REVIEW ARTICLE

Drugs 2004; 64 (2): 159-204
0012-6667/04/0002-0159/$34.00/0

Efflux-Mediated Drug Resistance in Bacteria

Xian-Zhi Li and *Hiroshi Nikaido*

Department of Molecular and Cell Biology, University of California, Berkeley, California, USA*

Contents

Abstract . . . 160
1. Drug-Specific and Multidrug Efflux Transporters of Bacteria . . . 161
   1.1 ATP-Binding Casssette Superfamily . . . 162
   1.2 Major Facilitator Superfamily . . . 162
   1.3 Multidrug and Toxic Compound Extrusion Family . . . 163
   1.4 Small Multidrug Resistance (MDR) Family . . . 163
   1.5 Resistance-Nodulation-Cell Division Superfamily . . . 163
2. Drug Efflux in Gram-Negative Bacteria . . . 163
   2.1 *Escherichia coli* and Other Members of Enterobacteriaceae . . . 163
   2.2 *Pseudomonas aeruginosa* and Other Non-Fermentative Gram-Negative Bacteria . . . 166
      2.2.1 MexAB-OprM . . . 166
      2.2.2 MexCD-OprJ . . . 167
      2.2.3 MexEF-OprN . . . 168
      2.2.4 MexXY-OprM . . . 168
      2.2.5 MexJK-OprM and MexHI-OpmD . . . 168
      2.2.6 Organisms Related to *P. aeruginosa* . . . 168
   2.3 *Neisseria* spp . . . 169
   2.4 *Vibrio cholerae* . . . 169
3. Drug Efflux in Gram-Positive Bacteria . . . 170
   3.1 *Staphylococcus aureus* . . . 170
   3.2 Streptococci . . . 172
   3.3 Enterococci . . . 172
   3.4 *Bacillus subtilis* . . . 173
   3.5 *Lactococcus lactis* . . . 173
4. Drug Efflux in Mycobacteria . . . 173
5. Recognition of Substrates by Multidrug Efflux Transporters . . . 175
6. Components of the Tripartite Drug Transporters and Efflux Mechanisms . . . 175
   6.1 Inner Membrane Efflux Transporters . . . 176
   6.2 Membrane Fusion Proteins . . . 177
   6.3 Outer Membrane (OM) Channels . . . 177
   6.4 Tripartite Transporter Complex and Efflux Mechanisms . . . 178
7. Regulation of Drug Efflux Pump Expression . . . 178
   7.1 Local Repressors and Activators . . . 179
   7.2 Two-Component Regulatory Systems . . . 181
   7.3 Global Regulators . . . 181
8. Development of Efflux-Mediated MDR Strains and Impact on Antibacterial Therapy . . . 182
   8.1 *In Vitro* Development of Efflux-Based Resistance . . . 183
   8.2 *In Vivo* Development of Efflux-Based Resistance . . . 183

* The current affiliation for Dr Li is: Veterinary Drugs Directorate, Health Canada, Ottawa, Ontario, Canada.

8.3 Which Drugs Are Important in the Development of Efflux-Based MDR? 184
9. Interplay Between Drug Efflux and Other Resistance Mechanisms 185
9.1 Interplay Between Efflux Pumps 185
9.2 Efflux Pumps and the OM Permeability Barrier 186
9.3 Efflux Pumps and Antibacterial-Inactivating Enzymes 186
9.4 Efflux Pumps and Antibacterial Target Alterations 187
10. Overcoming Efflux Activity: The Development of Efflux Pump Inhibitors and Antibacterials that Bypass Efflux Pumps 187
10.1 Inhibitors for Drug-Specific Pumps 187
10.2 Inhibitors for Multidrug Efflux Pumps 188
10.3 Bypassing Efflux Mechanisms 189
11. Conclusions 189

Abstract

Drug resistance in bacteria, and especially resistance to multiple antibacterials, has attracted much attention in recent years. In addition to the well known mechanisms, such as inactivation of drugs and alteration of targets, active efflux is now known to play a major role in the resistance of many species to antibacterials. Drug-specific efflux (e.g. that of tetracycline) has been recognised as the major mechanism of resistance to this drug in Gram-negative bacteria. In addition, we now recognise that multidrug efflux pumps are becoming increasingly important. Such pumps play major roles in the antiseptic resistance of *Staphylococcus aureus*, and fluoroquinolone resistance of *S. aureus* and *Streptococcus pneumoniae*. Multidrug pumps, often with very wide substrate specificity, are not only essential for the intrinsic resistance of many Gram-negative bacteria but also produce elevated levels of resistance when overexpressed. Paradoxically, 'advanced' agents for which resistance is unlikely to be caused by traditional mechanisms, such as fluoroquinolones and β-lactams of the latest generations, are likely to select for overproduction mutants of these pumps and make the bacteria resistant in one step to practically all classes of antibacterial agents. Such overproduction mutants are also selected for by the use of antiseptics and biocides, increasingly incorporated into consumer products, and this is also of major concern. We can consider efflux pumps as potentially effective antibacterial targets. Inhibition of efflux pumps by an efflux pump inhibitor would restore the activity of an agent subject to efflux. An alternative approach is to develop antibacterials that would bypass the action of efflux pumps.

