			
Protegrin-1	Protegrin-1 (PG-1) is synthesized in porcine leukocytes. PC-8 is a linearized synthetic variant lacking both disulfide bonds.	Ion channel-like structures with predominantly three to five subunits. The NMR structure suggested a membrane-inserted $\beta$ -barrel enclosing a water pore. In the POPC/cholesterol membrane, the N and C strands of PG-1 cluster into tetramers. It has two intramolecular disulfide bonds.	 PDB: 1PG1	18	+7	Qu et al. (1997), Mani et al. (2006), Capone et al. (2010)
Tachyplesin-1	TP-1 is a 17 amino acid AMP extracted from the hemocytes of the horseshoe crab.	Structure stabilized by two cross-strand disulfide linkages, forming a $\beta$ -hairpin structure, both in aqueous solution and in lipid-mimicking environments. It has two intramolecular disulfide bonds.	 PDB: 2MDB	17	+7	Kawano et al. (1990), Kushibiki et al. (2014).
			<b>Mixed</b>			
$\beta$ -defensins	Human HBD-1,-2,-3,-4. Defensins are synthesized as inactive precursors being activated through posterior proteolytic removal of the inhibitory propeptide. There is an analog in chinchilla called cDB-1.	$\beta$ -defensins are mostly $\beta$ -sheets with a short Nt $\alpha$ -helix. Able to dimerize to form pores through anionic membranes. HBD-2 was found in solution mainly as dimers although it is likely to form higher-order oligomers either in higher concentrations that are induced by pathogen attack or in interactions with the lipid membranes of the bacterial cells. They have three disulfide bonds.	 PDB: 1FD4	36 (HBD-1) 41 (HBD-2) 45 (HBD-3) 50 (HBD-4)	+4 (HBD1) +7 (HBD2) +11 (HBD3) +6 (HBD4)	Hoover et al. (2000), Harris et al. (2004), Shafee et al. (2017).

Table 1. Continued

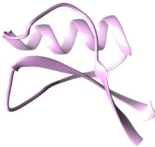
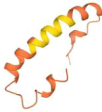


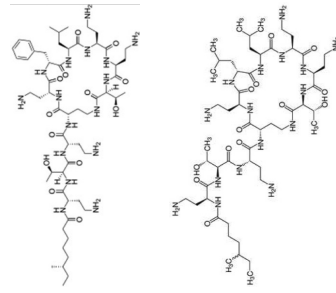
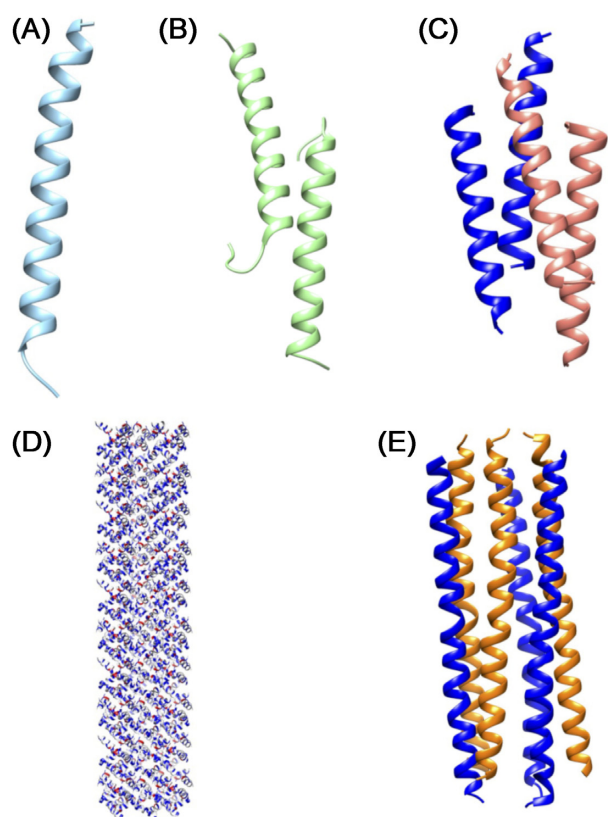
AMP	Organism	Known biophysical properties and mode of action	PDB	Amino acids	Charge	References
			<b>Ribosomal AMPs</b>			
			<b>Linear <math>\alpha</math>-helical</b>			
Thionins	The thionins are AMPs in plants.	Composed of two antiparallel $\alpha$ -helices and an antiparallel double-stranded $\beta$ -sheet with three or four conserved disulfide linkages. They have three or four disulfide bonds.		47	+9	Lucca et al. (2005), Nawrot et al. (2014).
Protamine	Protamines are short proteins that can contain up to 70% arginines found in the nuclei of sperm of different animal species. It participates in the packing of the DNA.	Protamine changes from a random coil to $\alpha$ -helix on binding tRNA. The DNA-bound protamine showed $\beta$ -turns and $\alpha$ -helix, but no $\beta$ -sheet was observed by infrared spectroscopy. They have two (bull) or none (piscine) disulfide bonds.	PDB: 1GPT 	50–110	~ +24	Wade et al. (1978), Aspedon and Groisman (1996), Roque et al. (2011).
			<b>Linear extended</b>			
Indolicidin	This AMP was isolated from the neutrophil blood cells of cows.	Extended structure located in the membrane interface when forming complexes with vesicles and detergent micelles.		13	+4	Rozek et al. (2000).
			PDB: 1G89			

Table 1. Continued

AMP	Organism	Known biophysical properties and mode of action	PDB	Amino acids	Charge	References
<p><b>Ribosomal AMPs</b></p> <p><b>Linear <math>\alpha</math>-helical</b></p>						
<p><b>Cyclic/complex topologies</b></p>						
Nisin	This lantibiotic is produced by <i>Lactococcus lactis</i> and is used as a food preservative. The two common forms of nisin are nisin A and Z, being recently isolated a new natural variant called nisin Q.	Structure consisting of two domains: an N-terminal domain containing three lanthionine rings, and a C-terminal domain containing two intertwined lanthionine rings. Nisin sequesters lipid II, resulting in prevention of cell wall biosynthesis, and forms nisin-lipid II complexes, which lead to pores in the bacterial cell membrane.	 <p>PDB: 1WGO</p>	34	+3	Van Den Hooven et al. (1996), Wiedemann et al. (2001), Hsu et al. (2004).
<p><b>Nonribosomal AMPs</b></p>						
Polymyxin B	These AMPs are used as last-resort antibiotics for the treatment of MDR Gram-negative infections.	The structure of polymyxin B consists of a polycationic cyclic heptapeptide with a branched fatty acid tail. It is a mixture of four closely related components, only differing in the fatty acid moiety. Colistin and Polymyxin B share a common scaffold and present only minor changes in their constituent amino acids. Both of them interact with the LPS of the OM of Gram-negative bacteria displacing divalent cations and promoting membrane disruption. They also disrupt the inner bacterial membranes.		10	+6 (PXB, COL)	Hancock (1997), Orwa et al. (2001), Kwa et al. (2007), Zavascki et al. (2007), Deris et al. (2014), Ayoub Moubareck (2020).
Colistin (Polymyxin E)						



**Figure 1.** 3D structures of full length and truncated LL-37 and dermcidin. (A)–(D) The different human LL-37 structures show their structural plasticity. (A) The monomeric structure of LL-37 (PDB 5NMN), (B) antiparallel dimer structure crystallized in the presence of detergents (PDB 5NNT), (C) tetrameric structure of LL-37 (7PDC), and (D) fiber of the core sequence of LL-37 peptide (residues 12–29; PDB 6S6M). (E) Hexameric channel structure of dermcidin (PDB 2YMK).

bial activity and include the subgroup of lanthionine containing peptide antibiotics known as lantibiotics (e.g. nisin; Table 1; Cotter et al. 2013). The lantibiotics are post-translationally modified and contain dehydrated amino acids (dehydrobutyrine and/or dehydroalanine) amongst other unusual amino acids (Willey and van der Donk 2007, Bierbaum and Sahl 2009). This has been recently reviewed in detail (Clemens et al. 2017, Kumariya et al. 2019). Another group of AMPs are the cyclic nonribosomal AMPs (e.g. colistin and polymyxin B). These AMPs are synthesized by bacteria (*Bacillus/Paenibacillus polymyxa*) and are currently used as a last-resort antibiotics for the treatment of MDR Gram-negative infections, but microbial resistance towards these antibiotics has already been reported (El-Sayed Ahmed et al. 2020). Even though these AMPs are not synthesized by multicellular organisms, the overview of the efflux of colistin and polymyxin B is included here with the aim of understanding whether efflux is a relevant mechanism of resistance to these AMP-based therapeutic agents.

Certain AMP properties such as size, amphipathicity, and especially their cationic nature allow them to target different molecules of the bacterial cell envelope and the cytoplasm, including the negatively charged LPS, the lipoteichoic acid (LTA) of the Gram-positive bacterial cell wall, phospholipids of the bacterial membranes, proteins, nucleic acids, or ribosomes (Ding et al. 2003, Malanovic and Lohner 2016, Macleod et al. 2019, Martynowycz et al. 2019).

The main killing mechanism used by the AMPs is the disruption of bacterial membranes through initial electrostatic interactions with LPS or LTA in the outer membrane (OM) or cell wall, respectively, after a threshold concentration of accumulated AMP is reached (Ding et al. 2003, Malanovic and Lohner 2016). Classically, the cytoplasmic membrane disruption mechanisms have been divided into the detergent-like carpet model, the toroidal pore model, and the barrel-stave model (Zasloff 2002, Brogden 2005, Bechinger and Lohner 2006). In the carpet model (e.g. cecropin B), the disruption of the membrane occurs after the formation of a layer of peptide monomers on the membrane surface destabilizing its phospholipid packing and leading to its disintegration (Gazit et al. 1996). In the toroidal pore model (e.g. melittin), the peptides interact with the head of the phospholipids in order to form a combined peptide–lipid pore (Lee et al. 2013). In the barrel-stave model (e.g. dermcidin), the peptides form a pore exclusively composed of peptides (Mihajlovic and Lazaridis 2010, Song et al. 2013, Sancho-Vaello et al. 2020). However, there are now also known to be intermediate mechanisms and combinations of these mechanisms (Wimley 2010, Nguyen et al. 2011, Sancho-Vaello et al. 2017).

The formation of pores in membranes can be transient or stable, as shown by the concentration-dependent pore formation in melittin. At nanomolar concentrations, it induces transient pores that allow transmembrane conduction of atomic ions, but not leakage of glucose or larger molecules. Beyond a critical peptide/lipid ratio, pores become stable and lead to leakage of cellular contents, the loss of transmembrane potential, and death of the bacteria (Terwilliger and Eisenberg 1982, Matsuzaki et al. 1997, Lee et al. 2013). If the OM pores are transient or their number is low, the peptides can access the periplasm, where they can interact with other proteins and/or accumulate on the surface of the inner membrane (IM). After a peptide/phospholipid threshold is reached in the cytoplasmic membrane, the peptides can oligomerize, form pores and access the cytoplasm where they can interact with cytoplasmic targets including ribosomes and nucleic acids (Graf et al. 2017, Cardoso et al. 2019). Proline-rich AMPs (e.g. oncocin, Api137) have been shown to bind to ribosomes and inhibit protein synthesis *in vivo* and *in vitro* (Krizsan et al. 2015, Mardirossian et al. 2018). Specifically, the Oncocin and Onc112 allow translation initiation but prevent the transition into the elongation phase (Seefeldt et al. 2015). Others, such as the Api137 arrests terminating ribosomes (Florin et al. 2017). Some AMPs (e.g. indolicidin) induce filamentation in *Escherichia coli* cells as a result of DNA synthesis inhibition (Subbalakshmi and Sitaram 1998). In this way, AMPs can interfere with vital intracellular processes, such as cell wall or protein synthesis (Le et al. 2017).

Although several mechanisms of AMP resistance have been shown *in vitro* (see the section 'Mechanisms of AMP resistance in Gram-negative bacteria' in this review) and by using *in vivo* models of infection (Mount et al. 2010, Hobbs et al. 2011, 2013, Bauer and Shafer 2015), it has been shown that AMP resistance evolves at a much lower rate than to antibiotics, except for the nonhost defence peptide colistin (Peschel and Sahl 2006, Spohn et al. 2019). Recently, other constraints on the evolution of AMP resistance have been proposed to explain their low rate of appearance. These evolutionary constraints are related to fitness trade-offs, functional compatibility, and the small fraction of AMP resistance genes linked to mobile genetic elements (Jangir et al. 2021). Specifically, it was shown that whereas AMP resistance genes are widespread in the gut microbiome, their rate of horizontal transfer is lower than that of antibiotic resistance genes. By gut microbiota culturing and functional metagenomics it was revealed that

AMP resistance genes originating from phylogenetically distant bacteria have only a limited potential to confer resistance in *E. coli* (Kintses et al. 2019). Related to the fitness trade-offs, it was shown that increased expression of *mcr-1* (a lipid A modifying enzyme that confers resistance to colistin) results in decreased growth rate, cell viability, competitive ability, and significant degradation in cell membrane and cytoplasmic structure (Yang et al. 2017).

A second advantage of using AMPs as antimicrobial agents is their ability to target the challenging nonpermeable double membrane of Gram-negative bacteria (Fjell et al. 2011). In particular, the synergy of some AMPs when used in combination with the classical antibiotics have opened the possibility for decreasing their therapeutic dose (Steenbergen et al. 2009, Wu et al. 2020, Kampshoff et al. 2019, Pizzolato-Cezar et al. 2019, Ruden et al. 2019). For example, the AMP DP7 in combination with vancomycin or azithromycin was more effective, especially against highly antibiotic-resistant strains (Wu et al. 2017).

All AMPs used currently in authorized treatments belong to the nonribosomally synthesized group (e.g. polymyxin B and colistin). However, none of the more than 3000 identified ribosomally encoded AMPs have been approved by the FDA (Browne et al. 2020, Liu et al. 2019, Chen and Lu 2020) because of issues with the AMP sensitivity to environmental conditions in particular proteolysis (Mahlpuu et al. 2016), the high production costs of the chemical modifications needed to overcome their instability (e.g. use of D-amino acids, macrocyclization by using disulfide bonds, and incorporation of noncanonical amino acids), and the toxicity against mammalian cells *in vivo* (Haney and Hancock 2013, Koo and Seo 2019). Despite these issues, the number of AMPs with activities related to membrane disruption, immunomodulation, or inhibition of intracellular functions is increasing in clinical and preclinical development (Browne et al. 2020, Koo and Seo 2019).

## Mechanisms of AMP resistance in Gram-negative bacteria

In order to avoid AMP accumulation on their surface and consequent membrane pore formation, bacteria have to protect their exposed surfaces (cell wall, OM, and IM) and the potential cytoplasmic targets (DNA and ribosomes) against AMP attachment. To do this, bacteria employ different defence mechanisms including extra- or intracellular proteolytic attack, the alteration of membrane charge and fluidity of the bacterial membrane, the use of extracellular matrices to entrap AMPs, and the active removal of AMPs through efflux pumps. Detailed reviews of other AMP resistance mechanisms have been recently published (Koprivnjak and Peschel 2011, Matamouros and Miller 2015, Cole and Nizet 2016, Joo et al. 2016, Bechinger and Gorr 2017) so in this review, we will only briefly describe the mechanisms employed by Gram-negative bacteria to resist the action of AMPs, but provide an in depth view on the available data concerning AMP efflux.

### (a) Proteolysis by extracellular and intracellular proteases

In Gram-negative bacteria, several proteases have been shown to confer AMP resistance by cleaving AMPs at the OM (Fig. 2). For example, *E. coli* OmpT, *Salmonella enterica* serovar Typhimurium PgtE, and *Yersinia pestis* Pla, belong to the ompT family of aspartate proteases and can cleave LL-37, C18G, CRAMP, and protamine (Galva'n et al. 2008, Guina et al. 2000, Stumpe et al. 1998, Thomassin et al. 2012). In *Proteus mirabilis*, the metalloprotease ZapA cleaves the human HBD-1, LL-37, and PG-1 (Belas et al. 2004). In *Burkholderia cenocepacia*, two zinc-dependent metalloproteases (ZmpA and ZmpB) can cleave and inactivate LL-37 and HBD-1, re-

spectively (Kooi and Sokol 2009). The *Pseudomonas aeruginosa* elastase completely degrades and inactivates LL-37 (Schmidtchen et al. 2002). Also, some proteases secreted by *Porphyromonas gingivalis* and *Prevotella spp.* can cleave cecropin B and brevinin (Devine et al. 1999). In *S. Typhimurium* and *Haemophilus influenzae*, the cytoplasmic proteases can also degrade AMPs via Sap transport, as explained in the section 'Sap system: importing and degrading AMPs as mechanism of resistance' of this review.

### (b) Entrapment of AMPs by bacterial biofilms and other extracellular matrices

Bacterial biofilms and other extracellular matrices can hinder the AMP attachment to the bacterial surface, by decreasing the penetration of AMPs through the matrix (Fig. 2; Mah and O'Toole 2001). In *P. aeruginosa*, the production of alginate polysaccharide induces aggregation in some AMPs (Chan et al. 2004, Foschiatti et al. 2009). The polysaccharide intercellular adhesin (PIA) produced by *E. coli* was shown to be responsible for HBD-3, LL-37, and dermcidin resistance (Wang et al. 2004).

Also, the capsule of *Klebsiella pneumoniae* is responsible for conferring resistance against polymyxin B, HNP-1, lactoferrin, and protamine likely by hindrance and electrostatic trapping (Campos et al. 2004). The same mechanism seems to work in the *Neisseria meningitidis* capsule that prevents AMP surface binding of LL-37, protegrins, defensins, polymyxin B, LL-37, and CRAMP (Jones et al. 2009, Spinosa et al. 2007).

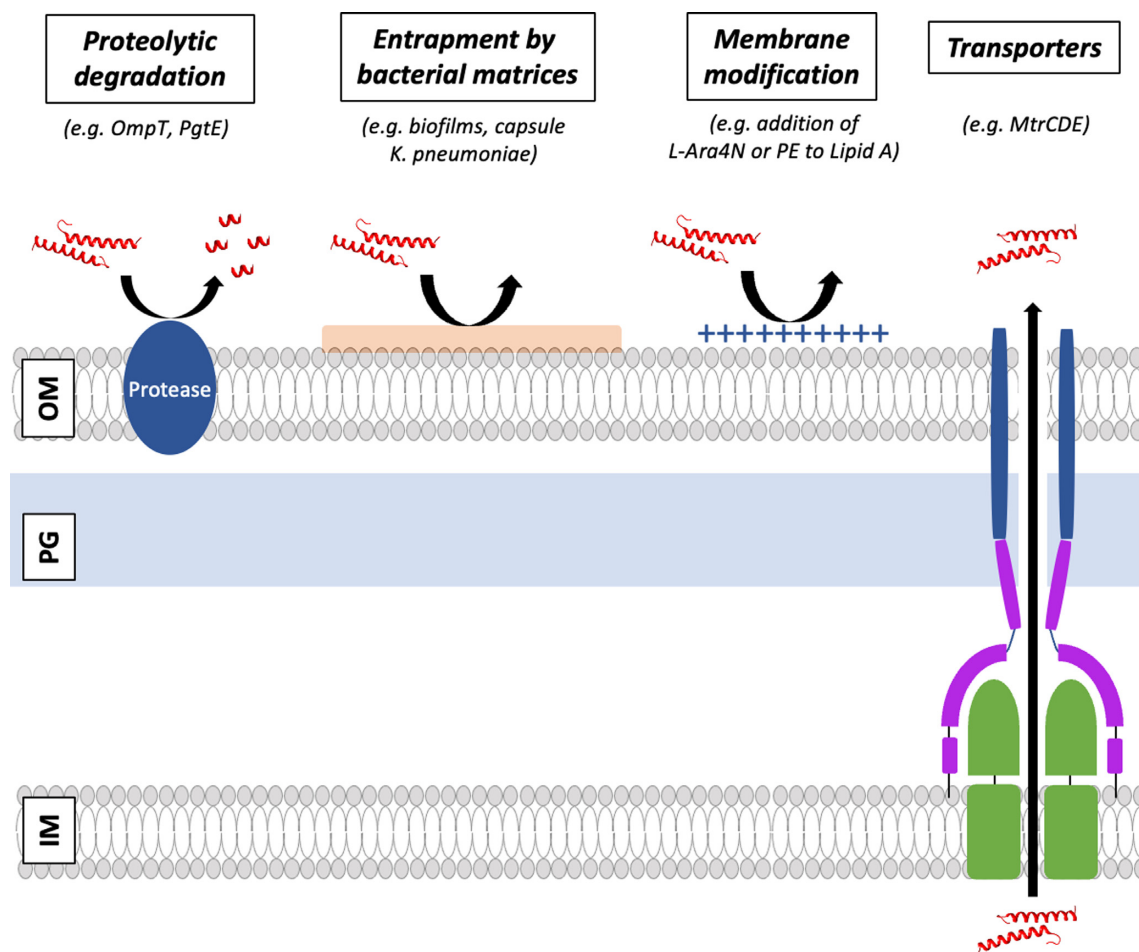
Another approach to entrap the cationic AMPs is to perform a proteolytic release of negatively charged elements belonging to the host epithelial cells. Some proteases in *P. aeruginosa* can degrade the proteoglycan decorin releasing dermatan sulfate, which can bind and inactivate the  $\alpha$ -defensin HNP-1 (Schmidtchen et al. 2001).