Antimicrobial resistance is currently of great concern. Particularly, concerns have heightened over the increasing numbers of pathogenic bacteria that display resistance to multiple antibacterials.[1,2] Biochemical mechanisms for antimicrobial resistance fall into three major categories: production of hydrolytic or modifying enzymes, alteration of targets such that they are no longer susceptible to antibacterial action, and modification of target accessibility, including permeability barrier and energy-dependent antibiotic efflux pumps. Resistance genes occur either on the chromosome of wild-type strains or are carried on elements of extraneous origin, such as R plasmids and transposons. Table I summarises the mode of action of, and resistance mechanisms for, important antibacterial agents.

Antibiotic efflux in bacteria was first reported in the late 1970s for tetracyclines,[3-6] although drug efflux, mediated by P-glycoprotein, was originally

Table I. Mode of action of antimicrobial agents and mechanisms of antimicrobial resistance

Drug	Bacterial target	Mechanism of resistance
β-Lactams	Cell wall synthesis (PBPs)	β-Lactamases, alteration of PBPs, permeability barrier, active efflux
Aminoglycosides	Protein synthesis (30S ribosome inhibitors)	Aminoglycoside-modifying enzymes (ANT, APH, AAC), alterations of ribosomes, permeability barrier, active efflux
Cationic peptides	Cell membranes	Target alterations, active efflux
Coumarins	DNA synthesis (DNA gyrase B)	Target alterations, active efflux
Chloramphenicol	Protein synthesis (50S ribosome inhibitors)	Acetyltransferase, active efflux
Isoniazid	Fatty acid synthesis	Loss of drug activation, target alteration, efflux?
Glycopeptides	Cell wall synthesis	Target alteration
Macrolides	Protein synthesis (50S ribosome inhibitors)	Target alteration, active efflux
Oxazolidinones	Protein synthesis (50S ribosome inhibitors)	Target alteration, active efflux
Polymyxins	Cell membranes	Alterations of LPS
Quinolones	DNA synthesis (DNA gyrase and topoisomerase IV)	Alterations of DNA gyrase and topoisomerase IV, active efflux
Rifamycins	RNA synthesis (DNA-dependent RNA polymerase)	Alteration of β subunit of RNA polymerase, active efflux
Streptogramins	Protein synthesis (50S ribosome inhibitors)	Target alteration
Sulfonamides	Folic acid metabolism	Target modification, target by-passing, active efflux
Tetracyclines	Protein synthesis (30S ribosome inhibitors)	Active efflux, alteration of ribosomes, drug modification
Trimethoprim	Folic acid metabolism	Target by-passing, active efflux

AAC = *N*-acetyltransferase(s); **ANT** = *O*-nucleotidyltransferase(s); **APH** = *O*-phosphotransferase(s); **LPS** = lipopolysaccharide; **PBPs** = penicillin-binding proteins.

discovered in mammalian cancer cells even earlier.[7] Since then, efflux-mediated resistance to a wide range of antibacterial agents has been reported in a variety of bacterial species, and a number of efflux determinants have been identified.

Over the past decade, efflux systems that accommodate multiple antibacterials or multidrug efflux pumps have gained much attention. When microbial genome sequences (as of 11 November 2003, 133 were available at the Institute of Genome Research (TIGR) website[8]) are inspected, very wide distribution of putative drug efflux genes is confirmed. Outside the realm of bacteria, efflux-mediated multidrug resistance (MDR) also occurs in other pathogens such as *Candida albicans* (CaMDR1) and *Plasmodium falciparum* (PfMDR). Many of the multidrug efflux systems actively pump out a variety of compounds that include not only the conventional classes of antimicrobials but also dyes, detergents and organic solvents. Thus, they provide broad defence for bacteria and contribute significantly to intrinsic and acquired MDR.

In recent years, efflux-mediated drug resistance has become one of the most intensively studied topics in the area of antimicrobial therapy and a literature search found more than 5000 references, so not all could be included in this article. In addition to many minireviews, there are reviews[9,10] and a book[11] on drug efflux by bacteria.

1. Drug-Specific and Multidrug Efflux Transporters of Bacteria

Antibacterial efflux systems are examples of larger classes of transporters involved in the uptake of essential nutrients and ions, excretion of metabolic end products and deleterious substances, and communication between cells and the environment. Bacterial drug efflux transporters fall into five families. Two of these are very large and ancient superfamilies known as the adenosine triphosphate (ATP)-binding cassette (ABC) superfamily[12,13] and the major facilitator superfamily (MFS).[14] The other three are smaller families: the multidrug and toxic compound extrusion (MATE) family,[15] the small MDR (SMR) family[16] and the resistance-nodula-

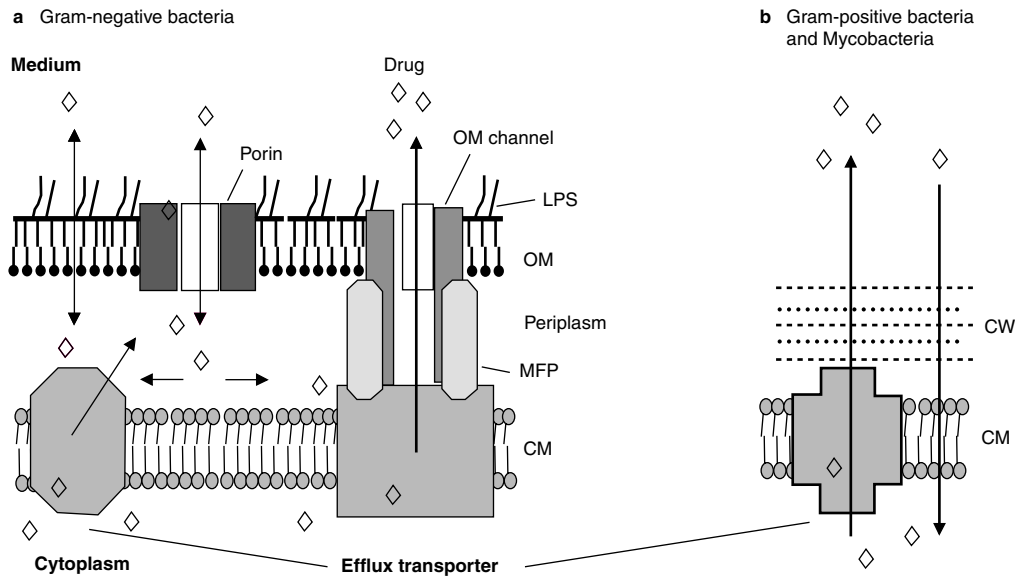

Fig. 1. Schematic models of bacterial drug efflux pumps. Organisation of typical drug efflux machinery in Gram-negative bacteria (**a**) and Gram-positive bacteria (**b**) is shown. In Gram-negative bacteria, drugs traverse the outer membrane (OM) either through the porin channel or through the lipopolysaacharide (LPS)/phospholipid asymmetric bilayer. Once the drug reaches the cytoplasm, they can be expelled into periplasm by simple transporters (shown as an octagon). Drugs are captured either from periplasm or from the outer leaflet of cytoplasmic membrane (CM) by tripartite transport complex that ejects drugs directly into the medium. In Gram-positive bacteria, simple transporters are able to pump out drugs into the medium, as the cell wall (CW) does not offer much resistance to diffusion of small molecules. **MFP** = membrane fusion proteins.

tion-cell division (RND) family.[17] On the other hand, we can see that the transporters are organised in one of the two ways.[18] Either they occur as single-component transporters catalysing the efflux of drugs across the cytoplasmic membrane (CM) (also called inner membrane [IM]), or as multiple-component systems containing not only the CM transporters but also the outer membrane (OM) channel proteins (OMP)[19] and periplasmic membrane fusion proteins (MFP).[20,21] The three components in the latter type function together to catalyse efflux across both CM and OM (figure 1).

1.1 ATP-Binding Casssette Superfamily

Driven by ATP hydrolysis, ABC transporters are involved in the transport of many substances including sugars, amino acids, ions, drugs, iron complexes, polysaccharides and proteins.[12,22,23] The ABC transporter of bacteria often consists of an integral membrane protein with typically 6 transmembrane α-helical segments (TMSs) and an ATP-binding subunit localised at the cytoplasmic face of the membrane.[12] The two proteins may be nonconvalently associated or covalently linked in a single polypeptide chain, and the complete system usually is a dimer, containing 12 (6 + 6) TMSs. ABC-type drug efflux systems are not very common in bacteria, but include a multidrug pump LmrA of *Lactococcus lactis*[24] and a drug-specific pump MacAB of *Escherichia coli*.[25]

1.2 Major Facilitator Superfamily

These transporters include symporters and antiporters, involved in the transport of sugars, metabolites, anions and drugs, and are driven by the electrochemical gradient, typically proton-motive force (PMF).[14,26,27] The transporters of this family usually function as single-component pumps (e.g. NorA of *Staphylococcus aureus*[28]), but some in Gram-negative bacteria function with MFP and OM com-

ponents as multiprotein pumps (e.g. the EmrAB-TolC multidrug efflux pump of *E. coli*[29]). Drug pumps usually belong to a few branches (families) of the superfamily, exhibiting 12- or 14-TMS.[14] Within each branch, both single drug and multidrug transporters occur, a finding that suggests that there is no fundamental distinction between these two types of pumps.

1.3 Multidrug and Toxic Compound Extrusion Family

The MATE family exhibits a membrane topology similar to the MFS family,[15] yet shows no homology to members of MFS. The family includes NorM of *Vibrio parahaemolyticus* and YdhE of *E. coli*,[30] which mediate resistance to cationic dyes, aminoglycosides and fluoroquinolones utilising an electrochemical gradient, often a Na^+ gradient, as the driving force.

1.4 Small Multidrug Resistance (MDR) Family

The SMR transporters are drug/proton antiporters driven by the PMF. They contain only about 110 amino acid residues and four TMSs, and possibly function in a trimeric form.[31,32] The well-characterised examples of this family include the Smr protein of *S. aureus*[33] and the EmrE protein of *E. coli*.[34] Although they are multidrug pumps, their substrate ranges are limited to lipophilic cations, including antiseptics and disinfectants.

1.5 Resistance-Nodulation-Cell Division Superfamily

Initially thought to be a bacteria-specific family,[17] the RND family is also found in eukaryotes.[35] The RND family consists of at least seven distinct subfamilies.[35] Some members of the RND transporters catalyse drug/proton antiport.[17] RND drug transporters are typically encoded by chromosomes but heavy metal efflux pumps are often encoded by plasmids.[36] A recent report identified the first example of RND drug transporter encoded by transmissible plasmids.[37] It has become clear that RND-type pumps play a major role in both intrinsic and acquired resistance of Gram-negative bacteria to a variety of clinically relevant antimicrobials.[38] RND transporters usually form complexes with MFP and OM components and function as multiprotein systems. They possess an unusual topology consisting of 12 TMS with two large, external (periplasmic) loops between TMS1 and 2 and TMS 7 and 8. All RND drug pumps are multidrug transporters, and include AcrB (organised as AcrAB-TolC system) of *E. coli*[39,40] and MexB (organised as MexAB-OprM system) of *Pseudomonas aeruginosa*.[41,42]