### (c) Modification of charge and fluidity of bacterial membranes

Perhaps the most common strategy to decrease the ionic attraction between the cationic AMPs and the negatively charged elements of the bacterial membranes is the bacterial surface charge modification (Fig. 2). The Gram-negative double membrane is a challenging structure to penetrate. It consists of an asymmetric OM with an inner leaflet containing phospholipids, and an outer leaflet mostly composed of LPS. Between the OM and the IM, there is a periplasmic space containing a thin layer of peptidoglycan. The IM is a phospholipid bilayer composed of phosphatidylethanolamine (PEA), phosphatidylglycerol (PG), phosphatidylserine (PS), and cardiolipin (Silhavy 2010).

One mechanism that bacteria employ to decrease the negative net charge of LPS is to add the positively charged 4-amino-4-deoxy-L-arabinose (L-Ara4N) or phosphoethanolamine (PEA) moieties to lipid A. In *Salmonella spp.*, this modification is regulated by the two-component system PmrAB, which senses the AMP presence *in vivo* and expresses *pmrC* and *pmrEHFJKLM* (Gunn and Miller 1996, Gunn et al. 2000, Tamayo et al. 2005). Specifically, in *Salmonella*, the PmrA-dependent modification of lipid A was shown to be responsible for polymyxin B resistance (Lee et al. 2004). The enhancement of AMP resistance by the addition of L-Ara4N has been also shown in *P. mirabilis* (McCoy et al. 2001), *Yersinia pseudotuberculosis* (Marceau et al. 2003), *K. pneumoniae* (Cheng et al. 2010), and *P. aeruginosa* (Moskowitz et al. 2004). The addition of pETN to dephosphorylated lipid A also involves an increase in AMP resistance. This has been shown in *Helicobacter pylori* under the regulation of the *lpxEHP* genes and showed an increase in MIC of polymyxin B (Tran et al. 2006). The polymyxin B resistance was also observed in *Neisseria gonorrhoeae* and *N. meningitidis* by the





**Figure 2.** Mechanisms of AMP resistance in Gram-negative bacteria, including (A) proteolysis by extracellular and intracellular proteases; (B) entrapment by bacterial biofilms and other extracellular matrices; (C) modification of charge and fluidity of bacterial membranes; and (D) removal of AMPs through transporters. The schematic representation and localization of the PG is based on (Ma et al. 2021).

mediation of the *lptA* gene (Lewis et al. 2009, Tzeng et al. 2005). This effect was also shown *in vivo*, by inoculating mice and men with mixtures of *N. gonorrhoeae* wild-type (WT) and an isogenic mutant lacking the PEA transferase *LptA* (Hobbs et al. 2013). In *Campylobacter jejuni*, mutants in *waaF*, *cstII*, *galT*, or *lgtF* genes abolished the lipooligosaccharide/LPS production, becoming resistant to polymyxin B or HD-5 (Keo et al. 2011, Naito et al. 2010).

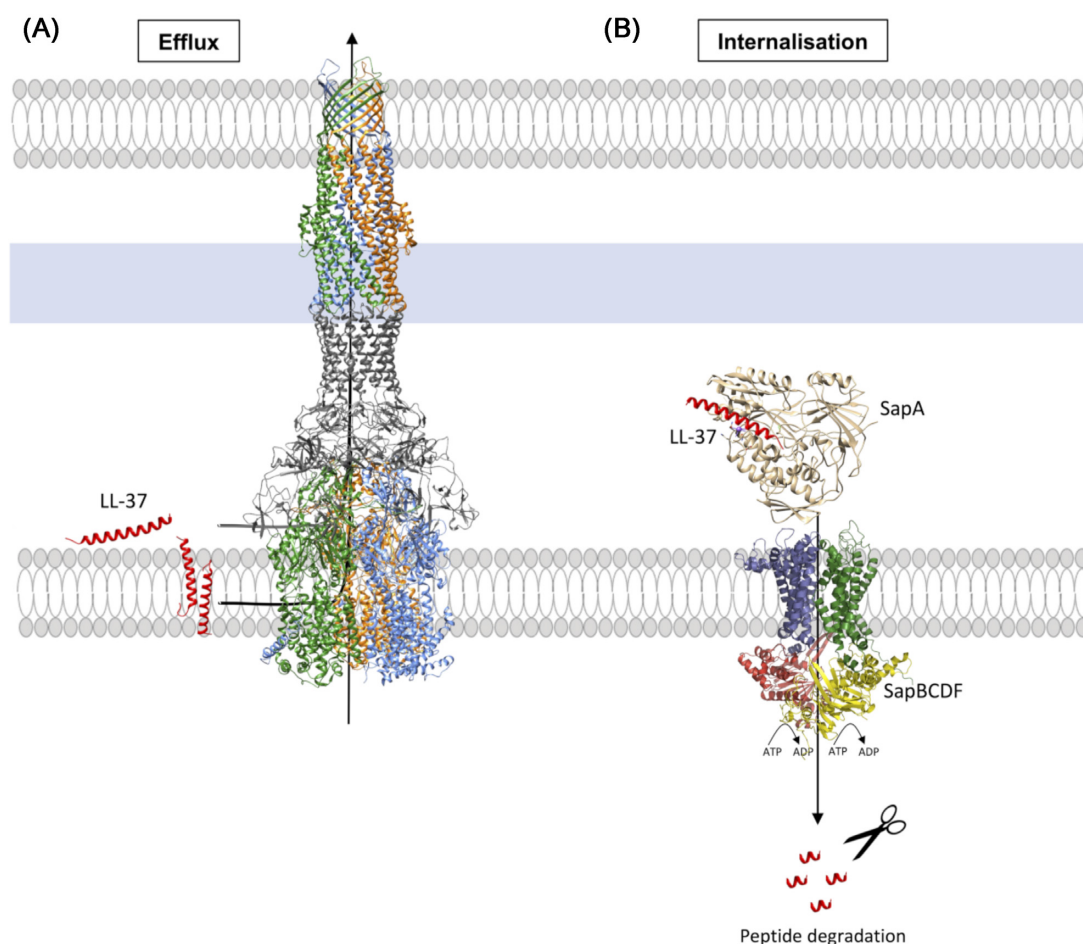
Also, the addition of palmitoyl groups to Lipid A by the acyl-transferase *PagP* increases the resistance to AMPs by reducing the OM permeability. In *S. Typhimurium*, this acylation is regulated by the two-component system *PhoPQ*, decreasing the fluidity and permeability of the OM (Bishop et al. 2000, Dalebroux and Miller 2014, Guo et al. 1998). This strategy promoted the resistance against AMPs such as C18G, protegrin, polymyxin B, LL-37, and magainin II (Guo et al. 1998). In *H. influenzae*, the lipooligosaccharide acylation mediated by the *htrB* gene was shown to be responsible for the resistance against HBD-2 (Stamer et al. 2002). The addition of phosphorylcholine to the oligosaccharide portion of LPS confers resistance against LL-37 in *H. influenzae* (Lysenko et al. 2000). Also, in *K. pneumoniae* the gene *lpxM* increases susceptibility to AMPs since it enhances OM permeability (Clements et al. 2007).

In *P. aeruginosa*, alanylation of PG (which does not change the overall net charge) by the multiple peptide resistance factor *MprF* has been reported as another mechanism to disrupt the ability

of protamine to bind and disrupt the bacterial membrane (Klein et al. 2009). In *Salmonella*, a *pmrAB*-regulated mechanism involving the change of O-antigen length has been linked to AMP resistance. In this way, mutants with long O-antigen chains showed a mild increase in susceptibility to polymyxin B (Farizano et al. 2012).

#### (d) Removal of AMPs mediated by transporters

If the previously explained mechanisms fail and AMPs accumulate on the surface of the bacterial membranes, they can change their conformation after contact with phospholipids and, after reaching a threshold, access the periplasm or cytoplasm via transient pore formation (Melo et al. 2009). In this case, the bacteria still have a last resort mechanism to remove them. This mechanism is conducted by efflux pumps and transporters normally responsible for the introduction of nutrients and the efflux of harmful molecules from the bacterial cytoplasm (Figs. 2 and 3; Alav et al. 2021, Henderson et al. 2021). Efflux pumps are an important mechanism of AMR because they export antimicrobials to keep the bacterial intracellular concentrations below toxic levels (Blair et al. 2014, Colclough et al. 2020). In terms of transport, bacteria have two main strategies for dealing with AMPs: (1) pump them out of the bacterial cell or (2) transport them into the cytoplasm to be degraded by cytoplasmic proteases (as done by the *Sap* transporter; Fig. 3). Both strategies will be discussed here in detail.



**Figure 3.** Transport-related mechanisms of AMP resistance. Efflux pumps such as MtrCDE and AcrAB–TolC can efflux AMPs out of bacterial cells, while the Sap transporter introduces the AMP into the cytoplasm in order to be degraded. (A) The structure of the *E. coli* AcrAB–TolC tripartite pump (PDB 5NG5). The protomers of AcrB and TolC are shown in green, orange, and blue, respectively. AcrA is coloured in dark grey. (B) Sap transporter. The figure contains a model of the *E. coli* Sap transporter (this review) showing SapB in blue, SapC in green, SapD in red, and SapF in yellow. This model has been based on the AlphaFold outputs for individual proteins and the assembly of the tetramer has been guided by the structure of the bacterial alginate ABC transporter (importer) AlgM1M2SS (a heterotetramer of AlgM1, AlgM2, and AlgS; PDB 4XTC), as per (Kaneko et al. 2017). This is a type I ABC transporter according to the latest nomenclature from (Thomas et al. 2020). The figure also contains the recently solved 3D structure of *H. influenzae* SapA (7OFZ). The AMP LL-37 (PDB 5MNM) is shown in ribbon representation. The figure was prepared in UCSF Chimera (Pettersen et al. 2004).

## Efflux as a mechanism of AMP resistance in Gram-negative bacteria

Efflux pumps are molecular machines able to export a wide range of antimicrobials and metabolites (Henderson et al. 2021), and multiple lines of evidence implicate them also in clearance of AMPs (Eswarappa et al. 2008, Feng et al. 2003, Lister et al. 2012, Shafer et al. 1998, Clemens et al. 2017, Honeycutt et al. 2020, Wang et al. 2016). Their capacity to recognize a broad range of structurally different compounds may be explained by their structural flexibility and multiple binding sites (Alav et al. 2021).

There are seven generally recognized classes of efflux pumps:

1. The ATP-binding cassette (ABC) superfamily.
2. The resistance–nodulation–division (RND) superfamily.
3. The major facilitator (MFS) superfamily.
4. The multidrug and toxic-compound extrusion (MATE) family.
5. The drug/metabolite transporter superfamily (DMT) including the small multidrug resistance (SMR) family.
6. The proteobacterial antimicrobial compound efflux (PACE) family.

7. the p-aminobenzoyl-glutamate transporter (AbgT) family.

The two main efflux pump families involved in AMP resistance in Gram-negative bacteria are the ABC-transporter and the RND efflux pump superfamilies (Table 2). Some members of the MFS family have also been proposed to be able to confer the AMP resistance phenotype.

## The ABC superfamily

ABC transporters are ubiquitous to all domains of life, and are considered the most abundant transporters on Earth (Alav et al. 2021, Elbourne et al. 2017). The ABC transporters can transport a broad range of substrates, including metabolites, vitamins, amino acids, lipids, peptides, ions, and drugs (Rees et al. 2009, Thomas and Tampé 2018). Based on their sequence homology and architecture, ABC transporters can be divided into seven types performing different physiological functions (Thomas and Tampé 2020, Thomas et al. 2020).

ABC transporters use ATP hydrolysis as their primary energy source (Lubelski et al. 2007), sharing a common modular architecture including two highly conserved nucleotide-binding domains (NBDs) that contain the conserved Walker A and Walker B mo-

**Table 2.** Transporter systems reported to confer AMP resistance in Gram-negative bacteria to date. PG-1, protegrin-1; PXB, polymyxin B; CO, colistin; cBD-1, chinchilla  $\beta$ -defensin-1; CRAMP-38, mouse CRAMP; PRO, protamine; POLY, polyphemus II; TAC, tachygrin. The  $\alpha$ -defensins include HNP-1, -2, -3, -4, HD-5, the  $\beta$ -defensins HBD-1, -2, -3, -4, and the rabbit defensins NP-1, -2.

Transporter family	Transportersystem	Microorganism	Substrates	No substrates	References		
RND	MtrCDE	<i>N. gonorrhoeae</i>	LL-37, PG-1, PC-8	HNP-2, NP-2	Shafer et al. (1998)		
			LL-37, CRAMP-38		Warner et al. (2008)		
		<i>N. meningitidis</i> <i>Haemophilus ducreyi</i> <i>K. pneumoniae</i> <i>Y. pestis</i> <i>E. coli</i>	CO, PXB	LL-37, PG-1, PXB		Handing et al. (2018)	
				LL-37, HBD-2, -3, -4	HNP-1, -2, HD-5	Chitsaz et al. (2019)	
			PXB, HNP-1, HBD-1, HBD-2		Tzeng et al. (2005)		
			PXB		Rinker et al. (2011)		
					Padilla et al. (2010)		
					Lister et al. (2012)		
					Rieg et al. (2009)		
					Warner and Levy (2010)		
		Weatherspoon-Griffin et al. (2014)					
			Rieg et al. (2009)				
ABC	MexAB-OprM	<i>P. aeruginosa</i>	LL-37, HBD-1, HNP-2*, PXB	LL-37, PXB, HNP-1, -2, -3, HD-5, HBD-2, -3	Masuda et al. (2000)		
			PRO	PXB	Masuda et al. (2000)		
		<i>P. aeruginosa</i> <i>P. aeruginosa</i>	PXB	PXB	Masuda et al. (2000)		
			PXB	PRO	Akiba et al. (2006)		
		<i>C. jejuni</i> <i>Vibrio cholerae</i>	PXB	PXB	Bina et al. (2008)		
			CO	CO	Cheng et al. (2018)		
		<i>Haemophilus ducreyi</i>	LL-37	LL-37	HNP-1, HNP-2, HD-5 & HBD-2, -3 and -4	Mount et al. (2010)	
						Rinker et al. (2012)	
		MFS	yejABEF	<i>S. Typhimurium</i>	LL-37	HD-5, HNP-2, HBD-2	Parra-Lopez et al. (1993)
					PRO, melittin	cecropin P-1, magainin-2, NP-1	Groisman et al. (1992)
NTHI	PRO, melittin				Mason et al. (2005)		
	cBD-1				Mason et al. (2006)		
<i>Erwinia chrysanthemi</i> <i>E. coli</i>	cBD-1, HBD-3, LL-37				Mason et al. (2011)		
	HBD-1, -2, -3, LL-37, HNP-1, melittin				Shelton et al. (2011)		
<i>Actinobacillus pleuropneumoniae</i> <i>Vibrio fischeri</i>	LL-37, HBD-3				López-Solamilla et al. (1998)		
	snakin-1, $\alpha$ -thionin			Plant defensins, PRO	Sugiyama et al. (2016)		
<i>Brucella melitensis</i> <i>Salmonella</i>	PXB			PXB	PG-1, nisin, JB-367, POLY, TAC	McCoy et al. (2001)	
	PXB, melittin, PRO, HBD-1, -2			PR-39	LL-37, PXB, PRO, CP11CN, CP26, CP28, CP29	Xie et al. (2017)	
<i>Salmonella</i> <i>Yersinia enterocolitica</i>	C18G			Lupp et al. (2002)			
	PXB, cecropin P1, melittin			Wang et al. (2016)			
<i>E. coli</i> <i>Acinetobacter baumannii</i>	PRO			Eswarappa et al. (2008)			
	CO			Honeycutt et al. (2020)			
K + transporters (SKT)	TtkG/H-TtkA	<i>Vibrio vulnificus</i>	PRO, PXB		Bengochea and Skumik (2000)		
					Weatherspoon-Griffin et al. (2014)		

\*In Warner and Levy (2010), only the *tolC* mutant was slightly susceptible to HNP-2, while the *acrAB* mutant was not susceptible to this AMP. The AMPs included in Table 2 exhibit one of the three classical mechanisms of membrane disruption (barrel-stave, toroidal, and carpet) or a combination of them. No correlation between mechanism of membrane disruption and ability to be effluxed by the efflux pumps/transporters have been found by the authors of this review.

tifs, and two variable transmembrane domains (TMDs) forming the translocation pathway (Rees et al. 2009, Schneider and Hunke 1998). In bacteria and archaea, the four domains are often distinct subunits, or they are fused into homo- or heterodimerizing half-transporters composed of one NBD and one TMD (Thomas and Tampé 2020). These seven types are present in bacteria acting as importers (types I–III), exporters (types IV and V), extractors (type VI), or operating as tripartite efflux pumps (VII category). Recently the cryo-EM structure of the MlaFEDB complex, involved in phospholipid transport across the bacterial envelope, has revealed distant relationships to other ABC transporters, suggesting that a separate group should be added to the ABC transporters classification (Coudray et al. 2020, Malinverni and Silhavy 2009).

Regarding the ABC importers, the type I and II conserve the overall topology of ABC transporters having 5–6 or 10–12 helices in their TMDs, respectively. TMDs and NBDs dimerize and assemble the minimal unit of the importer. Both type I and type II transporters utilize a substrate binding protein (SBP), located in the periplasm of the Gram-negative bacteria, to recognize and deliver substrates to its cognate ABC transporter located in the IM (Tanaka et al. 2018). Similar to type I and II transporters, type III transporters [also called energy-coupling factor (ECF) transporters] consist of two NBDs (EcfA and EcfA'), a TMD (EcfT), and a substrate-binding component (EcfS). The EcfA–EcfA'–EcfT complex forms the energizing module while the EcfS component is an integral membrane protein that binds substrate with nanomolar affinity (Duurkens et al. 2007, Hebbeln et al. 2007). In the view of the rapid advances in structural biology of the ABC transporters, their classification has been frequently revised, e.g. by Rice et al. (2014), and more recently by Thomas et al. (2020), which we would like to direct the reader to for further details.

The ABC-transporters involved in AMP transport present different structural architectures including tripartite structures in which the IM ABC-transporter associates with an accessory periplasmic protein and an OM protein, as in the MacAB–TolC transporter (Kobayashi et al. 2001). The Sensitive-to-antimicrobial-peptides SapABCDF transporter has been shown to act as the main transporter of AMPs into the cytoplasm in nontypeable *H. influenzae* (NTHI), *Haemophilus ducreyi*, *S. Typhimurium*, *Erwinia chrysanthemi*, *P. mirabilis*, and *Actinobacillus pleuropneumoniae*, while the *E. coli* orthologue has been associated with putrescine transport (Fig. 3B and Table 2; Groisman et al. 1992, López-Solanilla et al. 1998, Mason et al. 2005, 2006, 2011, McCoy et al. 2001, Mount et al. 2010, Parra-Lopez et al. 1993, Rinker et al. 2012, Shelton et al. 2011, Sugiyama et al. 2016, Xie et al. 2017). A subsequent proteolytic degradation step by bacterial cytoplasmic proteases was shown (Handing et al. 2018, Mason et al. 2005, 2006, 2011, Mount et al. 2010, Rinker et al. 2012, Shelton et al. 2011). In addition, the YejABEF transporter in *Salmonella* and *Brucella* and the tripartite pump MacAB–TolC in *Salmonella* appear to confer AMP resistance (Table 2; Eswarappa et al. 2008, Honeycutt et al. 2020, Wang et al. 2016).

## The RND Superfamily

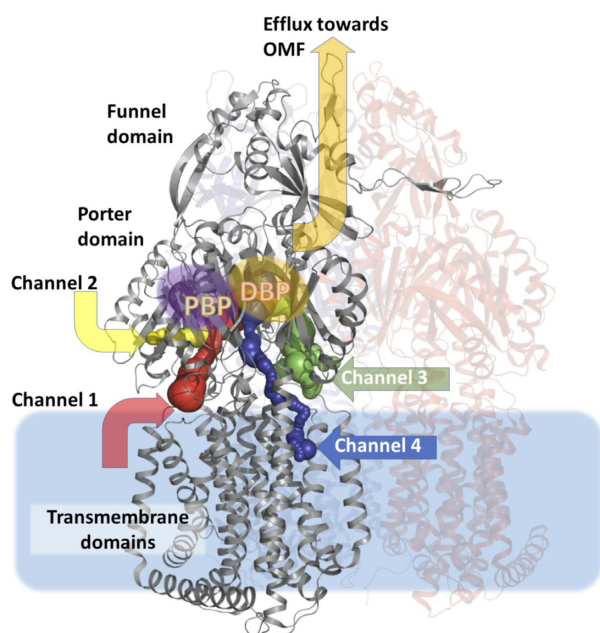
This superfamily has members in all three domains of life and contains the most clinically relevant efflux pumps associated with the MDR phenotype in bacteria (Saier et al. 2006). RND pumps are Proton Motive Force (PMF) driven and depend on the pH gradient over the IM. The export of amphiphilic and hydrophobic substrates is governed by the hydrophobic–amphiphilic efflux (HAE–RND) family, while the efflux of heavy metals relies on the heavy metal efflux (HME–RND) family (Alav et al. 2021, Colclough et al.