2. Drug Efflux in Gram-Negative Bacteria

Most Gram-negative bacteria are more inherently resistant to antibacterials than Gram-positive bacteria. Earlier, the only known molecular mechanism that could explain this 'intrinsic resistance' was the OM permeation barrier.[43] However, the OM alone is not a sufficient explanation because most drug molecules equilibrate, across even the rather impermeable OM of *P. aeruginosa*, in less than a minute.[43-45] Now it is recognised that the intrinsic drug resistance of Gram-negative bacteria is a result of the cooperation between the OM barrier and the expression of broad-specificity multidrug efflux pumps[46] (figure 1). Gram-negative bacteria also possess drug-specific efflux pumps which mediate resistance to certain classes of antibacterials.

2.1 *Escherichia coli* and Other Members of Enterobacteriaceae

Living in a natural habitat surrounded by high concentrations of bile salts and other antimicrobial inhibitors, *E. coli* cells are armed with the OM, as well as a wide range of efflux pumps. A survey of *E. coli* genome revealed the presence of at least 37 efflux transporters, either single drug or multidrug, putative or proven, which include 7 ABC, 19 MFS, 1 MATE, 5 SMR and 7 RND transporters.[47] Nevertheless, the tripartite RND-type AcrAB-TolC system is the predominant pump in terms of efflux of commonly used antibacterial agents.[18]

In 1965 Nakamura[48] discovered that the so-called *acr* mutation on the chromosome of *E. coli* results in hypersusceptibility to basic dyes, detergents and antibacterials, which have different struc-

tures and different cellular targets. This phenotype was thought to be due to the increased OM permeability in the mutant but subsequent studies failed to detect alterations in the OM. The cloning and sequencing of the *acrAB* loci eventually revealed that the *acrAB* genes form a single operon and encode a periplasmic, IM-associated lipoprotein AcrA and an integral IM protein AcrB, which together produced acriflavine efflux.[39] AcrAB functions with an OM protein TolC (which is encoded at a separate location of the chromosome) to form a tripartite transporter.[40] The AcrAB-TolC efflux system is perhaps the best-characterised RND drug efflux pump to date. The system displays unusually broad substrate specificity, including the majority of clinically important antibacterials and other toxicants (dyes, detergents and organic solvents) [table II]. The system catalyses drug efflux at the expense of PMF as demonstrated by proteoliposome reconstitution.[49]

E. coli also possesses other RND transporters such as AcrEF, AcrD, YhiUV and MdtABC, which were demonstrated to extrude antibacterials. All these systems (with the possible exception of AcrD[58]) require TolC as the OM component.[47] Inactivation of the *acrEF*, *yhiUV* and *mdtABCD* (*yegMNOB*) genes does not change drug susceptibility of wild-type *E. coli* under the standard laboratory growth conditions, indicating that these pumps are not expressed to a significant extent in wild type cells.[47,60,61,88] Originally reported to function as single component pump to efflux aminoglycosides,[58] the AcrD pump, like its homologue AcrB, was recently found to require AcrA and TolC to perform efflux of at least bile salts and novobiocin.[59] Deletion of *acrD* renders mutants hypersusceptible to aminoglycosides.[58] YhiUV-TolC overexpression is associated with resistance to doxorubicin, erythromycin, deoxycholate and crystal violet, and MdtABC mediates resistance to bile salts and novobiocin.[61,62] Intriguingly, the MdtABC system contains two RND transporters, MdtB and MdtC, which are both required for efflux.

A number of non-RND transporters are present in *E. coli*. Requiring TolC for its activity, EmrAB is an MFS efflux system that contributes to the intrinsic resistance of *E. coli* to nalidixic acid and carbonyl cyanide *m*-chlorophenylhydrazone (CCCP), a proton conductor. Its overexpression, due to either induction or mutational upregulation, causes increased resistance to nalidixic acid, thiolactomycin, proton uncouplers and ethidium.[29,89]

Another chromosomally encoded MFS transporter, MdfA (also known as CmlA, Cmr), is a multidrug pump but its substrate range is limited. It provides resistance to chloramphenicol,[90-92] a compound that is often inactivated enzymatically by a specific chloramphenicol acetlytransferase (CAT), coded by a plasmid gene. MdfA-mediated chloramphenicol/H^+ antiport has been experimentally demonstrated.[93] Homologues of MdfA are present, either chromosomally or plasmid-borne, in many Gram-negative bacteria including members of Enterobacteriaceae,[94-96] as well as *P. aeruginosa*,[97] where CmlA contributes high-level chloramphenicol resistance. As yet undefined is the role of CmlA in resistance to florfenicol, a fluorinated chloramphenicol analogue that is not subject to CAT inactivation. Florfenicol and chloramphenicol resistance in *Pasteurella piscicida* (a fish pathogen) was due to a transferable plasmid carrying *pp-flo* gene which encodes an MFS protein sharing 50% similarity with CmlA.[98] Almost identical to *pp-flo* (97% identity), the flo_{St} gene was found among *Salmonella enterica* serovar typhimurium isolates and contributed to the dual resistance of the isolates to florfenicol and chloramphenicol.[99,100] More recently, the flo_{Ec} gene was found on a large plasmid isolated from florfenicol/chloramphenicol-resistant *E. coli* associated with bovine diarrhoea.[101] Given the 21% identity between Flo_{Ec} and CmlA, Flo most likely functions as an exporter with altered substrate specificity for both florfenicol and chloramphenicol (unpublished observations).

An SMR transporter, EmrE, of *E. coli* is another well-studied efflux pump that provides intrinsic resistance to a number of lipophilic cations such as ethidium and methyl viologen.[102] A wealth of data exist regarding proton/drug antiport coupling and substrate recognition.[102-105] Besides EmrE, *E. coli* contains at least four other SMR transporters. Of

Table II. Resistance-nodulation-cell division (RND) family multidrug efflux pumps of Gram-negative bacteria

Organism	Efflux system component			Regulator	Substrate(s)	References
	MFP	RND	OMP			
Acinetobacter baumannii	AdeA	AdeB	AdeC	AdeT, AdeSR	AG, CM, EB, FQ, NO, TC, TM	50
Agrobacterium tumefaciens	IfeA	IfeB	?	IfeR	Coumestrol	51
	AmeA	AmeB	AmeC	AmeR	CB, DC, NO, SDS	52
Bradyrhizobium japonicum	RagD	RagC	?	RagAB	?	53
Berkholderia cepacia	CeoA	CeoB	OpcM		CM, FQ, TM	54
B. pseudomallei	AmrA	AmrB	OprA	AmrA	AG, ML	55
Campylobacter jejuni	CmeA	CmeB	CmeC	Cj0368c	AP, CM, CT, EB, EM, NA, FQ, PR, RF, TC	56
Enterobacter aerogenes	AcrA	AcrB	TolC	AcrR	AC, CM, FQ, MC, NO, SDS,TC	57
Escherichia coli	AcrA	AcrB	TolC	AcrR, MarA, SoxS, Rob, SdiA	AC, BL, BS, CM, CV, EB, FA, ML, NO, OS, RF, SDS, TX	39,40
	AcrA	AcrD	TolC	?	AG, DC, FU, NO	58,59
	AcrE	AcrF	TolC	AcrS	Similar to AcrAB-TolC	60
	MdtA (YegM)	MdtBC (YegNO)	TolC	BaeSR	DC, NO	61,62
	YhiU	YhiV	TolC	EvaAS	DC	63
Haemophilus influenzae	AcrA	AcrB	TolC	?	AC, CV, EB, EM, NO, RF, SDS	64
Neisseria gonorrhoeae	MtrC	MtrD	MtrE	MtrR, MtrA	EB, FA, TX	65,66
	FarA	FarB	MtrE	?	FA, TX	67
Porphyromonas gingivalis	XepA	XepB	XepC	?	AC, EB, PU, RF, SDS	68
Pseudomonas aeruginosa	MexA	MexB	OprM	MexR, NalC (PA3721)	AC, AG, BL, CM, CV, EB, ML, NO, OS, SDS, SF, TC, TM, TR	41,42,69
	MexC	MexD	OprJ	NfxB	CM, CP, FQ, TC, TR	70
	MexE	MexF	OprN	MexT	CM, FQ	71
	MexX (AmrA)	MexY (AmrB)	OprM	MexZ	AG, ML, TC	72-74
	MexH	MexI	OpmD	PA4203?	Vanadium	75
	MexJ	MexK	OprM	MexL	EM, TC, TR	76
P. putida	SrpA	SrpB	SrpC	SrpSR	OS	77
	TtgA/, ArpA/MepA	TtgB/ArpB/MepB	TtgC/ArpC/MepC	TtgR/ArpR/MepR	OS	77-79
	TtgD	TtgE	TtgF	?	OS	80
	TtgG	TtgH	TtgI	?	OS	81
Stenotrophomonas maltophilia	SmeA	SmeB	SmeC	SmeSR	AG, BL, FQ	82
	SmeD	SmeE	SmeF	SmeT	EM, FQ, OS, TC	83,84
Serratia marcescens	?	MexF-like	?	?	FQ	85
Salmonella typhimurium	AcrA	AcrB	TolC	AcrR (STM0477)	BL, FQ	86,87

AC = acriflavine; **AG** = aminoglycosides; **AP** = ampicillin; **BL** = β-lactams; **BS** = bile salts; **CB** = carbenicillin; **CM** = chloramphenicol; **CP** = cephalosporins; **CT** = cefotaxime; **CV** = crystal violet; **DC** = deoxylchloate; **EB** = ethidum bromide; **EM** = erythromycin; **FA** = fatty acids; **FQ** = fluoroquinolones; **FU** = fusidic acid; **MC** = mitomycin; **MFP** = membrane fusion proteins; **ML** = macrolides; **NA** = nalidixic acid; **NO** = novobiocin; **OMP** = outer membrane channel proteins; **OS** = organic solvents; **PR** = protamine; **PU** = puromycin; **RF** = rifampicin; **SDS** = sodium dodecyl sulfate; **SF** = sulfonamides; **TC** = tetracyclines; **TM** = trimethoprim; **TR** = triclosan; **TX** = Triton X-100; **?** = unknown.

these, SugE (initially known as a suppressor of *groEL* mutations) needs to be overexpressed to reveal its export function.[106]