2020, Klenotic et al. 2021). The HAE family members are IM proteins composed of ~1000 amino acids and organized into 12 transmembrane helices (TMHs) with two large periplasmic loops between helices 1 and 2 and 7 and 8 (Colclough et al. 2020). In order to export AMPs out of the cell, these IM proteins form a tripartite assembly alongside members of the periplasmic adaptor protein (PAP) family and an OM protein channel belonging to the outer membrane factor (OMF) family, such as TolC (Alav et al. 2021, Colclough et al. 2020, Kobylika et al. 2020). Experimental evidence shows a stoichiometry of 3:6:3, comprising an IM trimer, an accessory protein hexamer and a TolC trimer (Fig. 3; Du et al. 2014, Janganan et al. 2011, Wang et al. 2017). In some Gram-negative bacteria, notably Enterobacteriaceae, the drug exporting RND pumps associate with accessory proteins such as AcrZ in the case of the AcrAB–TolC efflux pump (Hobbs et al. 2012). AcrZ and its homologues seem to induce conformational changes to AcrB that alter drug specificity (Du et al. 2020), and is speculated to play a modulatory role (Henderson et al. 2021). A relay network of transporters has been proposed in which IM proteins belonging to the MFS and SMR families act in the IM by transporting toxic compounds from the cytoplasm, while RND-based tripartite efflux pumps remove these compounds from the periplasmic space out of the cell (Tal and Schuldiner 2009).

Tripartite efflux pumps of the RND superfamily include AcrAB–TolC in *E. coli* (Fig. 3A); *Pseudomonas* Mex systems (MexAB–OprM, MexCD–OprJ, MexEF–OprN, and MexXY–OprM), and *Acinetobacter* Ade systems (AdeABC and AdeIJK) (Alav et al. 2021, Colclough et al. 2020, Klenotic et al. 2021). Among the members of the RND family, the *multiple transferable resistance* (*mtr*) operon (Hagman et al. 1995) coding for the tripartite pump MtrCDE has been shown to be important for AMP resistance in *N. gonorrhoeae*, *N. meningitidis*, and *H. ducreyi* as well as AcrAB–TolC in *Klebsiella* and *Y. pestis* (Table 2; Chitsaz et al. 2019, Handing et al. 2018, Lister et al. 2012, Padilla et al. 2010, Rinker et al. 2011, Shafer et al. 1998, Tzeng et al. 2005, Warner et al. 2008). The role of AcrAB–TolC in *E. coli* is also discussed in this review, since its involvement in AMP resistance has been debated (Rieg et al. 2009, Warner and Levy 2010, Weatherspoon–Griffin et al. 2014). Other RND pumps shown to confer AMP resistance include VexAB–TolC from *Vibrio cholerae*, CmeDEF in *C. jejuni*, and a new RND pump found in *K. pneumoniae* (named H239\_3064), which has been related to colistin resistance (Table 2; Akiba et al. 2006, Bina et al. 2008, Cheng et al. 2018).

MtrCDE is composed of the PAP MtrC that bridges between the OM channel MtrE, and the IM-transporter MtrD (similar to AcrAB–TolC in Fig. 3A). The stoichiometry of the pump has been shown to be MtrD<sub>3</sub>–MtrC<sub>6</sub>–MtrE<sub>3</sub> (Janganan et al. 2011). MtrCDE is directly regulated by the TetR family protein MtrR (Beggs et al. 2019). This efflux pump is particularly important in *N. gonorrhoeae* and *H. influenzae* as these microorganisms only contain a single RND efflux system, which is unusual among the Gram-negative bacteria (Maness and Sparling 1973, Zwama et al. 2019). This importance is further highlighted as the ectopic expression of *mtrR*, can restore the sensitivity of *N. gonorrhoeae* to previously used antibiotics (Chen et al. 2019).

Members of the OMF family of proteins, such as MtrE and TolC, form an elongated homotrimeric channel-tunnel, which is embedded in the OM using a beta-barrel similar to the porin-fold, while a large  $\alpha$ -helical periplasmic domain extends nearly 130 Å long reaching the peptidoglycan layer (Koronakis et al. 2000, Lei et al. 2014). The periplasmic end of the OMF channels is sealed by specific charged interactions, which are thought to be broken upon engagement with the PAP partner (Bavro et al. 2008, Tamburrino et al. 2017). For MtrE, the stabilization of the channel in an



**Figure 4.** General organization of RND transporter trimer, shows oligomeric structure and principal substrate pathways. The distal binding pocket (DBP) and proximal binding pocket (PBP), as well as the entrance to channels 1–4 are indicated based on the *E. coli* AcrB structure [PDB ID: 5JMN; (Oswald et al. 2016)]. The L-protomer of the RND-transporter is shown and annotated, with the neighbouring protomers semitransparent. The blue rectangle delineates approximate limits of the IM.

open state seems to be related to the direct interaction with MtrC, similar to the stable assembled tripartite systems AcrAB–TolC and MexAB–OprM observed by cryo-EM microscopy (Wang et al. 2017; Tsutsumi et al. 2019; Glavier et al. 2020) and cryo-tomography *in situ* (Chen et al. 2021, Shi et al. 2019).

The PAP-component of the pumps—including MtrC, AcrA, and MexA, have a multidomain structure that can be likened to a ‘beads on a string arrangement’, including a long  $\alpha$ -hairpin domain, a lipoyl domain, a  $\beta$ -barrel domain, and a membrane-proximal domain (Akama et al. 2004, Higgins et al. 2004, Mikolosko et al. 2006, Symmons et al. 2009, 2015).

The RND component of the pump, e.g. MtrD, (Bolla et al. 2014, Chitsaz et al. 2019, Fairweather et al. 2021, Lyu et al. 2020, Murakami et al. 2002, Nakashima et al. 2013, Sennhauser et al. 2009), is a trimeric transporter, each protomer of which contains 12 TMHs. MtrD shares 48.9% sequence identity with the homologous *E. coli* AcrB protein (Fig. 4). MtrD exhibits a large periplasmic domain, which is formed from two lobes that are spliced between TM1/TM2 and TM7/TM8, respectively. The resulting periplasmic domain can be divided into six subdomains: four of which form the porter domain (PN1, PN2, PC1, and PC2), while two (FN and FC) form the funnel (formerly OMF-docking) domain of each protomer (Bolla et al. 2014, Fairweather et al. 2021). The porter domain recognizes and transports the pump substrates, which bind within it to the so-called proximal (access) and distal (deep) binding pockets (abbreviated PBP and DBP, respectively), which are separated by the gate G-loop, also known as a switch loop (Eicher et al. 2012, Nakashima et al. 2011, 2013, Tam et al. 2021, Zwama et al. 2018). There are a number of substrate channels that lead to the PBP and DBP, with some of them originating from the membrane leaflet (channels 1 and 4), while others (channels 2 and 3) syphon the

soluble substrates directly from the periplasmic space (Fig. 4; Oswald, et al. 2016, Tam et al. 2020, Zwama et al. 2018).

The availability of the numerous channels with varied specificities, alongside the distinct multidrug-binding pockets helps explain the remarkably wide substrate spectrum of the RND pumps, including MtrD (Bolla et al. 2014, Chitsaz et al. 2019, Eicher et al. 2012, Kobayashi et al. 2014, Lyu et al. 2020, Nakashima et al. 2011, Ramaswamy et al. 2017, Tam et al. 2020, 2021, Zwama et al. 2018). The majority of the substrates are thought to be first vetted in the PBP, and then transferred to the DBP, which is facilitated by the gating G-loop (Eicher et al. 2012, Fairweather et al. 2021, Vargiu and Nikaido 2012), although large molecular weight drugs may be able to bypass the DBP altogether (Tam et al. 2021). The RND pumps, including MtrD utilize a functionally rotating mechanism within the functionally assembled trimer, coupling three different conformational states, known as Access (A)/Loose (L), Bound (B)/Tight (T), and Extrusion (E)/Open (O) conformer respectively based on the capability of the individual protomers to bind the drug, keeping it associated with the protomer and being able to release it into the receiving OMF channel (Murakami et al. 2006, Seeger et al. 2006). These conformer motions are allosterically linked to substrate binding, PAP association and proton acceptance and release to ensure a directionality of cycling and effective substrate expulsion (Alav et al. 2021).

## The MFS superfamily

This superfamily has members in all domains of life, working as uniporters, symporters, or antiporters (Huang et al. 1999). These proteins are generally highly hydrophobic and are predicted to be formed by 12  $\alpha$ -helices and short loops (Foster et al. 1983). The minimum functional structural unit seems to be a monomer, but the existence of homo-oligomers has also been proposed (Yin et al. 2006). Some of them interact with other periplasmic and OM proteins, as in the case of the EmrAB–TolC system. Members of this superfamily are powered by the PMF, although alternative coupling energy has also been proposed (Krulwich et al. 2005). The MFS efflux pump EmrAB–TolC from *E. coli* and the RosAB pump from *Yersinia enterocolitica* are involved in conferring the AMP resistance phenotype (Table 2; Bengoechea and Skurnik 2000, Weatherspoon-Griffin et al. 2014).

## K<sup>+</sup> transporters (SKT)

One last system related to AMP resistance is the TrkG/H–TrkA potassium uptake system, widely found in bacteria and archaea, with the uptake of K<sup>+</sup> linked to H<sup>+</sup> symport (Durell et al. 1999). The homodimer TrkH, belonging to the superfamily of K<sup>+</sup> transporters (SKTs), is a transmembrane protein responsible for the transport of K<sup>+</sup> across the cell membrane (Bakker 1993, Cao et al. 2011). They coassemble with TrkA, a cytosolic partner protein containing NAD binding sites (Cao et al. 2013). The structures of the TrkH–TrkA complex in the presence of ADP or ATP have been recently reported (Zhang et al. 2020). In *E. coli*, the activity of the system is dependent on the association with the ATP-binding protein TrkE (also known as SapD), which also forms a part of the SapABCDF ABC transporter (Sugiyama et al. 2016). A *trkA* mutant has been shown to confer resistance against polymyxin B and protamine in *Vibrio vulnificus* (Table 2; Chen et al. 2004).

In summary, two main mechanisms of efflux are used by Gram-negative bacteria to confer resistance to AMPs: (1) the efflux of AMPs to the extracellular space, as exemplified by MtrCDE or AcrAB–TolC and (2) the internalization of AMPs to the cytoplasm

with subsequent proteolytic degradation, exemplified by the action of the Sap-transporter system (Fig. 3).

In the next sections, we will discuss these systems in more detail and delve into the evidence linking them to the AMP-resistant phenotype in Gram-negative bacteria.

## The MtrCDE efflux pump confers AMP resistance

MtrCDE is a tripartite efflux pump in *N. gonorrhoeae*, which confers broad-spectrum resistance to structurally diverse antimicrobial molecules such as AMPs, nonionic detergents, fatty acids, bile salts, macrolides,  $\beta$ -lactams, tetracycline, and the extended-spectrum cephalosporin ceftriaxone, which is the last option for gonorrhoea therapy (Golparian et al. 2014, Hagman et al. 1995, Shafer et al. 1998).

There is mounting evidence that the MtrCDE pump is capable of recognition and effluxing of AMPs, utilizing similar mechanisms to the ones described above. Indeed, in *N. gonorrhoeae*, the isogenic transformant strains bearing insertional inactivated *mtrC*, or *mtrE* genes, and a 10-bp deletion at the 3'-end of the *mtrD* gene resulting in a MtrD truncation (strain BR54) were significantly more susceptible to PG-1 than the parental strain FA19 (Shafer et al. 1998). In addition, an inactivated *mtrD* affecting *mtrE* expression as well (*mtrD/mtrE* deficient strain) was more susceptible to the structurally diverse peptides PG-1, PC-8, and LL-37.

In the same work, the importance of the electrostatic interactions as a first step in the AMP-membrane interaction mechanism was shown, since both the FA19 and *mtrD/mtrE* deficient strain were more sensitive to PG-1 and LL-37 under low salt conditions, showing the *mtrD/mtrE* deficient strain higher susceptibility (Shafer et al. 1998). At low salt concentration, the *mtrD/mtrE* deficient strain was also susceptible to PC-8, a linearized PG-1 synthetic variant lacking both disulfide bonds, and this AMP was almost inactive against the FA19 strain. This correlates with the hypothesis that the intramolecular disulfide bonds are crucial for PG-1 activity (Qu et al. 1997). Unexpectedly, the *mtrD/mtrE* inactivated strain showed resistance to the human HNP-2 and rabbit NP-2  $\alpha$ -defensins at any salt concentration (Table 2; Shafer et al. 1998). These results suggest that the MtrCDE system in general, and the MtrD transporter in particular, is not involved in the efflux of defensins, but confers resistance to LL-37, although both are found in the genitourinary mucosae where they are involved in host defence mechanisms (Feng et al. 2003). The MtrCDE role was confirmed by testing deficient efflux pump mutants in a female mouse model of genital tract infection (Jerse et al. 2003).

In Warner et al. (2008), the *N. gonorrhoeae* *mtrR* mutants were consistently more resistant to LL-37 and its murine homologue CRAMP-38 than the WT strain (Table 2). Also a *mtrE:cat* mutant designed by using a natural *mtrR* mutant as the parental strain (MS11) showed a higher susceptibility to these AMPs. The authors confirmed the MtrCDE role in AMP efflux since mutations in *mtrCDE* reduced gonococcal survival in the female murine genital tract, and mutations causing derepression of the *mtrCDE* operon enhanced gonococcal survival (Warner et al. 2008).

In a work using different antimicrobial proteins and peptides released by neutrophils, the MtrCDE specific defence mechanism was shown to be location- and component-specific depending on the molecule to be exported (Handing et al. 2018). LL-37 was proved to be MtrCDE-dependent, since the *mtrD* and *mtrE* mutants were significantly more sensitive to killing than the parental strain (Table 2). Conversely, the glycopeptide vancomycin showed

an MtrD-dependent but MtrE-independent sensitivity since the *mtrC* and *mtrD* mutants had a significantly lower MIC compared with the parent or *mtrE* mutant strains (Handing et al. 2018).

A third phenotype was shown in these experiments when using bigger cationic proteins as molecules to be extruded by efflux pumps. The 55 kDa bactericidal permeability-increasing protein (Elsbach 1998) was shown to be MtrE-dependent but MtrD-independent, since the *mtrE*, but not the *mtrD* mutant, was more sensitive to killing. The authors concluded that the Mtr system contributes to gonococcal survival after neutrophil challenge.

Transport of the cyclic peptides also appears to be MtrD-dependent, as the deletion of *mtrD* from *N. gonorrhoeae* FA19 resulted in a 2-fold reduction of the MICs for polymyxin B and colistin. The reduction in MIC for colistin was 4-fold in a KH15 background, maybe due to the higher level of *mtrCDE* expression in this strain due to the upregulation of the system by a single-base-pair deletion in the *mtrR* promoter (Table 2; Chitsaz et al. 2019), indicating that colistin B is an actively effluxed substrate of the pump. The MIC values were restored in the KH15  $\Delta mtrD \Delta norM$  derivative by reinsertion of *mtrD*. Mutagenesis studies in two aromatic residues located in the deep drug-binding pocket of MtrD (F176A and F623A) and in its switch loop (F612A) highlighted the importance of these residues for the binding to polymyxin B and other substrates (Chitsaz et al. 2019). The amino acids R714 and K823, engaged in the entrance and proximal substrate binding site within the periplasmic domain of MtrD, were also shown to be critical for polymyxin B resistance when MtrCDE is overexpressed in a KH15 background (Lyu et al. 2020).

By using a library of mariner transposon mutants generated in an *N. meningitidis* strain, mutations within *mtrD* or *lptA* genes and the *pilMNOPQ* operon showed increased susceptibility to the cyclic polymyxin B, the  $\alpha$ -helical LL-37 and the  $\beta$ -sheet protegrin-1 (Table 2; Tzeng et al. 2005). Thus, the AMP resistance in *N. meningitidis* was shown to involve multiple mechanisms including the MtrCDE efflux pump, lipid A modification as well as the type IV pili secretion system and the major OM porin PorB (Tzeng et al. 2005). The heteroresistance to polymyxin B, colistin, and LL-37 has also been recently observed in clinical isolates of *N. meningitidis* urethritis (Tzeng et al. 2019).

In 2007, *H. ducreyi* was shown to be naturally more resistant than *E. coli* to killing by LL-37, the  $\alpha$ -defensins (HPN-1, HNP-2, HNP-3, and HD-5) and  $\beta$ -defensins (HBD-2, -3, and -4), AMPs that *H. ducreyi* can encounter during infection, being still susceptible to be killed by the nonrelated PG-1 (Mount et al. 2007). Some years later, an orthologue of the MtrCDE efflux pump was identified in *H. ducreyi*, showing high similarity with the MtrD (31% identical and 37% similar) and MtrC (29% identical and 44% similar) proteins in *N. gonorrhoeae* (Rinker et al. 2011). In *H. ducreyi*, the deletion of the PAP *mtrC* rendered the bacteria more sensitive to LL-37 and  $\beta$ -defensins (especially against HBD-3), but not to the  $\alpha$ -defensins (Table 2; Rinker et al. 2011). The *mtrC* deletion also affected the OM protein profile, colony morphology, and activated the two-component system CpxRA. Despite the action of CpxRA, the authors showed that MtrCDE contributed to LL-37 and HBD-3 resistance in a CpxRA-independent way, with the MtrCDE transporter being the major determinant of resistance to HBD-3 in *H. ducreyi* (Rinker et al. 2011).

In any case, the mechanism of LL-37 resistance in *H. ducreyi* was shown to be multifactorial, since MtrCDE, the Sap transporter, and the Cpx regulon contribute to the resistant phenotype (Mount et al. 2010, Rinker et al. 2011). In spite of the *H. ducreyi* MtrCDE apparent ability to efflux  $\beta$ - but not  $\alpha$ -defensins, it was suggested that the MtrCDE pump could also efflux  $\alpha$ -defensins if increas-

ing the peptide concentration (Rinker et al. 2011). Also, the existence of another main mechanism conferring resistance against  $\alpha$ -defensins and masking the MtrCDE activity could not be ruled out. Some years later, cell envelope modification through the addition of PEA to the lipid A and the core oligosaccharide of LPS, was shown to be the main mechanism responsible for the  $\alpha$ -defensin resistance (HD-5), also affecting  $\beta$ -defensin resistance (HBD-3), but not resistance to LL-37 (Trombley et al. 2015).

## The AcrAB–TolC system

Similar to the aforementioned MtrCDE system, the AcrAB–TolC efflux pump has a wide range of substrates including dyes, detergents, different classes of antibiotics, and solvents (Figs 3A and 4; Anes et al. 2015, Kobyłka et al. 2020). AcrAB–TolC has been shown to be involved in conferring AMP resistance in *K. pneumoniae* and *Y. pestis* although this is contentious in *E. coli*.

In *K. pneumoniae*, the *acrB* knockout mutant was significantly more susceptible to polymyxin B,  $\alpha$ -defensin HNP-1, and  $\beta$ -defensins HBD-1 and HBD-2 (Table 2; Padilla et al. 2010). The authors confirmed that this susceptibility was not due to a reduced expression of the capsular polysaccharide or LPS, since the WT as well as the *acrB* knockout mutant expressed similar amounts of both polysaccharides (Padilla et al. 2010). Finally, the authors tested the ability of the *acrB* knockout to cause pneumonia in a mouse model, showing that lungs from mice infected with the *acrB* knockout presented significant lower bacterial loads than those infected with the WT strain (Padilla et al. 2010).

In *Y. pestis*, the  $\Delta$ *acrAB* and  $\Delta$ *tolC* mutants were more sensitive to polymyxin B than the WT, suggesting that polymyxin B is a substrate for AcrAB–TolC (Table 2). As the *tolC* deletion dramatically increased the susceptibility to polymyxin B, it was proposed that other pumps could also efflux polymyxin B by using TolC as the exit duct (Lister et al. 2012).