Drug-specific transporters occur in *E. coli*. The best-known examples are the plasmid-coded Tet pumps [e.g. TetA(B)] for tetracycline efflux[107] from the cytosol to the periplasm.[108] Tet proteins, which are MFS transporters, transport tetracycline-divalent cation complex[109] and are the principal resistance mechanism for this class of antimicrobials in Gram-negative bacteria. Recently, MacAB was identified as a macrolide-specific pump of *E. coli*, providing resistance to 14- and 15-membered macrolides.[25] MacA is an MFP and MacB is an ABC protein with four TMS and one nucleotide-binding domain. Thus, unlike all other drug pumps characterised in *E. coli*, this pump belongs to ABC transporters, representing the first example of an ABC transporter for drug efflux in Gram-negative bacteria. MacAB requires TolC for its function. Deletion of *macAB* (*ybjYZ*) genes from wild-type *E. coli* did not alter drug susceptibility,[88] a result suggesting little expression of MacAB in wild-type cells or the masking of the role of MacAB by the AcrAB-TolC pump, which also pumps out macrolides. Mdl, which probably consists of MdlA and MdlB,[110,111] is intriguing because the transmembrane domain and the ATPase domain are fused into a single polypeptide as in P-glycoprotein. However, overexpression of *mdlAB* did not increase minimum inhibitory concentrations (MICs) of various drugs.[47]

2.2 *Pseudomonas aeruginosa* and Other Non-Fermentative Gram-Negative Bacteria

A notoriously opportunistic human pathogen, *P. aeruginosa* causes severe infections, especially in immunocompromised patients. It also infects plants and insects. A well-known feature of *P. aeruginosa* is its high-level intrinsic resistance to a variety of antimicrobial agents. This phenotype requires its low permeability OM[112] but this is not a sufficient explanation. The discovery of the constitutive Mex AB-OprM multidrug efflux pump in *P. aeruginosa*[41,42,113] has substantially changed our views on the intrinsic resistance of *P. aeruginosa* and also served to emphasise the importance of efflux-mediated antibacterial resistance in general.[112] It is now accepted that the intrinsic and acquired MDR of *P. aeruginosa* involves both the multidrug efflux systems and the low OM permeability.[46,114,115]

To date, six RND-type multidrug efflux systems have been identified in *P. aeruginosa*: MexAB-OprM,[41] MexCD-OprJ,[70] MexEF-OprN,[71] MexXY (also referred as MexGH- or AmrAB)-OprM,[72-74] MexJK-OprM,[76] and MexGHI-OpmD.[75] Each is encoded by an efflux operon, and contains an RND transporter (MexB, MexD, MexF, MexX, MexK or MexI) in IM, an OM channel protein (OprM, OprJ, OprN or OpmD) and a periplasmic MFP (MexA, MexC, MexE, MexY, MexJ, MexH). In addition, the *P. aeruginosa* genome sequences reveal the presence of several additional RND-type systems.[116]

2.2.1 MexAB-OprM

The MexAB-OprM system was originally identified during a study of siderophore-mediated iron transport systems.[41,113] At the same time, accumulation assays of radiolabeled tetracycline, chloramphenicol, norfloxacin and β-lactams in intact cells of *P. aeruginosa* indicated that even wild-type strains can effectively pump out these antimicrobials, and that the efflux process is PMF-driven.[42,44,45] Inactivation of any components of MexAB-OprM inactivates the drug efflux and leads to a multidrug hypersusceptibility phenotype, indicating that this system is responsible for the intrinsic resistance.[42]

The overexpression of MexAB-OprM, causing acquired, elevated levels of MDR, is seen in at least two types of mutants, *nalB* and *nalC*, selected either *in vitro* or *in vivo*.[117-120] The MexAB-OprM system has the broadest substrate specificity amongst the known multidrug efflux pumps of *P. aeruginosa*. Those antimicrobial agents that have been confirmed as substrates include various β-lactams (including β-lactamase inhibitors), older and newer quinolones, macrolides, tetracyclines (including the recent derivative, tigecycline), chloramphenicol, novobiocin, sulfonamides, trimethoprim, cerulenin and thiolactomycin.[42,44,45,121-123] Moreover, the sub-

strates include non-antibiotic compounds such as dyes (acridine orange, acriflavine, crystal violet and ethidium bromide), detergents, triclosan and organic solvents.[123-127] The overproduction or deletion of MexAB system, however, does not strongly affect the MIC of imipenem, a carbapenem,[128] and this is in part due to the presence of the OprD channel in the OM which permits rapid penetration of imipenem,[129] a process that may overwhelm the efflux. However, the antipseudomonal activity of other carbapenems such as panipenem and meropenem can be strongly influenced by the MexAB-OprM pump.[117,130,131]

OprM, like its homologue TolC, can function in multiple efflux systems. Firstly, OprM works not only with MexAB[42] but also independently of MexAB.[132] In the latter case, OprM may function with MexXY[72-74] or MexJK.[76] Secondly, OprM can functionally replace the role of either OprJ of MexCD-OprJ or OprN of MexEF-OprN efflux systems, without affecting substrate profiles of these systems.[133,134] Some yet uncharacterised *P. aeruginosa* RND transporter genes lack linked genes for OM components[116] and perhaps OprM may work with those transporters as a universal OM channel.

Some scientists suspect that multidrug pumps function in the efflux not only of antimicrobial compounds but also of 'physiological' compounds made by the cells themselves. *P. aeruginosa*, like other bacteria, produces *N*-acyl homoserine lactones which diffuse into other cells of population and activate many processes, such as the production of pyocyanine, elastase, etc.;[135] thereby, serving as quorum-sensing signals.[136] Studies showing the connection between the MexAB-OprM and the diffusion in and out of *N*-acyl homoserine lactones therefore attracted much attention.

Pearson et al.[137] examined the entry of labelled *N*-(3-oxododecanoyl) homoserine lactone ($3OC_{12}$-HSL) and a shorter chain *N*-butyryl homoserine lactone (C_4-HSL) into non-growing *P. aeruginosa* cells. The entry of the latter was not affected by the presence of MexAB-OprM but that of the former, a more lipophilic compound, was limited by efflux catalysed by MexAB-OprM, as expected. (The authors' conclusion that "*P. aeruginosa* is not freely permeable to $3OC_{12}$-HSL" is phrased in an unfortunate, misleading manner. Any lipophilic, uncharged compound is capable of freely entering and leaving bacterial cytosol, as predicted by physical chemistry.) Perhaps the emphasis on MexAB-OprM-catalysed acceleration of secretion of a 'natural' compound[137] (a phenomon expected to occur but for which there are no data) created a (mistaken) impression, often cited,[75] that MexAB-OprM is necessary for, or at least enhances, the quorum sensing.

An earlier study by Evans et al.[138] shows the situation to be contrary to this interpretation. They found that a *growing population* of MexAB-OprM-overproducing strain was defective in producing quorum-sensing response, including pyocyanine and elastase production, and was incapable of accumulating high levels of $3OC_{12}$-HSL in the medium. The latter response occurs because in a successful quorum-sensing response, initially unstimulated cells become stimulated by the signal from the outside and then become producers of quorum sensing signals.

Thus, overproduction of MexAB-OprM, a very broad spectrum pump, prevents the entry of most lipophilic compounds (including, accidentally, quorum-sensing signals) from the medium. These data do not show that the efflux of endogenous quorum-sensing signals is the natural function of MexAB-OprM. Very interestingly, MexAB-OprM-deficient mutants were recently shown to be significantly less invasive *in vivo*.[139] Although Hirakata et al.[139] speculate that the pump extrudes "virulence factors", we cannot rule out other alternative interpretations at present.

2.2.2 MexCD-OprJ

Unlike MexAB-OprM, MexCD-OprJ is apparently not expressed in wild-type *P. aeruginosa* under normal laboratory conditions and disruption of the *mexCD-oprJ* operon does not alter antibacterial susceptibility.[70,124] Overexpression of this operon in *nfxB*-type mutants significantly increases resistance to quinolones, tetracycline, chloramphenicol and fourth-generation cephalosporins such as

cefepime and cefpirome.[70,128] Similar to MexAB-OprM, the substrates for MexCD-OprJ also include other compounds, including cerulenin,[123] triclosan,[140] acriflavine, ethidium bromide and rhodamine 6G,[141] and organic solvents.[125] Still, the *nfxB*-type mutants show variability in antibacterial resistance patterns which can be classified into type A and type B. Type A mutants are resistant to ofloxacin, erythromycin and new zwitterionic cephalosporins (i.e. cefpirome, cefclidin, cefozopran and cefoselis), and type B mutants are resistant to these agents as well as to tetracycline and chloramphenicol. However, type B mutants are four to eight times more susceptible to many conventional penicillins (e.g. carbenicillin), atypical β-lactams (e.g. moxalactam and aztreonam), carbapenems (e.g. imipenem and biapenem) and aminoglycosides (e.g. gentamicin and kanamycin) than the wild-type PAO1.[142] This hypersusceptibility is probably due to the down-regulation of the MexAB-OprM system[143,144] and the AmpC β-lactamase[145] in the MexCD-OprJ-overproducing mutants. Decreased MexAB-OprM expression would also decrease activity of the MexXY pump, which requires OprM for extruding aminoglycosides (see section 2.2.4). MexCD-OprJ expression can be induced by some non-antibiotic compounds including ethidium bromide, acriflavine, tetraphenylphophonium and rhodamine 6G, all of them substrates of MexCD-OprJ.[141]

2.2.3 MexEF-OprN

The *mexEF-oprN* system also is not expressed in wild-type strains of *P. aeruginosa* and disruption of the *mexEF-oprN* genes produces no alteration in antibacterial susceptibility.[71] MexEF-OprN is highly expressed in *nfxC* mutants, which show increased resistance to chloramphenicol, quinolones and trimethoprim.[71] Imipenem resistance is also seen in *nfxC* mutants, although this is apparently due to the downregulation of OprD expression.[146] Overproduction of MexEF-OprN affects quorum sensing, as we have seen with MexAB-OprM overexpression,[138] but interestingly the level of a short-chain signal, C_4-HSL, is more strongly affected in this system.[147]

2.2.4 MexXY-OprM

Without a gene for an OM protein linked to the *mexXY* operon, the MexXY system utilises OprM of the MexAB-OprM system.[72,73] Initial expression study in *E. coli* showed resistance to fluoroquinolones and macrolides,[73] but MexXY, together with OprM, was shown to provide natural resistance to aminoglycoside antibacterials in *P. aeruginosa*.[72] Overexpression of the MexXY-OprM pump is probably responsible for aminoglycoside resistance in 'impermeability type' clinical isolates.[74]

2.2.5 MexJK-OprM and MexHI-OpmD

MexJK pump is not expressed in wild-type cells.[76] While MexJK requires OprM for extruding ciprofloxacin, erythromycin and tetracycline, it functions apparently independently of OprM for triclosan resistance.