In *E. coli*, the involvement of AcrAB–TolC in the AMP resistance phenotype is controversial. In 2009, using *E. coli* strains with *acrAB* overexpressed or inactivated, it was shown that LL-37, polymyxin B,  $\alpha$ -defensins HNP-1–3 and HD-5, and  $\beta$ -defensin hBD-2 were not substrates of AcrAB–TolC (Table 2; Rieg et al. 2009). However, in 2010, Warner and Levy suggested that LL-37, polymyxin B, and the  $\beta$ -defensin HBD-1 were substrates of AcrAB–TolC since the *acrAB* deficient mutant showed susceptibility to these AMPs (Table 2; Warner and Levy 2010). As this susceptibility was even greater in the *tolC* deficient mutant, they proposed the existence of other efflux pumps using TolC as a mediator of the AMP efflux [as seen later for *Y. pestis* (Lister et al. 2012)]. The susceptibility to the defensins was increased in a *tolC* mutant, while an *acrAB* deletion mutant only showed an increase in susceptibility to  $\beta$ -defensin (HBD-1) but not to the  $\alpha$ -defensin HNP-2.

When using the *acrAB* and *tolC* mutants in the presence of the PMF inhibitor carbonyl cyanide-*m*-chlorophenylhydrazone (CCCP), the levels of LL-37 susceptibility increased in the *acrAB* mutant. Surprisingly, the strain containing the *tolC* deletion caused a 70-fold decrease in susceptibility compared to the non-CCCP-treated control. The authors proposed two hypotheses to explain this fact: (1) the presence of another AMP uptake system, which is normally masked by other TolC efflux pumps, or (2) the loss of PMF could alter the OM charge such that the AMPs were less attracted to the membrane (Warner and Levy 2010).

The differences obtained by Rieg et al. (2009) and Warner and Levy (2010) were shown to be related to the use of different microbiological media for the experiments because the different ion concentrations of the media could affect the AMP stability and

ability to bind negatively charged surfaces (D'Amato et al. 1975, Dorschner et al. 2006). This hypothesis was confirmed by Warner and Levy (2010) when performing parallel experiments with both media (MH broth and LB broth) comparing the AMP susceptibility for the WT, *acrAB*, and *tolC* mutants.

Since the deletion of *tolC* resulted in an increase in the levels of the transcriptional regulators MarA, SoxS, and Rob, and porins are among the many genes regulated by these transcriptional factors, Warner et al. proposed that the loss of AcrAB–TolC induced changes in membrane permeability beyond simply loss of efflux, which may also affect susceptibility (Saw et al. 2016, Warner and Levy 2010).

In 2014, it was shown that the deletion of *acrB*, as well as *emrB* in *E. coli*, rendered the bacteria more susceptible to protamine when compared with the isogenic WT strain, with the *tolC* mutant showing a more extreme phenotype (Table 2; Weatherspoon-Griffin et al. 2014). This observation suggested that an additional TolC-dependent efflux pump(s) contributes to the protamine resistance. In this work, the  $\Delta$ *acrD*,  $\Delta$ *acrE*,  $\Delta$ *acrF*,  $\Delta$ *mdtE*,  $\Delta$ *mdtF*,  $\Delta$ *macA*,  $\Delta$ *macB*,  $\Delta$ *emrK*, and  $\Delta$ *emrY* single mutants showed the same survival rate than the WT strain when exposed to protamine. Thus, AcrAB–TolC and EmrAB–TolC should contribute to TolC-dependent protamine resistance (Weatherspoon-Griffin et al. 2014). An interesting CpxR-dependent regulation was proposed for AMP resistance in *E. coli*, in which the two component system CpxR/CpxA activates (1) the transcription of the *mar* operon, which induces the expression of the tripartite multidrug efflux transporters, and (2) the transcription of the *aroK* gene, enhancing the production of metabolites able to release the repressor MarR from the *marO* site increasing *marA* expression and synthesis of efflux pumps (Weatherspoon-Griffin et al. 2014).

## Other RND-efflux pumps implicated in the AMP-resistance phenotype

In *C. jejuni* NCTC 11168, a single mutant affecting *cmeE*, the IM component of the CmeDEF efflux pump showed a 2-fold increase in the susceptibility to polymyxin B, but not to protamine. The same result was obtained for the 21190 strain isolated from chicken, and for the *cmeF/cmeB* double mutants, which also lack the IM component of the main efflux pump CmeABC (Akiba et al. 2006).

In VexAB–TolC, a RND-efflux pump in *V. cholerae*, the  $\Delta$ *vexB* strain, as well as the  $\Delta$ *tolC*, were shown to decrease the MIC for polymyxin B (Table 2; Bina et al. 2008).

Recently, a new locus affecting the resistance to colistin has been identified in *K. pneumoniae*. The locus, called H239\_3064 was predicted to be an RND-type efflux pump by homology, exhibiting a 49% amino acid identity with AcrB in *K. pneumoniae*, with unknown periplasmic adaptor and OM proteins. Deletion of H239\_3064 resulted in an 8-fold decrease in the MIC of colistin. However, it is not clear if this pump directly performs the efflux of colistin or the efflux of substrates that affect the bacterial surface charge (Table 2; Cheng et al. 2018).

In spite of the high similarity between MexA and AcrA, or MexB and AcrB (71%, and 89%, respectively), the *P. aeruginosa* MexAB–OprM pump seems to be only linked to the AMP resistance phenotype when biofilms are formed (Table 2). The authors suggested that colistin could have an intracellular target in addition to its membrane interfering activity (Pamp et al. 2008). However, it seems that Rieg's experiments (done in MH media) were not repeated using LB media, raising the possibility that the me-

dia could also selectively affect AMP resistance conferred by *P. aeruginosa* MexAB–OprM (Rieg et al. 2009, Warner and Levy 2010). Also other efflux pumps in *P. aeruginosa* such as MexCD–OprJ and MexXY–OprM were not able to extrude polymyxin B (Masuda et al. 2000).

## Sap system: importing and degrading AMPs as mechanism of resistance

In addition to the strategy of pumping AMPs out of the bacterial cell, another approach that some bacteria use to become AMP resistant is to transport the AMPs into the cytoplasm, where they are degraded. In Gram-negative bacteria, this transport is carried out by the ABC family transporter encoded by the *sapABCDF* operon (sensitive-to-antimicrobial-peptides; Groisman et al. 1992, López-Solanilla et al. 1998, Mason et al. 2005, 2006, 2011, McCoy et al. 2001, Parra-Lopez et al. 1993, 1994, Shelton et al. 2011). The multimeric system is composed of SapA acting as a periplasmic solute-binding protein, SapB and SapC as transmembrane proteins forming a pore in the IM, SapD, and SapF as ATPase subunits and SapZ as an integral membrane protein presumably associated with SapC (Parra-Lopez et al. 1993). SapA directly binds the AMP and shuttles it from the periplasm to the SapBCDF for transport into the bacterial cytoplasm, where the AMPs are further degraded and its amino acids are recycled (Mount et al. 2010, Parra-Lopez et al. 1993). This mechanism was confirmed in the NTHI by using HBD-3 and LL-37, showing that kinetics of uptake and cytoplasmic proteolytic degradation seem to be dependent on AMP structure and charge (Table 2; Shelton et al. 2011). In addition, the accumulation of AMPs in the periplasm of *sapBC* permease-deficient cells supported the mechanism whose main goal seems to be to avoid the direct interaction between the AMPs and the cytoplasmic membrane, which could be lethal for the bacteria (Shelton et al. 2011).

Another proof of AMP-SapA binding is related to the ability of the sap system to uptake iron-containing nutrients (e.g. heme) required for NTHI growth and survival. The heme–SapA binding was shown to be displaced by HBD-1, HBD-2, HBD-3, LL-37, HNP-1, and melittin (Table 2; Mason et al. 2011). The ability to bind and displace the heme group was proportional to the relative charge of the AMP, with HBD-3 being the most efficient (+11 net charge). This AMP ability to displace the heme group in SapA shows a hierarchy, where immune evasion supersedes the need for the iron acquisition function by the Sap system (Mason et al. 2011).

Recently, the *H. influenzae* SapA protein structure has been solved in an open (no ligand) and closed conformation (Figs 3B and 5; Lukacik et al. 2021). These structures show a cavity volume that could accommodate a small ligand such as a short peptide or an extended polypeptide chain that could protrude out of the narrow openings of the SapA ligand-binding cavity, but with no space to accommodate AMPs in their folded state (Lukacik et al. 2021). In addition, the cavity is formed mainly by hydrophobic or neutral residues, showing a lack of countercharges to accommodate the AMPs. Moreover, the authors did not find any crystallographic or biophysical evidence of the highly purified SapA protein being able to bind hBD1, hBD2, hBD3, or LL-37 (Lukacik et al. 2021).

In *H. ducreyi*, the multimeric Sap transporter was shown to be responsible for LL-37 resistance but the *sapA* nonpolar mutant did not confer any resistance towards  $\alpha$ - and  $\beta$ -defensins (Fig. 5B; Mount et al. 2010). A nonpolar *sapBC* mutant lacking both IM per-

meases of the Sap transporter, exhibited greater sensitivity than the *sapA* mutant to killing by LL-37, but it did not affect the resistance of *H. ducreyi* to human defensins (Table 2; Rinker et al. 2012). The lack of sensitivity against defensins exhibited by the Sap transporter was also confirmed by the inactivation of the *sapA* locus in *E. chrysanthemi* (renamed to *Dickeya dadantii* in 2005), an important phytopathogenic bacterium (Table 2; López-Solanilla et al. 1998). In addition, the SapBC channel in *H. ducreyi* retained activity when *sapA* is removed, suggesting that the specificity of the Sap system does not rely exclusively on the interaction with the periplasmic solute-binding SapA, and raising the idea that other unknown SapA-independent mechanisms can exist in *H. ducreyi* (Rinker et al. 2012). This mechanism could be the interaction with other periplasmic solute-binding components different to SapA or even the efflux of AMPs by MtrCDE (Letoffe et al. 2006).

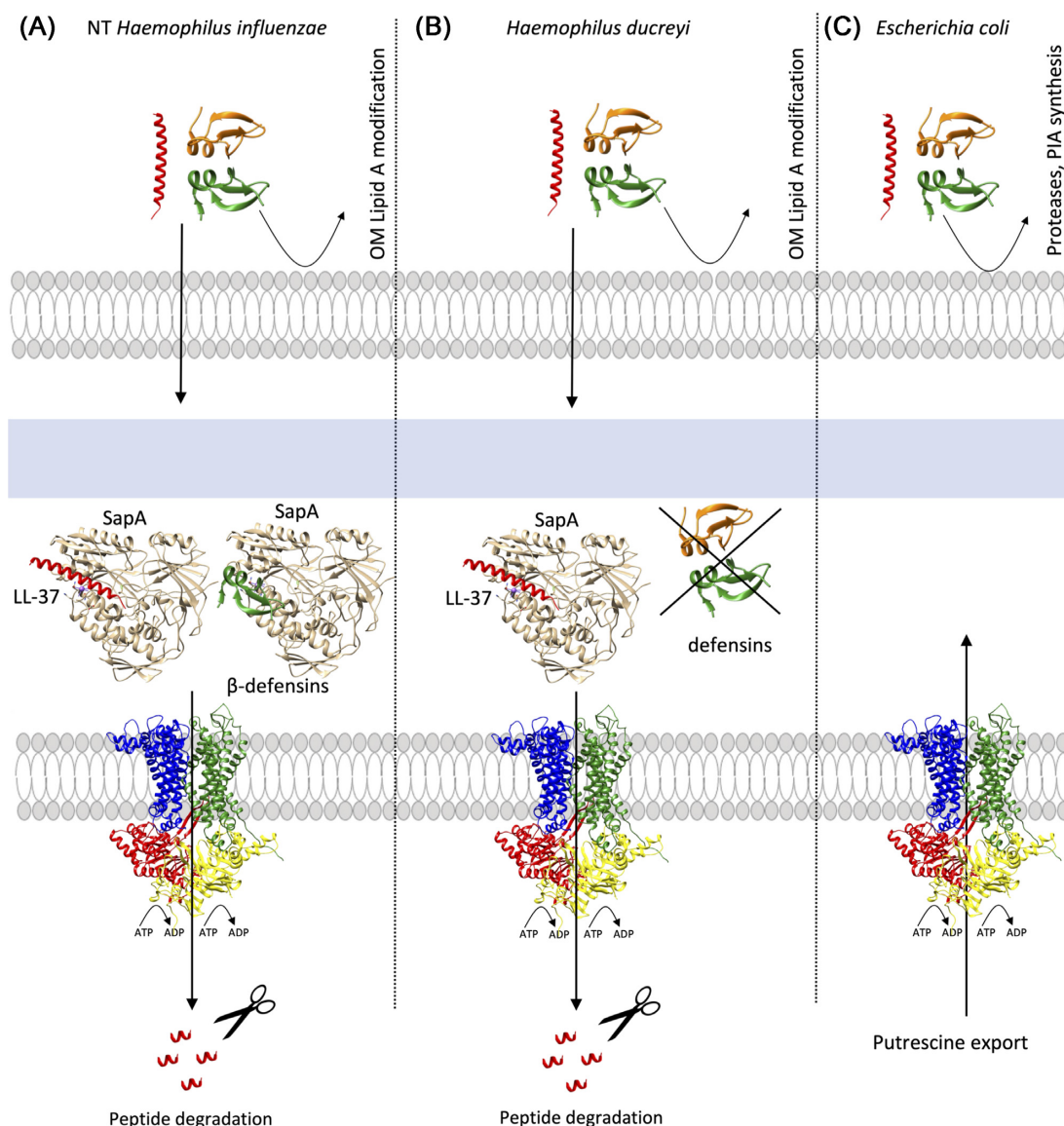
As seen, the *H. ducreyi* Sap transporter cannot confer the resistant phenotype when attacked by defensins. In contrast, the *sapA* nonpolar mutant in the NTHI Sap transporter was approximately 8-fold more sensitive than the parent strain to killing by recombinant chinchilla  $\beta$ -defensin-1 (cBD-1), an orthologue of human  $\beta$ -defensin-3 (HBD-3; Fig. 5A and Table 2; Mason et al. 2005). In addition, it showed a significantly attenuated ability to survive in a chinchilla model of otitis media compared with the parent strain (Mason et al. 2005). Also the *H. influenzae sapD* mutant was sensitive to killing by cDB-1, HBD-3, and LL-37 (Mason et al. 2006). In addition, HBD-2, HBD-3, LL-37, hNP-1, and melittin were shown to be able to bind and displace the heme group bound to SapA. They were also shown to be susceptible to degradation by cytoplasmic proteolysis (Table 2; Mason et al. 2011, Shelton et al. 2011).

In *H. ducreyi*, the modification of Lipid A in the OM confers resistance by means of electrostatic repulsion. Specifically, the phosphoethanolamine (PEA) transferase genes confer resistance to the  $\alpha$ -defensin HD-5 and the  $\beta$ -defensin HBD-3, but not to cathelicidins, such as LL-37 (Trombley et al. 2015). The resistance to LL-37 would come from the MtrCDE efflux pump and Sap transporter activity, and these two mechanisms could mask others such as the PEA modification (Trombley et al. 2015). In the case of NTHI, low concentrations of AMPs (including LL-37) could be counteracted by the modification of the OM (Lysenko et al. 2000), but increasing concentrations would increase the production of the Sap transporter, with the corresponding binding of SapA to the AMP and cytoplasmic membrane transport for the proteolytic degradation in the cytoplasm (Shelton et al. 2011).

Although the main mechanism of resistance to AMP attack in *P. mirabilis* is LPS modification, the *sapD* mutant showed that the Sap transporter is also involved in conferring resistance to polymyxin B, but not to the  $\beta$ -sheet protegrin, its analogue IB-367, nisin, tachygrin A, and polyphemusin (Table 2; McCoy et al. 2001). In *A. pleuropneumoniae*, a Gram-negative bacterial pathogen responsible for porcine pleuropneumonia, the  $\Delta sapA$  mutant showed increased sensitivity to PR-39, a linear porcine AMP with a high proline content (Table 2; Xie et al. 2017). In *Vibrio fischeri*, a marine Gram-negative bacteria, the polar mutation within the *sapABCDF* operon does not confer resistance to LL-37, polymyxin, protamine, the indolicidin derivative CP11CN, and the hybrid cecropin/melittin CP26, CP28, and CP29 peptides, but seems to be required for normal growth (Table 2; Chen et al. 2000, Lupp et al. 2002).

Groisman et al. (1992) identified eight distinct protamine resistance loci in a collection of *S. Typhimurium* mutants. The *sapC* and *sapD* mutants were also shown to confer resistance to melittin, and the crude granulocyte extract (Table 2; Groisman et al. 1992, Parra-Lopez et al. 1993). Also, the *sapG* gene was shown to





**Figure 5.** Representation of the Sap transporters in different microorganisms showing that behaviour against AMPs in the Sap transporters is microorganism-dependent. (A) The sap transporter in NTHI can introduce AMPs to the cytoplasm to be degraded. (B) The Sap transporter in *H. ducreyi* can introduce LL-37 into the cytoplasm but not defensins. In this microorganism, the PEA transferase genes confer resistance to the  $\alpha$ -defensin HD-5 and the  $\beta$ -defensin HBD-3 but not to cathelicidins, such as LL-37. (C) The Sap transporter in *E. coli* is not related to AMP transport. LL-37 and defensins are represented as red helices and green/orange ribbons, respectively. The figure contains a model of the *E. coli* Sap transporter (this review) showing SapB in blue, SapC in green, SapD in red, and SapF in yellow (Fig. 3 for details).

be a NAD<sup>+</sup> binding protein 99% identical to the *E. coli* low-affinity potassium uptake component TrkA, showing the *sapG* mutants a hypersensitivity to protamine (Table 2; Groisman et al. 1992, Parra-Lopez et al. 1994). In this paper, the authors proposed that SapG, SapJ, and the SapABCD transporter function as a complex to mediate both peptide and K<sup>+</sup> transport (Parra-Lopez et al. 1994). In this complex, SapABCD would be the peptide pore while SapJ (also known as TrkH) would form the K<sup>+</sup> pore, although an alternative model in which both SapJ and SapABCD form a single pore for K<sup>+</sup> and peptide transport could not be ruled out. In this model, SapG would act to coordinate the peptide and K<sup>+</sup> transport functions of the complex (Parra-Lopez et al. 1994), as shown by the *E. coli* TrkH protein which is dependent on the ATP-binding protein SapD (also known as TrkE), which is part of the SapABCD ABC transporter, although not all the Trk systems are dependent on

SapD and can utilize AP-binding proteins from other ABC transporters (Nakamura et al. 1998).

In *Vibrio*, the potassium uptake system consists of two proteins: the integral membrane K<sup>+</sup>-translocating protein TrkH (or TrkG), and the NAD-binding peripheral membrane protein TrkA (Bakker 1993, Cao et al. 2011). The *trkA* inactivated mutant showed a higher sensitivity to protamine and polymyxin B compared with the WT strain (Table 2; Chen et al. 2004).

An important exception occurs in *E. coli*, whose Sap transporter does not seem to be involved in conferring the AMP resistant phenotype since the *sapBCDF* knockout strain did not confer resistance to LL-37 (Fig. 5C and Table 2; Sugiyama et al. 2016). This fact is striking since it was reported that the  $\Delta$ *sapABCD* strain in *S. Typhimurium* was more sensitive to protamine than the parental strain (Parra-Lopez et al. 1993). While the amino acid identity of SapABCD in *Salmonella* and *E. coli* is very high (between 90% and

98% for all the genes in the operon), its gene organization is different since *sapABCDF* in *Salmonella* are expressed polycistronically, but in *E. coli* the promoter of *sapA* is located independently of that of *sapBCDF* (Sugiyama et al. 2016). In addition, the predicted sigma factor is different for *sapA* and *sapBCDF* ( $\sigma 70$  and  $\sigma 54$ , respectively; Sugiyama et al. 2016). In this work, the authors succeed in assigning a function related to putrescine export to the *E. coli* Sap transporter (Sugiyama et al. 2016). In *E. coli*, other resistant mechanisms, such as the synthesis of PIA or the activity of the omptin family of aspartate proteases seem to be responsible for AMP resistance (Stumpe et al. 1998, Thomassin et al. 2012, Wang et al. 2004).

### Other ABC-transporters implicated in the AMP-resistance phenotype

The ABC transporter YejABEF is composed of four genes: *yejA*, which encodes a putative periplasmic binding protein; *yejB* and *yejE*, which encode putative permease components; and *yejF*, which encodes the ATPase component of this transporter (Eswarappa et al. 2008). Partial and complete deletion of the operon in *Brucella melitensis* showed a significantly increased sensitivity to acidic stress.  $\Delta yejE$  and  $\Delta yejABEF$  mutants were also more sensitive than the WT to polymyxin B (Table 2; Zhen Wang et al. 2016). Moreover, cell and mouse infection assays indicated that both deletions have restricted invasion and replication abilities inside macrophages. In *Salmonella*, the *yejF* knockout showed increased sensitivity to protamine, melittin, polymyxin B, HBD-1, and HBD-2, and was compromised in its capacity to proliferate inside activated macrophages and epithelial cells (Table 2; Eswarappa et al. 2008).

In the tripartite ABC transporter MacAB–TolC, MacB hydrolyses cytoplasmic ATP and the molecules are translocated through MacA and TolC from the periplasm to the extracellular space (Crow et al. 2017, Fitzpatrick et al. 2017, Greene et al. 2018). Recently, a role in AMP resistance has been proposed for MacAB–TolC in *Salmonella* as constitutive expression of *macAB* conferred resistance against C18G, a synthetic  $\alpha$ -helical peptide derived from human platelet factor IV (Table 2; Honeycutt et al. 2020).

### MFS-efflux pumps implicated in the AMP-resistance phenotype

In *Y. enterocolitica*, *rosAB* encodes a temperature-regulated MFS efflux pump, i.e. coupled to a potassium antiporter (Table 2; Bengoechea and Skurnik 2000). This efflux pump has been shown to

confer resistance against polymyxin B, cecropin P1 and melittin (Bengoechea and Skurnik 2000). The mechanism seems to involve the efflux of AMPs by the IM protein RosA after the AMPs enter the cytoplasm, using the energy provided by RosB. Once the AMPs reach the periplasmic space, an OM protein such as TolC would be needed to transport the substrates to the extracellular environment (Bengoechea and Skurnik 2000).

The tripartite *E. coli* EmrAB–TolC efflux pump, belonging to the MFS superfamily was shown to confer protamine resistance. The percentage of survival in the presence of protamine for the  $\Delta emrB$  and  $\Delta tolC$  mutants was 20% and 0%, respectively, suggesting that those deletions rendered bacteria more susceptible to protamine (Table 2; Weatherspoon-Griffin et al. 2014). Also in *Acinetobacter baumannii* the *emrB* knockout mutant was shown to be more susceptible to colistin than the WT strain by using time kill assays and minimal inhibitory concentration determination (Lin et al. 2017).

### Open questions

As shown in this review, Gram-negative bacteria have developed two main mechanisms to get rid of the AMPs using transport, including the efflux of AMPs to the extracellular space by tripartite efflux pumps including MtrCDE or AcrAB–TolC, and the internalization of AMPs to the cytoplasm and posterior proteolytic digestion, by the SapABCDF transporter.

In this section, we will expose some questions that arose while writing this review.

### How do efflux pumps recognize and process a broad variety of substrate peptides unrelated in structure and sequence?

The most intriguing question that comes from this review is how the efflux pumps can recognize a broad structural substrate range (e.g. the *N. gonorrhoeae* MtrCDE can efflux AMPs as different as the  $\alpha$ -helical LL-37, the  $\beta$ -sheet PG-1, and the cyclic PXB). A potential explanation would be that IM proteins, such as MtrD or the *Staphylococcus aureus* phenol-soluble modulins (Pmt) transporter are supposed to be able to export peptides when fully inserted in the IM (Chang 2003, Cheung et al. 2018). In their membrane-bound conformation, the structural differences between the  $\alpha$ -helical or  $\beta$ -sheet AMPs would be reduced since they would be restricted by the environmental constraints imposed by the phospholipid membrane. In a recent molecular dynamic simulation using a hBD-3 analog, it was revealed that the membrane-binding rigidifies the peptide, enhancing its structural polymorphism, and promoting  $\beta$ -strand conformation (Kang et al. 2019). Also other factors such as the presence of secondary metabolites or reactive oxygen species (ROS) could contribute to any structural similarities between AMPs. Besides being able to capture IM-inserted peptides, most tripartite efflux pumps are likely to intercept substrates from the periplasm or outer leaflet of the IM (Alav et al. 2021). In this case, an option to transport peptides differing in their 3D structures would be to transport them as unfolded peptides. Although there is no available information regarding unfolding mechanisms for AMPs, there is evidence of similar peptides (e.g. amyloid peptides A $\beta$ -42 and A $\beta$ -40) being transported in a partial or complete disordered state by the ABC transporter P-glycoprotein (McCormick et al. 2021). Regrettably, the authors in this study could not conclude with certainty whether P-glycoprotein was able to transport or disrupt folded A $\beta$  monomers or whether it could facilitate the folding of the peptides during the

transport process. Also the existence of the translocon complex component SecDF, a member of the RND superfamily, could support this hypothesis. In *E. coli*, SecDF moves proteins (including unfolded polypeptides and other diverse substrates) through the IM towards the periplasmic space (Rahman et al. 2017, Tsukazaki et al. 2011). Sec recognizes their substrates by a Sec signal that does not show sequence similarities, but contains a conserved tripartite overall structure consisting of a cationic N-terminal region, a central hydrophobic core, and a polar C-terminus (Rusch and Kendall 2007). By analysing the AMPs sequences, we could not find any conserved sequence, and even less a hydrophobic core, since AMPs are, by nature, amphipathic, but an unknown recognition sequence to direct the AMPs to the efflux pump cannot be ruled out. Finding this sequence would allow modification of AMPs to avoid their recognition by the efflux pump and posterior removal to the extracellular space.

Recently, the *S. aureus* ABC transporter PmtABCD, responsible for the secretion of all the known phenol soluble modulins (PSMs), has been shown to be also able to export important human AMPs such as LL-37 and hBD3 (R. Chatterjee et al. 2013, Cheung et al. 2018, Wang et al. 2007, Zeytuni et al. 2020). This dual transport is not difficult to understand since PSMs and AMPs share structural features [e.g. PSM $\alpha$  and LL-37 are helical and a high sequence similarity exists between the short PSM $\alpha$ 3 and the core of LL-37 (Cheung et al. 2018, Engelberg and Landau 2020)]. As other substrates of the Pmt transporter are PSM $\beta$ 3 and hBD3 with more complicated structures in solution, it is assumed that the transporter is also able to capture the membrane-inserted peptides as well (Cheung et al. 2018). This ability could be explained by the proposed ‘vacuum cleaner’ mechanism in which the efflux pumps can take hydrophobic molecules embedded in the membrane to efflux them (Chang 2003, Raviv et al. 1990). In this way, the Pmt transporter could accept membrane embedded peptides coming from the periplasm (AMPs) and PSMs coming from the cytoplasm (Cheung et al. 2018). A similar mechanism to accept AMPs coming from the cytoplasm cannot be ruled out for other efflux pumps.

As a general rule, it seems that any efflux pump is able to efflux almost all the structurally different AMPs, but that the effect is masked by other dominant mechanisms to avoid AMP attack (e.g.  $\alpha$ -defensins are not effluxed by MtrCDE in *H. ducreyi* even though the pump is able to do it, because PEA modification is the main mechanism for  $\alpha$ -defensin resistance; Trombley et al. 2015).

In the case of the AMP-SapA binding, it is assumed that the AMPs will be intercepted in the periplasm in their membrane-unbound conformation. Thus, the different AMP structures that SapA can bind (e.g. the NTHI SapA protein can bind the  $\beta$ -sheet HBD-3, the  $\alpha$ -helix LL-37, and even the heme-group) could depend on the SapA general architecture (Shelton et al. 2011). The recent *H. influenzae* SapA protein structure solved in an open (no ligand) and closed conformation, shows a cavity volume (and lack of negative charge) unable to accommodate a complete folded AMP (Lukacik et al. 2021). The binding of the folded AMP protruding out of the narrow openings of the SapA ligand-binding cavity was proposed by the authors. Moreover, the authors could not obtain any SapA-AMP complexes when using pure SapA protein, but by contrast were able to obtain complexes of SapA bound to the heme group and dsRNA (Lukacik et al. 2021). This may suggest that an additional protein, acting as a chaperone, would be needed for the AMPs-SapA binding before transport to the cytoplasm by the SacBCDF transporter. This process may possibly be associated with at least partial unfolding of the AMPs to facilitate transport. Such a possibility is also supported by the fact that the *H. ducreyi* SapBC channel retains activity even when SapA is not

present, suggesting that other unknown SapA-independent mechanisms may exist in this microorganism, including the AMP interaction with other periplasmic solute-binding components different to SapA (Rinker et al. 2012).

## How do efflux pumps process such large molecules as AMPs?

Another striking question is related to the size of the molecules to be extruded. Aside from the AMPs, the maximum molecule size transported by AcrAB-TolC are the macrolides, with a molecular weight smaller than 1000 Da (Ababou and Koronakis 2016, Tam et al. 2021). The cyclic AMPs colistin and polymyxin B are a similar size (approx MW 1200 Da), but this is not the case for bigger AMPs such as LL-37 (4493 Da) or defensins (3000-5000Da). So, how could they interact and pass through the narrow channels in the IM components (e.g. MtrD) of the efflux pumps?

Recently, a new path for high molecular weight drugs (e.g. macrolides and ansamycins) has been proposed in AcrB (Tam et al. 2021). In this path, the high molecular weight drugs would be initially captured in the access pocket (via channel 2), where the switch loop would accommodate their binding. After that, the drug would be accommodated in the deep binding pocket region, and subsequently be exported through the O protomer exit tunnel (Tam et al. 2021). As polymyxins share structural features with macrolides, it is tempting to think about a similar recognition mechanism.

Molecular dynamics studies have shown the movement of molecules inside efflux pumps (e.g. progesterone in the *N. gonorrhoeae* MtrCDE efflux pump; Chitsaz et al. 2019). The computational approach would be an interesting tool to clear up structural questions related to the AMP-efflux pumps complexes, with the limitations of using short AMPs in these simulations (Ramesh et al. 2016). Also, structures of the AMP-efflux pump IM protein could be obtained by structural approaches, such as X-ray crystallography, as done recently by crystallizing MexB in the presence of high-molecular-mass compounds (Sakurai et al. 2019). The structure of complexes containing AMPs have been obtained as in the case of proline-rich peptides bound to the *Thermus thermophilus* 70S ribosome or short AMPs (11–13 amino acids) bound to *P. aeruginosa* lectin Lec B (Baeriswyl et al. 2019, Gagnon et al. 2016).

## Are the OMF proteins potential entry channels for AMPs?

Loss of *mtrE* enhanced, not reduced, gonococcal survival after exposure to azurocidin (37 kDa) raising the question if MtrE could be a portal across the OM for some antimicrobials, although other possibilities related to secondary effects linked to *mtrE* loss could not be ruled out (e.g. the *tolC* deletion increases the activity of the transcriptional regulators MarA, SoxS, and Rob, being these regulators responsible for the porin regulation and producing potential changes in membrane permeability; Handing et al. 2018). The possibility that MtrE can act as an AMP entry portal is supported by the recent finding that TolC is able to import bacteriocins (MW 60 kDa) in Gram-negative bacteria (Housden et al. 2021). If the OM proteins act as AMP access channels, then AMP entry can possibly be potentiated by blocking the OM channel in open state as done by using MtrE mutants and vancomycin (MW 1449 Da; Janganan et al. 2013). Such approach was also employed for TolC, where the introduction of mutations caused an increased susceptibility to vancomycin suggesting that these mutations cause disruption of

the OM permeability barrier at the level of TolC gating (Marshall and Bavro 2020). It is plausible that other cyclic AMPs such as polymyxin B or colistin could also use the OM as entry channel.

We could also take advantage of the ability of the SapABCDF transporter to introduce AMPs in the cytoplasm by introducing proteolytic resistant AMPs (Lu et al. 2020). Many efforts have been made to deal with this weakness of AMPs (e.g. D-amino acid substitutions, introduction of disulfide bonds, immobilize them on surfaces, use non natural amino acids incorporation, cyclization, and nano delivery systems), but further research is needed (Biswaro et al. 2018, Gentilucci et al. 2010, Jia et al. 2017).

### Could we use AMPs as scaffolds to design efflux pump inhibitors?

A significant global effort is underway to develop efflux pump inhibitors (EPIs) to potentiate the use of the existing antibiotics (Marshall et al. 2020). If we can better understand AMP–efflux pump interactions, similar strategies could be followed to design peptide-based EPIs. Thus, understanding the specific AMP–efflux pump interactions is critical for informed design of potential EPIs. While there are no current experimental 3D structures of efflux pumps in complex with AMPs, such approaches have been followed successfully in similar scenarios, e.g. by using pore-blocking toxins that inhibit voltage-dependent K<sup>+</sup> channels (Banerjee et al. 2013). The 1-(1-naphthylmethyl)-piperazine (NMP), an AcrAB–TolC inhibitor, was shown to interact with the critically important residue (Phe610) within the deep binding pocket of AcrB and causing conformational change in AcrB (Bohnert and Kern 2005, Vargiu et al. 2014). The structure of the complex formed by AcrB and the pyridopyrimidine derivative inhibitor D13-9001 in *E. coli* and MexB of *P. aeruginosa* showed the binding of the inhibitor to the distal pocket, preventing the binding of the substrates (Nakashima et al. 2013), while the binding of phenylalanylarginine- $\beta$ -naphthylamide (PA $\beta$ N) and other inhibitors to AcrB has been shown by computational approaches (Vargiu et al. 2014). Importantly, pyranopyridines, such as MBX2319, which bind within a phenylalanine-rich cage that branches from the deep binding pocket of AcrB, form extensive hydrophobic interactions within it, which allowed for an effective computational derivation of the structure of the lead compounds, increasing further the affinity of interaction and providing a template for an effective pump inhibition (Sjuts et al. 2016).

In addition, the use of peptides able to change their conformation depending on the pH or temperature could be useful in order to plug a channel. For doing this, the use of self-assembling peptides, whose huge variety of structures depends on the environmental conditions, would be a good option (Lee et al. 2019, Lombardi et al. 2019). Furthermore, peptide tectons, defined as peptide building blocks exhibiting structural complementarity at the interacting interfaces, can self-assemble into defined supramolecular structures promoted by these complementary interactions (Lou et al. 2019). Some of these supramolecular structures (e.g. peptide-based cages) have shown their ability in drug delivery while others (e.g. flexible fibres of indefinite length or large colloidal-scale assemblies) have been considered as new biomaterials with applications in biotechnology (Boyle et al. 2012, Fletcher et al. 2013).

Another approach would be to target and disrupt the dimerization interface as done by some peptide-based EPIs of the *P. aeruginosa* small multidrug resistance (SMR) efflux protein (Mitchell et al. 2019).

## Conclusions/future perspectives

Understanding in more detail the physical interaction between AMPs and efflux pumps/transporters will help us to develop novel strategies to take advantage of or inhibit the efflux process. First, the structural information of the complexes could guide us to design AMPs able to avoid the action of efflux pumps and/or proteolytic degradation in the cytoplasm. Second, the structural information could inspire the design of AMP-based EPIs able to plug the efflux pump channels. Third, we could design drugs fused to AMPs and use these peptides to transport them to the cytoplasm via the Sap transporter system. Once there, the drug could be released from the AMPs by bacterial proteases. Crystal structures of the AMP–efflux pump complexes will be crucial for the rational design of new drugs using these innovative approaches.

Lastly, by understanding the transport of the ribosomally synthesized and the nonribosomally synthesized AMPs we could answer two interesting questions. Future work on the nonribosomally synthesized AMPs, currently licenced for therapeutic use (e.g. colistin), should clarify whether efflux is a relevant mechanism of resistance to AMP-based medicines. On the other hand, future research into the ribosomally synthesized AMPs is required to understand how bacteria deal with the host immune response during infection. Given the emerging prominent role that efflux appears to play in resistance to both types of AMPs, efflux inhibitors have the potential to be an important addition to the physician's arsenal in the post antibiotic era.

## Acknowledgments

We gratefully acknowledge all members of Dr. Blair Group (Institute of Microbiology and Infection, University of Birmingham) for valuable scientific discussions.

**Conflict of interest.** None declared.

## Funding

This work was supported by the European Union's Horizon 2020 Research and Innovation Programme through the Marie Skłodowska-Curie Grant, grant number 839036 given to E.S.-V. J.M.A.B. was supported by a BBRSC David Phillips fellowship (BB/M02623X/1).

## References

- Ababou A, Koronakis V. Structures of gate loop variants of the AcrB drug efflux pump bound by erythromycin substrate. *PLoS ONE* 2016;**11**:e0159154.
- Akama H, Matsuura T, Kashiwagi S et al. Crystal structure of the membrane fusion protein, MexA of the multidrug transporter in *Pseudomonas aeruginosa*. *J Biol Chem* 2004; **279**:25939–42. Worldwide Protein Data Bank. DOI: [10.2210/pdb1vf7/pdb](https://doi.org/10.2210/pdb1vf7/pdb).
- Akiba M, Lin J, Barton Yi-W et al. Interaction of CmeABC and CmeDEF in conferring antimicrobial resistance and maintaining cell viability in *Campylobacter jejuni*. *J Antimicrob Chemother* 2006;**57**:52–60.
- Alav I, Kobylka J, Kuth MS et al. Structure, assembly, and function of tripartite efflux and type 1 secretion systems in Gram-negative bacteria. *Chem Rev* 2021;**121**:5479–596.
- Altenbach C, Hubbell WL. The aggregation state of spin-labeled melittin in solution and bound to phospholipid membranes: Evidence that membrane-bound melittin is monomeric. *Proteins: Struct Funct Bioinf* 1988;**3**:230–42.

- Anes J, McCusker MP, Fanning S et al. The ins and outs of RND efflux pumps in *Escherichia coli*. *Front Microbiol* 2015;**6**. DOI: [10.3389/fmicb.2015.00587](https://doi.org/10.3389/fmicb.2015.00587).
- Aspedon A, Groisman EA. The antibacterial action of protamine: evidence for disruption of cytoplasmic membrane energization in *Salmonella typhimurium*. *Microbiology* 1996;**142** (Pt 12):3389–97.
- Ayoub Moubarek C. Polymyxins and Bacterial Membranes: A Review of Antibacterial Activity and Mechanisms of Resistance. *Membranes* 2020;**10**: DOI: [10.3390/membranes10080181](https://doi.org/10.3390/membranes10080181).
- Baeriswyl S, Gan B-Ha, Siriwardena TN et al. X-ray crystal structures of short antimicrobial peptides as *Pseudomonas aeruginosa* lectin B complexes. *ACS Chem Biol* 2019;**14**:758–66.
- Bakker EP. Low-affinity K<sup>+</sup> uptake systems. In: *Alkali Cation Transport Systems in Prokaryotes*. Boca Raton: CRC Press, 1993,253–76.
- Banerjee A, Lee A, Campbell E et al. Structure of a pore-blocking toxin in complex with a eukaryotic voltage-dependent K(+) channel. *Elife* 2013;**2**:e00594.
- Bauer ME, Shafer WM. On the in vivo significance of bacterial resistance to antimicrobial peptides. *Biochim Biophys Acta Biomemb* 2015;**1848**:3101–11.
- Bavro VN, Pietras Z, Furnham N et al. Assembly and channel opening in a bacterial drug efflux machine. *Mol Cell* 2008;**30**:114–21.
- Bechinger B, Gorr S-U. Antimicrobial peptides: mechanisms of action and resistance. *J Dent Res* 2017;**96**:254–60.
- Bechinger B, Lohner K. Detergent-like actions of linear amphipathic cationic antimicrobial peptides. *Biochim Biophys Acta Biomemb* 2006;**1758**:1529–39.
- Beggs GA, Zalucki YM, Brown NG et al. Structural, biochemical, and in vivo characterization of MtrR-mediated resistance to innate antimicrobials by the human pathogen *Neisseria gonorrhoeae*. *J Bacteriol* 2019;**201**. DOI: [10.1128/JB.00401-19](https://doi.org/10.1128/JB.00401-19).
- Belas R, Manos J, Suvanasthi R. *Proteus mirabilis* ZapA metalloprotease degrades a broad spectrum of substrates, including antimicrobial peptides. *Infect Immun* 2004;**72**:5159–67.
- Bengoechea JA, Skumik M. Temperature-regulated efflux pump/potassium antiporter system mediates resistance to cationic antimicrobial peptides in *Yersinia*. *Mol Microbiol* 2000;**37**:67–80.
- Bierbaum G, Sahl H-G. Lantibiotics: mode of action, biosynthesis and bioengineering. *Curr Pharm Biotechnol* 2009;**10**:2–18.
- Bina XR, Provenzano D, Nguyen N. *Vibrio cholerae* RND family efflux systems are required for antimicrobial resistance, optimal virulence factor production, and colonization of the infant mouse small intestine. *Infection* 2008;**76**:3595–605. <https://iaa.asm.org/content/76/8/3595.short>.
- Bishop RE, Gibbons HS, Guina T et al. Transfer of palmitate from phospholipids to lipid a in outer membranes of Gram-negative bacteria. *EMBO J* 2000;**19**:5071–80.
- Biswalo LS, Sousa MGC, Rezende TMB et al. Antimicrobial peptides and nanotechnology, recent advances and challenges. *Front Microbiol* 2018;**9**. DOI: [10.3389/fmicb.2018.00855](https://doi.org/10.3389/fmicb.2018.00855).
- Blair JMA, Richmond GE, Piddock LJV. Multidrug efflux pumps in Gram-negative bacteria and their role in antibiotic resistance. *Fut Microbiol* 2014;**9**:1165–77.
- Bland JM, De Lucca AJ, Jacks TJ et al. All-D-cecropin B: synthesis, conformation, lipopolysaccharide binding, and antibacterial activity. *Mol Cell Biochem* 2001;**218**:105–11.
- Bohner JA, Kern WV. Selected arylpiperazines are capable of reversing multidrug resistance in *Escherichia coli* overexpressing RND efflux pumps. *Antimicrob Agents Chemother* 2005;**49**:849–52.
- Bolla JR, Su C-C, Do SV et al. Crystal structure of the *Neisseria gonorrhoeae* MtrD inner membrane multidrug efflux pump. *PLoS ONE* 2014;**9**:e97903.
- Boyle AL, Bromley EHC, Bartlett GJ et al. Squaring the circle in peptide assembly: from fibers to discrete nanostructures by de novo design. *J Am Chem Soc* 2012;**134**:15457–67.
- Brogden KA. Antimicrobial peptides: pore formers or metabolic inhibitors in bacteria?. *Nat Rev Microbiol* 2005;**3**:238–50.
- Browne K, Chakraborty S, Chen R et al. A new era of antibiotics: the clinical potential of antimicrobial peptides. *Int J Mol Sci* 2020;**21**. DOI: [10.3390/ijms21197047](https://doi.org/10.3390/ijms21197047).
- Butler MS, Paterson DL. Antibiotics in the clinical pipeline in October 2019. *J Antibiot* 2020;**73**:329–64.
- Campos MA, Vargas MA, Regueiro V et al. Capsule polysaccharide mediates bacterial resistance to antimicrobial peptides. *Infect Immun* 2004;**72**:7107–14.
- Cao Y, Jin X, Huang H et al. Crystal structure of a potassium ion transporter, TrkH. *Nature* 2011;**471**:336–40. DOI: [10.1038/nature09731](https://doi.org/10.1038/nature09731).
- Cao Y, Pan Y, Huang H et al. Gating of the TrkH ion channel by its associated RCK protein TrkA. *Nature* 2013;**496**:317–22.
- Capone R, Mustata M, Jang H et al. Antimicrobial protegrin-1 forms ion channels: molecular dynamic simulation, atomic force microscopy, and electrical conductance studies. *Biophys J* 2010;**98**:2644–52.
- Cardoso MH, Meneguetti BT, Costa BO et al. Non-lytic antibacterial peptides that translocate through bacterial membranes to act on intracellular targets. *Int J Mol Sci* 2019;**20**. DOI: [10.3390/ijms20194877](https://doi.org/10.3390/ijms20194877).
- Chan C, Burrows LL, Deber CM. Helix induction in antimicrobial peptides by alginate in biofilms. *J Biol Chem* 2004;**279**:38749–54.
- Chang G. Multidrug resistance ABC transporters. *FEBS Lett* 2003;**555**:102–5.
- Chatterjee SS, Joo H-S, Duong AC et al. Essential *Staphylococcus aureus* toxin export system. *Nat Med* 2013;**19**:364–67.
- Chen CH, Lu TK. Development and challenges of antimicrobial peptides for therapeutic applications. *Antibiotics* 2020;**9**. DOI: [10.3390/antibiotics9010024](https://doi.org/10.3390/antibiotics9010024).
- Chen H-Y, Weng S-F, Lin J-W. Identification and analysis of the sap genes from *Vibrio fischeri* belonging to the ATP-binding cassette gene family required for peptide transport and resistance to antimicrobial peptides. *Biochem Biophys Res Commun* 2000;**269**:743–48.
- Chen M, Shi X, Yu Z et al. In situ structure of the AcrAB-TolC efflux pump at subnanometer resolution. *Structure* 2021;**30**:107–113.e3.
- Chen S, Connolly KL, Rouquette-Loughlin C et al. Could dampening expression of the *Neisseria gonorrhoeae* mtrCDE-encoded efflux pump be a strategy to preserve currently or resurrect formerly used antibiotics to treat gonorrhea?. *Mbio* 2019;**10**. DOI: [10.1128/mBio.01576-19](https://doi.org/10.1128/mBio.01576-19).
- Chen Y-C, Chuang Y-C, Chang C-C et al. A K<sup>+</sup> uptake protein, TrkA, is required for serum, protamine, and polymyxin B resistance in *Vibrio vulnificus*. *Infect Immun* 2004;**72**:629–36.
- Cheng H-Y, Chen Y-F, Peng H-L. Molecular characterization of the PhoPQ-PmrD-PmrAB mediated pathway regulating polymyxin B resistance in *Klebsiella pneumoniae* CG43. *J Biomed Sci* 2010;**17**:60.
- Cheng Y-H, Lin T-L, Lin Y-T et al. A putative RND-type efflux pump, H239\_3064, contributes to colistin resistance through CrrB in *Klebsiella pneumoniae*. *J Antimicrob Chemother* 2018;**73**:1509–16.
- Cheung GYC, Fisher EL, McCausland JW et al. Antimicrobial peptide resistance mechanism contributes to *Staphylococcus aureus* infection. *J Infect Dis* 2018;**217**. DOI: [10.1093/infdis/jiy024](https://doi.org/10.1093/infdis/jiy024).
- Chitsaz M, Booth L, Blyth MT et al. Multidrug resistance in *Neisseria gonorrhoeae*: identification of functionally important residues in the MtrD efflux protein. *Mbio* 2019;**10**. DOI: [10.1128/mbio.02277-19](https://doi.org/10.1128/mbio.02277-19).

- Clemens R, Zschke-Kriesche J, Khosa S et al. Insight into two ABC transporter families involved in lantibiotic resistance. *Front Mol Biosci* 2017;**4**:91.
- Clements A, Tull D, Jenney AW et al. Secondary acylation of *Klebsiella pneumoniae* lipopolysaccharide contributes to sensitivity to antibacterial peptides. *J Biol Chem* 2007;**282**. DOI: [10.1074/jbc.m701454200](https://doi.org/10.1074/jbc.m701454200).
- Colclough AL, Alav I, Whittle EE et al. RND efflux pumps in Gram-negative bacteria; regulation, structure and role in antibiotic resistance. *Fut Microbiol* 2020;**15**:143–57.
- Cole JN, Nizet V. Bacterial evasion of host antimicrobial peptide defenses. *Microbiol Spectr* 2016;**4**. DOI: [10.1128/microbiolspec.VMBF-0006-2015](https://doi.org/10.1128/microbiolspec.VMBF-0006-2015).
- Cotter PD, Ross RP, Hill C. Bacteriocins - a viable alternative to antibiotics?. *Nat Rev Microbiol* 2013;**11**:95–105.
- Coudray N, Isom GL, MacRae MR et al. Structure of bacterial phospholipid transporter MlaFEDB with substrate bound. *Elife* 2020;**9**:e62518. November.
- Crow A, Greene NP, Kaplan E et al. Structure and mechanotransmission mechanism of the MacB ABC transporter superfamily. *Proc Natl Acad Sci* 2017;**114**:12572–77.
- D'amato RF, Thornsberry C, Baker CN et al. Effect of calcium and magnesium ions on the susceptibility of *Pseudomonas* species to tetracycline, gentamicin polymyxin B, and carbenicillin. *Antimicrob Agents Chemother* 1975;**7**:596–600.
- Dalebroux ZD, Miller SI. Salmonellae PhoPQ regulation of the outer membrane to resist innate immunity. *Curr Opin Microbiol* 2014;**17**:106–13.
- Deris ZZ, Swarbrick JD, Roberts KD et al. Probing the penetration of antimicrobial polymyxin lipopeptides into gram-negative bacteria. *Bioconjug Chem* 2014;**25**:750–60.
- Devine DA, Marsh PD, Percival RS et al. Modulation of antibacterial peptide activity by products of *Porphyromonas gingivalis* and *Prevotella* spp. *Microbiology* 1999;**145**:965–71.
- Diamond G, Beckloff N, Weinberg A et al. The roles of antimicrobial peptides in innate host defense. *Curr Pharm Des* 2009;**15**:2377–92.
- Ding L, Yang L, Weiss TM et al. Interaction of antimicrobial peptides with lipopolysaccharides. *Biochemistry* 2003;**42**:12251–59.
- Dorschner RA, Lopez-Garcia B, Peschel A et al. The mammalian ionic environment dictates microbial susceptibility to antimicrobial defense peptides. *FASEB J* 2006;**20**:35–42.
- Du D, Neuberger A, Orr MW et al. Interactions of a bacterial RND transporter with a transmembrane small protein in a lipid environment. *Structure* 2020;**28**:625–34.e6.
- Du D, Wang Z, James NR et al. Structure of the AcrAB-TolC multidrug efflux pump. *Nature* 2014;**509**:512–15.
- Durell SR, Hao Y, Nakamura T et al. Evolutionary relationship between K(+) channels and symporters. *Biophys J* 1999;**77**:775–88.
- Duurkens RH, Tol MB, Geertsma ER et al. Flavin binding to the high affinity riboflavin transporter ribU. *J Biol Chem* 2007;**282**:10380–86.
- Eicher T, Cha Hi-J, Seeger MA et al. Transport of drugs by the multidrug transporter AcrB involves an access and a deep binding pocket that are separated by a switch-loop. *Proc Natl Acad Sci* 2012;**109**:5687–92.
- El-Sayed Ahmed MAE, Zhong L-L, Shen C et al. Colistin and its role in the era of antibiotic resistance: an extended review (2000–2019). *Emerg Microb Infect* 2020;**9**:868–85.
- Elbourne LDH, Tetu SG, Hassan KA et al. TransportDB 2.0: a database for exploring membrane transporters in sequenced genomes from all domains of life. *Nucleic Acids Res* 2017;**45**:D320–24.
- Elsbach P The bactericidal/permeability-increasing protein (BPI) in antibacterial host defense. *J Leukocyte Biol* 1998;**64**:14–18.
- Engelberg Y, Landau M. The human LL-37(17–29) antimicrobial peptide reveals a functional supramolecular structure. *Nat Commun* 2020;**11**:3894.
- Eswarappa SM, Panguluri KK, Hensel M et al. The yjeABEF operon of *Salmonella* confers resistance to antimicrobial peptides and contributes to its virulence. *Microbiology* 2008;**154**:666–78.
- Fairweather SJ, Gupta V, Chitsaz M et al. Coordination of substrate binding and protonation in the *N. gonorrhoeae* MtrD efflux pump controls the functionally rotating transport mechanism. *ACS Infect Dis* 2021;**7**:1833–47.
- Farizano JV, Pescaretti MM, López FE et al. The PmrAB system-inducing conditions control both lipid a remodeling and O-antigen length distribution, influencing the *Salmonella typhimurium*-host interactions. *J Biol Chem* 2012;**287**:38778–89.
- Feng Y, Xiaoling P, Ning H et al. The human beta-defensins expression in female genital tract and pregnancy-related tissues. *Sichuan Da Xue Xue Bao Yi Xue Ban* 2003;**34**:217–19.
- Fitzpatrick AWP, Llabres S, Neuberger A et al. Structure of the MacAB-TolC ABC-type tripartite multidrug efflux pump. *Nat Microbiol* 2017; **2**:17070. Worldwide Protein Data Bank.
- Fjell CD, Hiss JA, Hancock REW et al. Designing antimicrobial peptides: form follows function. *Nat Rev Drug Discov* 2011;**11**:37–51.
- Fletcher JM, Harniman RL, Barnes FRH et al. Self-assembling cages from coiled-coil peptide modules. *Science* 2013;**340**:595–99.
- Florin T, Maracci C, Graf M et al. An antimicrobial peptide that inhibits translation by trapping release factors on the ribosome. *Nat Struct Mol Biol* 2017;**24**. DOI: [10.1038/nsmb.3439](https://doi.org/10.1038/nsmb.3439).
- Foschiatti M, Cescutti P, Tossi A et al. Inhibition of cathelicidin activity by bacterial exopolysaccharides. *Mol Microbiol* 2009;**72**:1137–46.
- Foster DL, Boublik M, Kaback HR. Structure of the lac carrier protein of *Escherichia coli*. *J Biol Chem* 1983;**258**:31–34.
- Gagnon MG, Roy RN, Lomakin IB et al. Structures of proline-rich peptides bound to the ribosome reveal a common mechanism of protein synthesis inhibition. *Nucleic Acids Res* 2016;**44**:2439–50.
- Gallo RL, Kim KJ, Bernfield M et al. Identification of CRAMP, a cathelin-related antimicrobial peptide expressed in the embryonic and adult mouse. *J Biol Chem* 1999;**272**:13088–93.
- Galva'n EM, Lasaro MAS, Schifferli DM. Capsular antigen fraction 1 and Pla modulate the susceptibility of *Yersinia pestis* to pulmonary antimicrobial peptides such as cathelicidin. *Infect Immun* 2008;**76**. DOI: [10.1128/iai.01197-07](https://doi.org/10.1128/iai.01197-07).
- Gazit E, Miller IR, Biggin PC et al. Structure and orientation of the mammalian antibacterial peptide cecropin P1 within phospholipid membranes. *J Mol Biol* 1996;**258**:860–70.
- Gentilucci L, De Marco R, Cerisoli L. Chemical modifications designed to improve peptide stability: incorporation of non-natural amino acids, pseudo-peptide bonds, and cyclization. *Curr Pharm Des* 2010;**16**:3185–203.
- Glavier M, Puvanendran D, Salvador D et al. Antibiotic export by MexB multidrug efflux transporter is allosterically controlled by a MexA-OprM chaperone-like complex. *Nat Commun* 2020;**11**:4948.
- Golparian D, Shafer WM, Ohnishi M et al. Importance of multidrug efflux pumps in the antimicrobial resistance property of clinical multidrug-resistant isolates of *Neisseria gonorrhoeae*. *Antimicrob Agents Chemother* 2014;**58**:3556–59.
- Goto Y, Hagihara Y. Mechanism of the conformational transition of melittin. *Biochemistry* 1992;**31**:732–8.
- Graf M, Mardirossian M, Nguyen F et al. Proline-rich antimicrobial peptides targeting protein synthesis. *Nat Prod Rep* 2017;**34**:702–11.

- Greene NP, Kaplan E, Crow A et al. Corrigendum: antibiotic resistance mediated by the MacB ABC transporter family: a structural and functional perspective. *Front Microbiol* 2018;**9**:2318.
- Groisman EA, Parra-Lopez C, Salcedo M et al. Resistance to host antimicrobial peptides is necessary for *Salmonella* virulence. *Proc Natl Acad Sci* 1992;**89**:11939–43.
- Guina T, Yi EC, Wang H et al. A phop-regulated outer membrane protease of *Salmonella enterica* serovar typhimurium promotes resistance to alpha-helical antimicrobial peptides. *J Bacteriol* 2000;**182**. DOI: [10.1128/jb.182.14.4077-4086.2000](https://doi.org/10.1128/jb.182.14.4077-4086.2000).
- Gunn JS, Miller SI. PhoP-PhoQ activates transcription of pmrAB, encoding a two-component regulatory system involved in *Salmonella typhimurium* antimicrobial peptide resistance. *J Bacteriol* 1996;**178**:6857–64.
- Gunn JS, Ryan SS, Van Velkinburgh JC et al. Genetic and functional analysis of a pmra-pmrB-regulated locus necessary for lipopolysaccharide modification, antimicrobial peptide resistance, and oral virulence of *Salmonella enterica* serovar typhimurium. *Infect Immun* 2000;**68**. DOI: [10.1128/.68.11.6139-6146.2000](https://doi.org/10.1128/.68.11.6139-6146.2000).
- Guo L, Lim KB, Poduje CM et al. Lipid acylation and bacterial resistance against vertebrate antimicrobial peptides. *Cell* 1998;**95**:189–98.
- Hagman KE, Spratt BG, Balthazar JT et al. Resistance of *Neisseria gonorrhoeae* to antimicrobial hydrophobic agents is modulated by the mtrRCDE efflux system. *Microbiology* 1995;**141**. DOI: [10.1099/1350-0872-141-3-611](https://doi.org/10.1099/1350-0872-141-3-611).
- Hancock REW. Peptide antibiotics. *The Lancet* 1997;**349**:418–22.
- Handing JW, Ragland SA, Bharathan UV et al. The MtrCDE efflux pump contributes to survival of *Neisseria gonorrhoeae* from human neutrophils and their antimicrobial components. *Front Microbiol* 2018;**9**:2688.
- Haney EF, Hancock REW. Peptide design for antimicrobial and immunomodulatory applications. *Biopolymers* 2013;**100**:572–83.
- Harris F, Dennison SR, Phoenix DA. Anionic antimicrobial peptides from eukaryotic organisms. *Curr Protein Pept Sci* 2009;**10**:585–606.
- Harris RH, Wilk D, Bevins CL et al. Identification and characterization of a mucosal antimicrobial peptide expressed by the chinchilla (*Chinchilla lanigera*) airway. *J Biol Chem* 2004;**279**:20250–6.
- Hebbeln P, Rodionov DA, Alfandega A et al. Biotin uptake in prokaryotes by solute transporters with an optional ATP-binding cassette-containing module. *Proc Natl Acad Sci* 2007;**104**:2909–14.
- Henderson PJF, Maher C, Elbourne LDH et al. Physiological functions of bacterial ‘Multidrug’ efflux pumps. *Chem Rev* 2021;**121**. March. DOI: [10.1021/acs.chemrev.0c01226](https://doi.org/10.1021/acs.chemrev.0c01226).
- Higgins MK, Bokma E, Koronakis E et al. Structure of the periplasmic component of a bacterial drug efflux pump. *Proc Natl Acad Sci* 2004;**101**:9994–99.
- Hill CP, Yee J, Selsted ME et al. Crystal structure of defensin HNP-3, an amphiphilic dimer: mechanisms of membrane permeabilization. *Science* 1991;**251**:1481–5.
- Hobbs EC, Yin X, Paul BJ et al. Conserved small protein associates with the multidrug efflux pump AcrB and differentially affects antibiotic resistance. *Proc Natl Acad Sci* 2012;**109**:16696–701.
- Hobbs M, Sparling P, Cohen M et al. Experimental gonococcal infection in male volunteers: cumulative experience with *Neisseria gonorrhoeae* strains FA1090 and MS11mkC. *Front Microbiol* 2011;**2**. DOI: [10.3389/fmicb.2011.00123](https://doi.org/10.3389/fmicb.2011.00123).
- Hobbs MM, Anderson JE, Balthazar JT et al. Lipid A's structure mediates *Neisseria gonorrhoeae* fitness during experimental infection of mice and men. *Mbio* 2013;**4**:e00892–13.
- Honeycutt JD, Wenner N, Li Y et al. Genetic variation in the MacAB-TolC efflux pump influences pathogenesis of invasive *Salmonella* isolates from Africa. *PLoS Pathog* 2020;**16**. DOI: [10.1371/journal.ppat.1008763](https://doi.org/10.1371/journal.ppat.1008763).
- Hoover DM, Rajashankar KR, Blumenthal R et al. The structure of human beta-defensin-2 shows evidence of higher order oligomerization. *J Biol Chem* 2000;**275**:32911–8.
- Housden NG, Webby MN, Lowe ED et al. Toxin import through the antibiotic efflux channel tolC. *Nat Commun* 2021;**12**:4625.
- Hsu S-TD, Breukink E, Tischenko E et al. The nisin-lipid II complex reveals a pyrophosphate cage that provides a blueprint for novel antibiotics. *Nat Struct Mol Biol* 2004;**11**:963–7.
- Huang SC, Jack DL, Jahn PS et al. The major facilitator superfamily. *Microbiol Biotechnol* 1999;**1**:257–79. <https://www.caister.com/backlist/jmmb/v/v1/v1n2/09.pdf>.
- Hultmark D, Engström A, Bennich H et al. Hultmark D, Engström A, Bennich H et al. Insect immunity: isolation and structure of cecropin D and four minor antibacterial components from *Cecropia* pupae. *Eur J Biochem* 1982;**127**:207–17.
- Janganan TK, Bavro VN, Zhang Li et al. Evidence for the assembly of a bacterial tripartite multidrug pump with a stoichiometry of 3:6:3. *J Biol Chem* 2011;**286**:26900–912.
- Janganan TK, Bavro VN, Zhang Li et al. Tripartite efflux pumps: energy is required for dissociation, but not assembly or opening of the outer membrane channel of the pump. *Mol Microbiol* 2013;**88**:590–602.
- Jangir PK, Ogunlana L, MacLean RC. Evolutionary constraints on the acquisition of antimicrobial peptide resistance in bacterial pathogens. *Trends Microbiol*, 2021;**29**. April. DOI: [10.1016/j.tim.2021.03.007](https://doi.org/10.1016/j.tim.2021.03.007).
- Jerse AE, Sharma ND, Simms AN et al. A gonococcal efflux pump system enhances bacterial survival in a female mouse model of genital tract infection. *Infect Immun* 2003;**71**:5576–82.
- Jia F, Wang J, Peng J et al. D-amino acid substitution enhances the stability of antimicrobial peptide Polybia-CP. *Acta Biochim Biophys Sin* 2017;**49**:916–25.
- Jones A, Geörg M, Maudsdotter L et al. Endotoxin, capsule, and bacterial attachment contribute to *Neisseria meningitidis* resistance to the human antimicrobial peptide LL-37. *J Bacteriol* 2009;**191**:3861–68.
- Joo H-S, Fu C-I, Otto M. Bacterial strategies of resistance to antimicrobial peptides. *Philos Trans R Soc Lond Ser B Biol Sci* 2016;**371**:371. DOI: [10.1098/rstb.2015.0292](https://doi.org/10.1098/rstb.2015.0292).
- Kampshoff F, Willcox MDP, Dutta D. A pilot study of the synergy between two antimicrobial peptides and two common antibiotics. *Antibiotics* 2019;**8**. DOI: [10.3390/antibiotics8020060](https://doi.org/10.3390/antibiotics8020060).
- Kaneko Ai, Uenishi K, Maruyama Y et al. A solute-binding protein in the closed conformation induces ATP hydrolysis in a bacterial ATP-binding cassette transporter involved in the import of alginate. *J Biol Chem* 2017;**292**:15681–90.
- Kang X, Elson C, Penfield J et al. Integrated solid-state NMR and molecular dynamics modeling determines membrane insertion of human  $\beta$ -defensin analog. *Commun Biol* 2019;**2**:1–10.
- Kawano K, Yoneya T, Miyata T et al. Antimicrobial peptide, tachyplesin I, isolated from hemocytes of the horseshoe crab (*Tachyplesus tridentatus*). NMR determination of the beta-sheet structure. *J Biol Chem* 1990;**265**:15365–7.
- Keo T, Collins J, Kunwar P et al. Campylobacter capsule and lipooligosaccharide confer resistance to serum and cationic antimicrobials. *Virulence* 2011;**2**:30–40.
- Kintses B, Méhi O, Ari E et al. Phylogenetic barriers to horizontal transfer of antimicrobial peptide resistance genes in the human gut microbiota. *Nat Microbiol* 2019;**4**:447–58.
- Klein S, Lorenzo C, Hoffmann S et al. Adaptation of *Pseudomonas aeruginosa* to various conditions includes tRNA-dependent for-

- mation of alanyl-phosphatidylglycerol. *Mol Microbiol* 2009;**71**. DOI: 10.1111/j.1365-2958.2008.06562.x.
- Klenotic, PA, Moseng MA, Morgan CE et al. Structural and functional diversity of resistance-nodulation-cell division transporters. *Chem Rev* 2021;**121**:5378–416.
- Kobayashi N, Nishino K, Yamaguchi A. Novel macrolide-specific ABC-type efflux transporter in *Escherichia coli*. *J Bacteriol* 2001;**183**: 5639–44. <https://jb.asm.org/content/183/19/5639.short>.
- Kobayashi N, Tamura N, van Veen HW et al.  $\beta$ -lactam selectivity of multidrug transporters AcrB and AcrD resides in the proximal binding pocket. *J Biol Chem* 2014;**289**:10680–90.
- Kobylka J, Kuth MS, Müller RT et al. AcrB: a mean, keen, drug efflux machine. *Ann NY Acad Sci* 2020;**1459**:38–68.
- Koehbach J, Craik DJ. The vast structural diversity of antimicrobial peptides. *Trends Pharmacol Sci* 2019;**40**:517–28.
- Koo HB, Seo J. Antimicrobial peptides under clinical investigation. *Pept Sci* 2019;**111**:715.
- Kooi C, Sokol PA. *Burkholderia cenocepacia* zinc metalloproteases influence resistance to antimicrobial peptides. *Microbiology* 2009;**155**:2818–25.
- Koprivnjak T, Peschel A. Bacterial resistance mechanisms against host defense peptides. *Cell Mol Life Sci* 2011;**68**:2243–54.
- Koronakis V, Sharff A, Koronakis E et al. Crystal structure of the bacterial membrane protein TolC central to multidrug efflux and protein export. *Nature* 2000;**405**:914–19.
- Kovach MA, Ballinger MN, Newstead MW et al. Cathelicidin-related antimicrobial peptide is required for effective lung mucosal immunity in Gram-negative bacterial pneumonia. *J Immunol* 2012;**189**:304–11.
- Krizsan A, Knappe D, Hoffmann R. Influence of the yjIL-mdtM gene cluster on the antibacterial activity of proline-rich antimicrobial peptides overcoming *Escherichia coli* resistance induced by the missing SbmA transporter system. *Antimicrob Agents Chemother* 2015;**59**:5992–98.
- Krulwich TA, Lewinson O, Padan E et al. Do physiological roles foster persistence of drug/multidrug-efflux transporters? A case study. *Nat Rev Microbiol* 2005;**3**:566–72.
- Kumariya R, Garsa AK, Rajput YS et al. Bacteriocins: classification, synthesis, mechanism of action and resistance development in food spoilage causing bacteria. *Microb Pathog* 2019;**128**:171–77.
- Kushibiki T, Kamiya M, Aizawa T et al. Interaction between tachyplesin I, an antimicrobial peptide derived from horseshoe crab, and lipopolysaccharide. *Biochim Biophys Acta* 2014;**1844**:527–34.
- Kwa A, Kasiakou SK, Tam VH et al. Polymyxin B: similarities to and differences from colistin (polymyxin E). *Expert Rev Anti Infect Ther* 2007;**5**:811–21.
- Lai Y, Villaruz AE, Li M et al. The human anionic antimicrobial peptide dermcidin induces proteolytic defence mechanisms in staphylococci. *Mol Microbiol* 2007;**63**:497–506.
- Le C-F, Fang C-M, Sekaran SD. Intracellular targeting mechanisms by antimicrobial peptides. *Antimicrob Agents Chemother* 2017;**61**. DOI: 10.1128/AAC.02340-16.
- Lee H, Hsu F-Fu, Turk J et al. The PmrA-regulated PmrC gene mediates phosphoethanolamine modification of lipid a and polymyxin resistance in *Salmonella enterica*. *J Bacteriol* 2004;**186**:4124–33.
- Lee M-T, Sun T-L, Hung W-C et al. Process of inducing pores in membranes by melittin. *Proc Natl Acad Sci* 2013;**110**:14243–48.
- Lee S, Trinh THT, Yoo M et al. Self-assembling peptides and their application in the treatment of diseases. *Int J Mol Sci* 2019;**20**. DOI: 10.3390/ijms20235850.
- Lei H-T, Chou T-H, Su C-C et al. Crystal structure of the open state of the *Neisseria gonorrhoeae* MtrE outer membrane channel. *PLoS ONE* 2014;**9**:e97475.
- Letoffe S, Delepelaire P, Wandersman C. The housekeeping dipeptide permease is the *Escherichia coli* heme transporter and functions with two optional peptide binding proteins. *Proc Natl Acad Sci* 2006;**103**. DOI: 10.1073/pnas.0605440103.
- Lewis LA, Choudhury B, Balthazar JT. Phosphoethanolamine substitution of lipid a and resistance of *Neisseria gonorrhoeae* to cationic antimicrobial peptides and complement-mediated killing by normal human serum. *Infection* 2009;**77**:1112–20. <https://iai.asm.org/content/77/3/1112.short>.
- Lin M-F, Lin Y-Y, Lan C-Yu. Contribution of EmrAB efflux pumps to colistin resistance in *Acinetobacter baumannii*. *J Microbiol* 2017;**55**:130–36.
- Lister IM, Raftery C, Mecsas J et al. *Yersinia pestis* AcrAB-TolC in antibiotic resistance and virulence. *Antimicrob Agents Chemother* 2012;**56**:1120–23.
- Liu L, Ganz T. The pro region of human neutrophil defensin contains a motif that is essential for normal subcellular sorting. *Blood* 1995;**85**:1095–103.
- Liu Y, Ding S, Shen J et al. Nonribosomal antibacterial peptides that target multidrug-resistant bacteria. *Nat Prod Rep* 2019;**36**:573–92.
- Lombardi L, Falanga A, Del Genio V et al. A new hope: self-assembling peptides with antimicrobial activity. *Pharmaceutics* 2019;**11**. DOI: 10.3390/pharmaceutics11040166.
- López-Solanilla E, García-Olmedo F, Rodríguez-Palenzuela P. Inactivation of the sapA to sapF locus of *Erwinia chrysanthemi* reveals common features in plant and animal bacterial pathogenesis. *Plant Cell* 1998;**10**:917–24.
- Lou S, Wang X, Yu Z et al. Peptide tectonics: encoded structural complementarity dictates programmable self-assembly. *Adv Sci* 2019;**6**:1802043.
- Lu J, Xu H, Xia J et al. D- and Unnatural amino acid substituted antimicrobial peptides with improved proteolytic resistance and their proteolytic degradation characteristics. *Front Microbiol* 2020;**11**:563030. DOI: 10.3389/fmicb.2020.563030.
- Lubelski J, Konings WN, Driessen AJM. Distribution and physiology of ABC-type transporters contributing to multidrug resistance in bacteria. *Microbiol Mol Biol Rev* 2007;**71**. DOI: 10.1128/mmr.00001-07.
- Lucca AJD, De Lucca AJ, Cleveland TE et al. Plant-derived antifungal proteins and peptides. *Canadian Journal of Microbiology* 2005;**51**:1001–14.
- Lukacik P, Owen CD, Harris G et al. The structure of nontypeable *Haemophilus influenzae* SapA in a closed conformation reveals a constricted ligand-binding cavity and a novel RNA binding motif. *PLoS ONE* 2021;**16**:e0256070.
- Lupp C, Hancock REW, Ruby EG. The *Vibrio fischeri* sapABCDF locus is required for normal growth, both in culture and in symbiosis. *Arch Microbiol* 2002;**179**:57–65.
- Lysenko ES, Gould J, Bals R et al. Bacterial phosphorylcholine decreases susceptibility to the antimicrobial peptide LL-37/hCAP18 expressed in the upper respiratory tract. *Infect Immun* 2000;**68**:1664–71.
- Lyu M, Moseng MA, Reimche JL et al. Cryo-EM structures of a gonococcal multidrug efflux pump illuminate a mechanism of drug recognition and resistance. *Mbio* 2020;**11**. DOI: 10.1128/mbio.00996-20.
- Ma M, Lustig M, Salem M et al. MexAB-OprM efflux pump interaction with the peptidoglycan of *Escherichia coli* and *Pseudomonas aeruginosa*. *Int J Mol Sci* 2021;**22**. DOI: 10.3390/ijms22105328.
- Macleod T, Ward J, Alase AA et al. Antimicrobial peptide LL-37 facilitates intracellular uptake of RNA aptamer apt 21-2 without inducing an inflammatory or interferon response. *Front Immunol* 2019;**10**:857.



- Mah TF, O'Toole GA. Mechanisms of biofilm resistance to antimicrobial agents. *Trends Microbiol* 2001;**9**:34–39.
- Mahlapuu M, Håkansson J, Ringstad L et al. Antimicrobial peptides: an emerging category of therapeutic agents. *Front Cell Infect Microbiol* 2016;**6**:194.
- Malanovic N, Lohner K. Antimicrobial peptides targeting Gram-positive bacteria. *Pharmaceuticals* 2016;**9**. DOI: [10.3390/ph9030059](https://doi.org/10.3390/ph9030059).
- Malinverni JC, Silhavy TJ. An ABC transport system that maintains lipid asymmetry in the Gram-negative outer membrane. *Proc Natl Acad Sci* 2009;**106**:8009–14.
- Maness MJ, Sparling PF. Multiple antibiotic resistance due to a single mutation in *Neisseria gonorrhoeae*. *J Infect Dis* 1973;**128**:321–30.
- Mani R, Cady SD, Tang M et al. Membrane-dependent oligomeric structure and pore formation of a beta-hairpin antimicrobial peptide in lipid bilayers from solid-state NMR. *Proceedings of the National Academy of Sciences* 2006;**103**:16242–7.
- Marceau M, Sebbane F, Collyn F et al. Function and regulation of the Salmonella-like pmrF antimicrobial peptide resistance operon in *Yersinia pseudotuberculosis*. *Adv Exp Med Biol* 2003;**529**:253–56.
- Mardirossian M, Pérébaskine N, Benincasa M et al. The dolphin proline-rich antimicrobial peptide Tur1A inhibits protein synthesis by targeting the bacterial ribosome. *Cell Chem Biol* 2018;**25**:530–39.e7.
- Marshall RL, Bavro VN. Mutations in the TolC periplasmic domain affect substrate specificity of the AcrAB-TolC pump. *Front Mol Biosci* 2020;**7**:166.
- Marshall RL, Lloyd GS, Lawler AJ et al. New multidrug efflux inhibitors for Gram-negative bacteria. *Mbio* 2020;**11**. DOI: [10.1128/mBio.01340-20](https://doi.org/10.1128/mBio.01340-20).
- Martynowycz MW, Rice A, Andreev K et al. Salmonella membrane structural remodeling increases resistance to antimicrobial peptide LL-37. *ACS Infect Dis* 2019;**5**:1214–22.
- Mason KM, Bruggeman ME, Munson RS et al. The non-typeable *Haemophilus influenzae* Sap transporter provides a mechanism of antimicrobial peptide resistance and SapD-dependent potassium acquisition. *Mol Microbiol* 2006;**62**:1357–72.
- Mason KM, Munson RS, Bakaletz LO. A mutation in the Sap operon attenuates survival of nontypeable *Haemophilus influenzae* in a chinchilla model of otitis media. *Infect Immun* 2005;**73**:599–608.
- Mason KM, Raffel FK, Ray WC et al. Heme utilization by nontypeable *Haemophilus influenzae* is essential and dependent on sap transporter function. *J Bacteriol* 2011;**193**:2527–35.
- Masuda N, Sakagawa E, Ohya S et al. Substrate specificities of MexAB-OprM, MexCD-OprJ, and MexXY-oprM efflux pumps in *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* 2000;**44**:3322–27.
- Matamouros S, Miller SI. *S. Typhimurium* strategies to resist killing by cationic antimicrobial peptides. *Biochim Biophys Acta Biomemb* 2015;**1848**:3021–25.
- Matsuzaki K, Yoneyama S, Miyajima K. Pore formation and translocation of melittin. *Biophys J* 1997;**73**:831–38.
- McCormick JW, Ammerman L, Chen G et al. Transport of Alzheimer's associated amyloid- $\beta$  catalyzed by P-glycoprotein. *PLoS ONE* 2021;**16**:e0250371.
- McCoy AJ, Liu H, Falla TJ et al. Identification of *Proteus mirabilis* mutants with increased sensitivity to antimicrobial peptides. *Antimicrob Agents Chemother* 2001;**45**:2030–37.
- Melo MN, Ferre R, Castanho MARB. Antimicrobial peptides: linking partition, activity and high membrane-bound concentrations. *Nat Rev Microbiol* 2009;**7**:245–50.
- Mihajlovic M, Lazaridis T. Antimicrobial peptides in toroidal and cylindrical pores. *Biochim Biophys Acta Biomemb* 2010;**1798**:1485–93.
- Mikolosko J, Bobyk K, Zgurskaya HI et al. Conformational flexibility in the multidrug efflux system protein acrA. *Structure* 2006;**14**:577–87.
- Mitchell CJ, Stone TA, Deber CM. Peptide-based efflux pump inhibitors of the small multidrug resistance protein from *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* 2019;**63**. DOI: [10.1128/AAC.00730-19](https://doi.org/10.1128/AAC.00730-19).
- Morizane S, Yamasaki K, Kabigting FD et al. Kallikrein Expression and Cathelicidin Processing Are Independently Controlled in Keratinocytes by Calcium, Vitamin D3, and Retinoic Acid. *J Invest Dermatol* 2010;**130**:1297–306.
- Moskowitz SM, Ernst RK, Miller SI. PmrAB, a two-component regulatory system of *Pseudomonas aeruginosa* that modulates resistance to cationic antimicrobial peptides and addition of aminoarabinose to lipid A. *J Bacteriol* 2004;**186**:575–79.
- Mount KLB, Townsend CA, Bauer ME. *Haemophilus ducreyi* is resistant to human antimicrobial peptides. *Antimicrob Agents Chemother* 2007;**51**:3391–93.
- Mount KLB, Townsend CA, Rinker SD et al. *Haemophilus ducreyi* SapA contributes to cathelicidin resistance and virulence in humans. *Infect Immun* 2010;**78**:1176–84.
- Murakami S, Nakashima R, Yamashita E et al. Crystal structure of bacterial multidrug efflux transporter acrB. *Nature* 2002;**419**:587–93.
- Murakami S, Nakashima R, Yamashita E et al. Crystal structures of a multidrug transporter reveal a functionally rotating mechanism. *Nature* 2006;**443**:173–79.
- Naito M, Fridrich E, Fields JA et al. Effects of sequential *Campylobacter jejuni* 81-176 lipooligosaccharide core truncations on biofilm formation, stress survival, and pathogenesis. *J Bacteriol* 2010;**192**:2182–92.
- Nakamura T, Yamamuro N, Stumpe S et al. Cloning of the trkAH gene cluster and characterization of the trk k<sup>-</sup>uptake system of *Vibrio alginolyticus*. *Microbiology* 1998;**144**. DOI: [10.1099/00221287-144-8-2281](https://doi.org/10.1099/00221287-144-8-2281).
- Nakashima R, Sakurai K, Yamasaki S et al. Structural basis for the inhibition of bacterial multidrug exporters. *Nature* 2013;**500**:102–6.
- Nakashima R, Sakurai K, Yamasaki S et al. Structures of the multidrug exporter AcrB reveal a proximal multisite drug-binding pocket. *Nature* 2011;**480**. DOI: [10.1038/nature10641](https://doi.org/10.1038/nature10641).
- Nawrot R, Barylski J, Nowicki G et al. Plant antimicrobial peptides. *Folia Microbiol* 2014;**59**:181–96.
- Nguyen LT, Haney EF, Vogel HJ. The expanding scope of antimicrobial peptide structures and their modes of action. *Trends Biotechnol* 2011;**29**:464–72.
- Orwa JA, Govaerts C, Busson R et al. Isolation and structural characterization of polymyxin B components. *J Chromatogr A* 2001;**912**:369–73.
- Oswald C, Tam H-K, Pos KM. Transport of lipophilic carboxylates is mediated by transmembrane helix 2 in multidrug transporter acrB. *Nat Commun* 2016;**7**:13819.
- Padilla E, Llobet E, Doménech-Sánchez A et al. *Klebsiella pneumoniae* AcrAB efflux pump contributes to antimicrobial resistance and virulence. *Antimicrob Agents Chemother* 2010;**54**:177–83.
- Pamp SJ, Gjermansen M, Johansen HK et al. Tolerance to the antimicrobial peptide colistin in *Pseudomonas aeruginosa* biofilms is linked to metabolically active cells, and depends on the pmr and mexAB-oprM genes. *Mol Microbiol* 2008;**68**. DOI: [10.1111/j.1365-2958.2008.06152.x](https://doi.org/10.1111/j.1365-2958.2008.06152.x).

- Parra-Lopez C, Baer MT, Groisman EA. Molecular genetic analysis of a locus required for resistance to antimicrobial peptides in *Salmonella typhimurium*. *EMBO J* 1993;**12**:4053–62.
- Parra-Lopez C, Lin R, Aspedon A et al. A salmonella protein that is required for resistance to antimicrobial peptides and transport of potassium. *EMBO J* 1994;**13**:3964–72.
- Peschel A, Sahl H-G. The co-evolution of host cationic antimicrobial peptides and microbial resistance. *Nat Rev Microbiol* 2006;**4**:529–36.
- Pettersen EF, Goddard TD, Huang CC et al. UCSF chimera—a visualization system for exploratory research and analysis. *J Comput Chem* 2004;**25**:1605–12.
- Pizzolato-Cezar LR, Okuda-Shinagawa NM, Teresa Machini M. Combinatory therapy antimicrobial peptide-antibiotic to minimize the ongoing rise of resistance. *Front Microbiol* 2019;**10**:1703.
- Porcelli F, Verardi R, Shi L et al. NMR structure of the cathelicidin-derived human antimicrobial peptide LL-37 in dodecylphosphocholine micelles. *Biochemistry* 2008;**47**:5565–72.
- Qu XD, Harwig SS, Shafer WM et al. Protegrin structure and activity against *Neisseria gonorrhoeae*. *Infect Immun* 1997;**65**:636–9.
- Rahman T, Yarnall B, Doyle DA. Efflux drug transporters at the forefront of antimicrobial resistance. *Eur Biophys J* 2017;**46**:647–53.
- Ramaswamy VK, Vargiu AV, Mallocci G et al. Molecular rationale behind the differential substrate specificity of bacterial RND multidrug transporters. *Sci Rep* 2017;**7**:8075.
- Ramesh S, Govender T, Kruger HG et al. Short antimicrobial peptides (SAMPs) as a class of extraordinary promising therapeutic agents. *J Pept Sci* 2016;**22**:438–51.
- Raviv Y, Pollard HB, Bruggemann EP et al. Photosensitized labeling of a functional multidrug transporter in living drug-resistant tumor cells. *J Biol Chem* 1990;**265**:3975–80.
- Rees DC, Johnson E, Lewinson O. ABC transporters: the power to change. *Nat Rev Mol Cell Biol* 2009;**10**:218–27.
- Rice AJ, Park A, Pinkett HW. Diversity in ABC transporters: type I, II and III importers. *Crit Rev Biochem Mol Biol* 2014;**49**:426–37.
- Rieg S, Huth A, Kalbacher H et al. Resistance against antimicrobial peptides is independent of *Escherichia coli* AcrAB, *Pseudomonas aeruginosa* MexAB and *Staphylococcus aureus* NorA efflux pumps. *Int J Antimicrob Agents* 2009;**33**. DOI: [10.1016/j.ijantimicag.2008.07.032](https://doi.org/10.1016/j.ijantimicag.2008.07.032).
- Rinker SD, Gu X, Fortney KR et al. Permeases of the Sap transporter are required for cathelicidin resistance and virulence of *Haemophilus ducreyi* in humans. *J Infect Dis* 2012;**206**:1407–14.
- Rinker SD, Trombley MP, Gu X et al. Deletion of MtrC in *Haemophilus ducreyi* increases sensitivity to human antimicrobial peptides and activates the CpxRA regulon. *Infect Immun* 2011;**79**:2324–34.
- Rios AC, Moutinho CG, Pinto FC et al. Alternatives to overcoming bacterial resistances: state-of-the-Art. *Microbiol Res* 2016;**191**: 51–80.
- Roque A, Ponte I, Suau P. Secondary structure of protamine in sperm nuclei: an infrared spectroscopy study. *BMC Struct Biol* 2011;**11**:14.
- Rozek A, Friedrich CL, Hancock RE. Structure of the bovine antimicrobial peptide indolicidin bound to dodecylphosphocholine and sodium dodecyl sulfate micelles. *Biochemistry* 2000;**39**: 15765–74.
- Ruden S, Rieder A, Ster IC et al. Synergy pattern of short cationic antimicrobial peptides against multidrug-resistant *Pseudomonas aeruginosa*. *Front Microbiol* 2019;**10**:2740.
- Rusch SL, Kendall DA. Interactions that drive Sec-dependent bacterial protein transport. *Biochemistry* 2007;**46**:9665–73.
- Saier MH, Jr CVT, Barabote RD. TCDB: the transporter classification database for membrane transport protein analyses and information. *Nucleic Acids Res* 2006;**34**:D181–86.
- Sakurai K, Yamasaki S, Nakao K et al. Crystal structures of multidrug efflux pump MexB bound with high-molecular-mass compounds. *Sci Rep* 2019;**9**:4359.
- Sancho-Vaello E, François P, Bonetti E-J et al. Structural remodeling and oligomerization of human cathelicidin on membranes suggest fibril-like structures as active species. *Sci Rep* 2017;**7**:15371.
- Sancho-Vaello E, Gil-Carton D, François P et al. The structure of the antimicrobial human cathelicidin LL-37 shows oligomerization and channel formation in the presence of membrane mimics. *Sci Rep* 2020;**10**:17356.
- Saw HTH, Webber MA, Mushtaq S et al. Inactivation or inhibition of AcrAB-TolC increases resistance of carbapenemase-producing enterobacteriaceae to carbapenems. *J Antimicrob Chemother* 2016;**71**:1510–19.
- Schitteck B, Hipfel R, Sauer B et al. Dermcidin: a novel human antibiotic peptide secreted by sweat glands. *Nat Immunol* 2001;**2**:1133–37.
- Schmidtchen A, Frick I-M, Andersson E et al. Proteinases of common pathogenic bacteria degrade and inactivate the antibacterial peptide LL-37. *Mol Microbiol* 2002;**46**:157–68.
- Schmidtchen A, Frick I-M, Björck L. Dermatan sulphate is released by proteinases of common pathogenic bacteria and inactivates antibacterial alpha-defensin. *Mol Microbiol* 2001;**39**. DOI: [10.1046/j.1365-2958.2001.02251.x](https://doi.org/10.1046/j.1365-2958.2001.02251.x).
- Schneider E, Hunke S. ATP-Binding-Cassette (ABC) transport systems: functional and structural aspects of the ATP-hydrolyzing subunits/domains. *FEMS Microbiol Rev* 1998;**22**:1–20.
- Seefeldt AC, Nguyen F, Antunes S et al. The proline-rich antimicrobial peptide onc112 inhibits translation by blocking and destabilizing the initiation complex. *Nat Struct Mol Biol* 2015;**22**:470–75.
- Seeger MA, Schiefner A, Eicher T et al. Structural asymmetry of AcrB trimer suggests a peristaltic pump mechanism. *Science* 2006;**313**:1295–98.
- Segura A, Moreno M, Madueño F et al. Snakin-1, a peptide from potato that is active against plant pathogens. *Mol Plant Microbe Interact* 1999;**12**:16–23.
- Sennhauser G, Bukowska MA, Briand C et al. Crystal structure of the multidrug exporter MexB from *Pseudomonas aeruginosa*. *J Mol Biol* 2009;**389**:134–45.
- Shafee TMA, Lay FT, Phan TK et al. Convergent evolution of defensin sequence, structure and function. *Cell Mol Life Sci* 2017;**74**:663–82.
- Shafer WM, Qu X, Waring AJ et al. Modulation of *Neisseria gonorrhoeae* susceptibility to vertebrate antibacterial peptides due to a member of the Resistance/nodulation/division efflux pump family. *Proc Natl Acad Sci* 1998;**95**:1829–33.
- Shahmiri M, Enciso M, Adda CG et al. Membrane Core-Specific Antimicrobial Action of Cathelicidin LL-37 Peptide Switches Between Pore and Nanofibre Formation. *Sci Rep* 2016;**6**: 38184.
- Shelton CL, Raffel FK, Beatty WL et al. Sap transporter mediated import and subsequent degradation of antimicrobial peptides in *haemophilus*. *PLoS Pathog* 2011;**7**:e1002360.
- Shi X, Chen M, Yu Z et al. In situ structure and assembly of the multidrug efflux pump AcrAB-TolC. *Nat Commun* 2019;**10**:2635.
- Silhavy TJ, Kahne D, Walker S. The bacterial cell envelope. *Cold Spring Harb Perspect Biol* 2010;**2**:a000414.
- Sjuts H, Vargiu AV, Kwasny SM et al. Molecular basis for inhibition of AcrB multidrug efflux pump by novel and powerful pyranopyridine derivatives. *Proc Natl Acad Sci* 2016;**113**:3509–14.
- Soltani S, Hammami R, Cotter PD et al. Bacteriocins as a new generation of antimicrobials: toxicity aspects and regulations. *FEMS Microbiol Rev* 2021;**45**. DOI: [10.1093/femsre/fuaa039](https://doi.org/10.1093/femsre/fuaa039).

- Son DJ, Lee JW, Lee YH et al. Therapeutic application of anti-arthritis, pain-releasing, and anti-cancer effects of bee venom and its constituent compounds. *Pharmacol Ther* 2007;**115**:246–70.
- Song C, Weichbrodt C, Salnikow ES et al. Crystal structure and functional mechanism of a human antimicrobial membrane channel. *Proc Natl Acad Sci* 2013;**110**:4586–91.
- Sørensen OE, Follin P, Johnsen AH et al. Sørensen OE, Follin P, Johnsen AH et al. Human cathelicidin, hCAP-18, is processed to the antimicrobial peptide LL-37 by extracellular cleavage with proteinase 3. *Blood* 2001;**97**:3951–9.
- Spinosa MR, Progida C, Talà A et al. The *Neisseria meningitidis* capsule is important for intracellular survival in human cells. *Infect Immun* 2007;**75**:3594–603.
- Spohn R, Daruka L, Lázár V et al. Integrated evolutionary analysis reveals antimicrobial peptides with limited resistance. *Nat Commun* 2019;**10**:4538.
- Stamer TD, Swords WE, Apicella MA et al. Susceptibility of non-typeable *Haemophilus influenzae* to human  $\beta$ -defensins is influenced by lipooligosaccharide acylation. *Infect Immun* 2002;**70**. DOI: [10.1128/iai.70.9.5287-5289.2002](https://doi.org/10.1128/iai.70.9.5287-5289.2002).
- Steenbergen JN, Mohr JF, Thorne GM. Effects of daptomycin in combination with other antimicrobial agents: a review of in vitro and animal model studies. *J Antimicrob Chemother* 2009;**64**:1130–38.
- Steiner H, Hultmark D, Engström Å et al. Sequence and specificity of two antibacterial proteins involved in insect immunity. *Nature* 1981;**292**:246–8.
- Stumpe S, Schmid R, Stephens DL et al. Identification of OmpT as the protease that hydrolyzes the antimicrobial peptide protamine before it enters growing cells of *Escherichia coli*. *J Bacteriol* 1998;**180**:4002–6.
- Subbalakshmi C, Sitaram N. Mechanism of antimicrobial action of indolicidin. *FEMS Microbiol Lett* 1998;**160**:91–96.
- Sugiyama Y, Nakamura A, Matsumoto M et al. A novel putrescine exporter SapBCDF of *Escherichia coli*. *J Biol Chem* 2016;**291**:26343–51.
- Symmons MF, Bokma E, Koronakis E et al. The assembled structure of a complete tripartite bacterial multidrug efflux pump. *Proc Natl Acad Sci* 2009;**106**:7173–78.
- Symmons MF, Marshall RL, Bavro VN. Architecture and roles of periplasmic adaptor proteins in tripartite efflux assemblies. *Front Microbiol* 2015;**6**:513.
- Tal N, Schuldiner S. A coordinated network of transporters with overlapping specificities provides a robust survival strategy. *Proc Natl Acad Sci* 2009;**106**:9051–56.
- Tam H-K, Foong WE, Oswald C et al. Allosteric drug transport mechanism of multidrug transporter AcrB. *Nat Commun* 2021;**12**:3889.
- Tam H-K, Malviya VN, Foong W-E et al. Binding and transport of carboxylated drugs by the multidrug transporter acrB. *J Mol Biol* 2020;**432**:861–77.
- Tamayo R, Choudhury B, Septer A et al. Identification of CptA, a PmrA-regulated locus required for phosphoethanolamine modification of the *Salmonella enterica* serovar typhimurium lipopolysaccharide core. *J Bacteriol* 2005;**187**:3391–99.
- Tamburrino G, Llabrés S, Vickery ON et al. Modulation of the *Neisseria gonorrhoeae* drug efflux conduit mtrE. *Sci Rep* 2017;**7**:17091.
- Tanaka KJ, Song S, Mason K et al. Selective substrate uptake: the role of ATP-Binding Cassette (ABC) importers in pathogenesis. *Biochim Biophys Acta Biomemb* 2018;**1860**:868–77.
- Terwilliger TC, Eisenberg D. The structure of melittin. II. Interpretation of the structure. *J Biol Chem* 1982;**257**:6016–22. <https://www.ncbi.nlm.nih.gov/pubmed/7076662>.
- Theuretzbacher U, Outtersson K, Engel A et al. The global preclinical antibacterial pipeline. *Nat Rev Microbiol* 2019;**18**:275–85.
- Thomas C, Aller SG, Beis K et al. Structural and functional diversity calls for a new classification of ABC transporters. *FEBS Lett* 2020;**594**:3767–75.
- Thomas C, Tampé R. Structural and mechanistic principles of ABC transporters. *Annu Rev Biochem* 2020;**89**:605–36.
- Thomas C, Tampé R. Multifaceted structures and mechanisms of ABC transport systems in health and disease. *Curr Opin Struct Biol* 2018;**51**. DOI: [10.1016/j.sbi.2018.03.016](https://doi.org/10.1016/j.sbi.2018.03.016).
- Thomassin J-L, Brannon JR, Gibbs BF et al. OmpT outer membrane proteases of enterohemorrhagic and enteropathogenic *Escherichia coli* contribute differently to the degradation of human LL-37. *Infect Immun* 2012;**80**:483–92.
- Tran AnX, Whittimore JD, Wyrick PB et al. The lipid a 1-phosphatase of *Helicobacter pylori* is required for resistance to the antimicrobial peptide polymyxin. *J Bacteriol* 2006;**188**:4531–41.
- Trombley MP, Post DMB, Rinker SD et al. Phosphoethanolamine transferase LptA in *Haemophilus ducreyi* modifies lipid a and contributes to human defensin resistance in vitro. *PLoS ONE* 2015;**10**. DOI: [10.1371/journal.pone.0124373](https://doi.org/10.1371/journal.pone.0124373).
- Tsakazaki T, Mori H, Echizen Y et al. Structure and function of a membrane component SecDF that enhances protein export. *Nature* 2011;**474**:235–38.
- Tsutsumi K, Yonehara R, Ishizaka-Ikeda E et al. Structures of the wild-type MexAB-OprM tripartite pump reveal its complex formation and drug efflux mechanism. *Nat Commun* 2019;**10**:1520.
- Tzeng Y-L, Ambrose KD, Zughayer S et al. Cationic antimicrobial peptide resistance in *Neisseria meningitidis*. *J Bacteriol* 2005;**187**:5387–96.
- Tzeng Y-L, Berman Z, Toh E et al. Heteroresistance to the model antimicrobial peptide polymyxin b in the emerging *Neisseria meningitidis* lineage 11.2 urethritis clade: mutations in the pilMNOPQ operon. *Mol Microbiol* 2019;**111**:254–68.
- Valore EV, Martin E, Harwig SS et al. Intramolecular inhibition of human defensin HNP-1 by its propiece. *J Clin Invest* 1996;**97**:1624–9.
- Van Den Hooven HW, Doeland CC, Van De Kamp M et al. Three-dimensional structure of the lantibiotic nisin in the presence of membrane-mimetic micelles of dodecylphosphocholine and of sodium dodecylsulphate. *Eur J Biochem* 1996;**235**:382–93.
- Vandamme D, Landuyt B, Luyten W et al. A comprehensive summary of LL-37, the factotum human cathelicidin peptide. *Cell Immunol* 2012;**280**:22–35.
- Vargiu AV, Nikaido H. Multidrug binding properties of the AcrB efflux pump characterized by molecular dynamics simulations. *Proc Natl Acad Sci* 2012;**109**:20637–42.
- Vargiu AV, Ruggerone P, Opperman TJ et al. Molecular mechanism of MBX2319 inhibition of *Escherichia coli* AcrB multidrug efflux pump and comparison with other inhibitors. *Antimicrob Agents Chemother* 2014;**58**. DOI: [10.1128/aac.03283-14](https://doi.org/10.1128/aac.03283-14).
- Wade Warrant R, Kim S-H.  $\alpha$ -Helix-double helix interaction shown in the structure of a protamine-transfer RNA complex and a nucleoprotamine model. *Nature* 1978;**271**:130–5.
- Wang G, Zietz CM, Mudgapalli A et al. The evolution of the antimicrobial peptide database over 18 years: milestones and new features. *Protein Sci* 2022;**31**:92–106.
- Wang G. Structures of human host defense cathelicidin LL-37 and its smallest antimicrobial peptide KR-12 in lipid micelles. *J Biol Chem* 2008;**283**:32637–43.
- Wang R, Braughton KR, Kretschmer D et al. Identification of novel cytolytic peptides as key virulence determinants for community-associated MRSA. *Nat Med* 2007;**13**:1510–14.

- Wang W, Smith DK, Moulding K et al. The dependence of membrane permeability by the antibacterial peptide cecropin B and its analogs, CB-1 and CB-3, on liposomes of different composition. *J Biol Chem* 1998;**273**:27438–48.
- Wang X, Preston JF, Romeo T. The pgaABCD locus of *Escherichia coli* promotes the synthesis of a polysaccharide adhesin required for biofilm formation. *J Bacteriol* 2004;**186**:2724–34.
- Wang Z, Bie P, Cheng J et al. The ABC transporter YejABEF is required for resistance to antimicrobial peptides and the virulence of *Brucella melitensis*. *Sci Rep* 2016;**6**:31876. DOI: [10.1038/srep31876](https://doi.org/10.1038/srep31876).
- Wang Z, Fan G, Hryc CF et al. An allosteric transport mechanism for the AcrAB-TolC multidrug efflux pump. *Elife* 2017;**6**. DOI: [10.7554/eLife.24905](https://doi.org/10.7554/eLife.24905).
- Wang Z, Wang G. APD: the antimicrobial peptide database. *Nucleic Acids Res* 2004;**32**:D590–92.
- Warner DM, Levy SB. Different effects of transcriptional regulators MarA, SoxS and rob on susceptibility of *Escherichia coli* to cationic antimicrobial peptides (CAMPs): rob-dependent CAMP induction of the marRAB operon. *Microbiology* 2010;**156**:570–78.
- Warner DM, Shafer WM, Jerse AE. Clinically relevant mutations that cause derepression of the *Neisseria gonorrhoeae* MtrC-MtrD-MtrE efflux pump system confer different levels of antimicrobial resistance and in vivo fitness. *Mol Microbiol* 2008;**70**:462–78.
- Weatherspoon-Griffin N, Yang D, Kong W et al. The CpxR/CpxA two-component regulatory system up-regulates the multidrug resistance cascade to facilitate *Escherichia coli* resistance to a model antimicrobial peptide. *J Biol Chem* 2014;**289**:32571–82.
- Wiedemann I, Breukink E, van Kraaij C et al. Specific binding of nisin to the peptidoglycan precursor lipid II combines pore formation and inhibition of cell wall biosynthesis for potent antibiotic activity. *J Biol Chem* 2001;**276**:1772–9.
- Willey JM, van der Donk WA. Lantibiotics: peptides of diverse structure and function. *Annu Rev Microbiol* 2007;**61**:477–501.
- Wimley WC Describing the mechanism of antimicrobial peptide action with the interfacial activity model. *ACS Chem Biol* 2010;**5**:905–17.
- Wu C-L, Hsueh Ju-Y, Yip B-S et al. Antimicrobial peptides display strong synergy with vancomycin against vancomycin-resistant *E. faecium*, *S. aureus*, and wild-type *E. coli*. *Int J Mol Sci* 2020;**21**. DOI: [10.3390/ijms21134578](https://doi.org/10.3390/ijms21134578).
- Wu X, Li Z, Li X et al. Synergistic effects of antimicrobial peptide DP7 combined with antibiotics against multidrug-resistant bacteria. *Drug Des Dev Ther* 2017;**11**:939–46.
- Xie F, Wang Y, Li G et al. The SapA protein is involved in resistance to antimicrobial peptide PR-39 and virulence of *Actinobacillus pleuropneumoniae*. *Front Microbiol* 2017;**8**:811.
- Yang Q, Li M, Spiller OB et al. Balancing mcr-1 expression and bacterial survival is a delicate equilibrium between essential cellular defence mechanisms. *Nat Commun* 2017;**8**:2054.
- Yin Y, He X, Szweczyk P et al. Structure of the multidrug transporter EmrD from *Escherichia coli*. *Science* 2006;**312**:741–44.
- Zaslöf M Antimicrobial peptides of multicellular organisms. *Nature* 2002;**415**:389–95.
- Zavascki AP, Goldani LZ, Li J et al. Polymyxin B for the treatment of multidrug-resistant pathogens: a critical review. *Journal of Antimicrobial Chemotherapy* 2007;**60**:1206–15.
- Zeth K, Sancho-Vaello E. Structural plasticity of LL-37 indicates elaborate functional adaptation mechanisms to bacterial target structures. *Int J Mol Sci* 2021;**22**:5200.
- Zeytuni N, Dickey SW, Hu J et al. Structural insight into the *Staphylococcus aureus* ATP-Driven exporter of virulent peptide toxins. *Sci Adv* 2020;**6**. DOI: [10.1126/sciadv.abb8219](https://doi.org/10.1126/sciadv.abb8219).
- Zhang H, Pan Y, Hu L et al. TrkA undergoes a tetramer-to-dimer conversion to open TrkH which enables changes in membrane potential. *Nat Commun* 2020;**11**:547.
- Zhang Y, Lu W, Hong M. The membrane-bound structure and topology of a human  $\alpha$ -defensin indicate a dimer pore mechanism for membrane disruption. *Biochemistry* 2010;**49**:9770–82.
- Zwama M, Yamaguchi A, Nishino K. Phylogenetic and functional characterisation of the *Haemophilus influenzae* multidrug efflux pump acrB. *Commun Biol* 2019;**2**:340.
- Zwama M, Yamasaki S, Nakashima R et al. Multiple entry pathways within the efflux transporter AcrB contribute to multidrug recognition. *Nat Commun* 2018;**9**:124.