MexGHI-OpmD contains an MFP MexH (mistakenly called an RND transporter), an RND transporter MexI (mistakenly called an MFP member), an OM channel OpmD and, in addition, a small integral membrane protein of unknown function, MexG.[75] This system is operative in wild-type cells and mediates resistance to vanadium[75] (vanadyl cation (VO^{2+}) was added to the medium). Interestingly, *mexGHI-opmD* null mutants show increased resistance to tetracycline, netilmicin and ticarcillin plus clavulanic acid, and this may be due to the compensating overexpression of other MDR pumps.[75,143] Possibly the same explanation applies to the lowered production in the growing culture of *N*-acyl homoserine lactones[75] and the data do not support the conclusion of Aendekerk et al.[75] that this system is involved in the transport of these signalling compounds.

2.2.6 Organisms Related to *P. aeruginosa*

Stenotrophomonas maltophilia, *Burkholderia cepacia* and *B. pseudomallei* display high-level intrinsic resistance to many antibacterial agents. Multidrug efflux systems were found in *S. maltophilia*.[148,149] Many MDR mutants selected *in vitro* and several clinical isolates were shown to overexpress an OM protein (SmeM),[149] which cross-reacted with antibodies against OprM of *P. aeruginosa*. Zhang et al.[83] recently demonstrated

that the SmeM-overproducing strains overexpress SmeDEF, a tripartite multidrug efflux system[83,84,150] that contains an RND pump. This system is expressed in wild-type cells, and contributes to the intrinsic resistance to fluoroquinolones, tetracyclines, chloramphenicol, macrolides and a limited number of β-lactams, as well as dyes.[83] In addition, five genes arranged in two operons (*smeABC* encoding an RND-type efflux pump complex and *smeSR*, a two-component regulatory system) were identified in *S. maltophilia*.[82] Overproduction of SmeC, possibly in conjunction with another, as yet unidentified efflux system but not SmeAB, is associated with elevated resistance to β-lactams, aminoglycosides and fluoroquinolones.[82]

Multiple drug-resistant strains of *B. cepacia*, an opportunistic human pathogen, were selected with chloramphenicol and trimethoprim/sulfamethazole, and alterations in the OM protein profiles of these mutants were noted. Previously attributed to the low OM permeability,[151] the resistance of these mutants is now known to be mediated via an efflux system, CeoAB-OpcM, which is highly homologous to MexAB-OprM.[54] Recently, two types of MDR mutants of *B. cepacia* were obtained *in vitro*.[152] Type I mutants were similar to the CeoAB-OpcM overproducers and resistant to quinolones, chloramphenicol and trimethoprim, whereas type II mutants were resistant to quinolones and β-lactams.[152] *B. cepacia* probably also possesses other MDR pumps.

A multidrug efflux system, AmrAB-OprA, which is responsible for aminoglycoside and macrolide resistance, was identified in *B. pseudomallei*.[55] Inactivation of the efflux system rendered the mutants 8- to 120-fold more susceptible to aminoglycosides and macrolides,[55] suggesting that it provides intrinsic resistance to these antibacterials.

P. putida, a soil bacterium, was shown to possess up to four RND-type efflux pumps, (SrpABC,[77] TtgABC,[78,153] TtgDEF,[80] and TtgGHI[81]) [table II], that mediate organic solvent tolerance. Given that these proteins display strong similarity to components of MexAB-OprM of *P. aeruginosa*, it is likely that some of these efflux pumps also provide resistance to antibacterials. Indeed, a *ttgB* mutant showed increased susceptibility to ampicillin, chloramphenicol and tetracycline.[78]

2.3 *Neisseria* spp

Mutations in multiple transferable resistance gene (*mtrR*) provide the human pathogen *Neisseria gonorrhoeae* with resistance to antibacterials, detergents and dyes.[154] Contrary to the initial assumption of increased OM barrier function,[155,156] it is now known that *mtrR* mutations increase expression of the MtrCDE efflux system,[65,157] which contains an RND family transporter (table II). Using the PMF, MtrCDE expels many hydrophobic agents, including antibacterials (e.g. penicillins, macrolides and rifamycins), detergents (Triton X-100, spermicide nonoxynol-9), bile salts and steroid hormones.[65,157-160] A survey of 51 consecutive clinical isolates obtained from males with acute gonococcal urethritis revealed that about half of these isolates displayed decreased susceptibility to azithromycin and erythromycin due to elevated MtrCDE expression.[161] Isolates from rectal infections often have the resistance profile mediated by the *mtr* system,[162,163] supporting the notion that at this site the presence of free fatty acids and bile salts promotes the selection of the MtrCDE overproducers.[65] *N. gonorrhoeae* possesses another RND efflux system, FarAB-MtrE, which is homologous to MtrCDE and is involved in the fatty acid resistance.[67] Not surprisingly, the homologues of the MtrCDE efflux systems were also identified in *N. meningitidis*.[164]

2.4 *Vibrio cholerae*

A Gram-negative enteric pathogen, *V. cholerae* is the causative agent of cholera. Antimicrobial resistance, including MDR, is already known in *V. cholerae*.[165] Two different drug-efflux pumps, VceAB (an MFS pump) and VcmA (a MATE pump), have been described (table III),[166,167] and these two pumps, when expressed in *E. coli*, were able to extrude quinolones and other antibacterial agents. Increased norfloxacin efflux was identified in fluoroquinolone-resistant clinical isolates.[168]

Table III. Non-resistance-nodulation-cell division family multidrug efflux pumps of Gram-negative bacteria

Organism	Family	Efflux system	Substrate(s)	References
Bacteroides thetaiotaomicron	MATE	BexA	EB, FQ	169
Burkholderia cepacia	MFS	BcrA	NA, TC	170
B. vietnamiensis	MATE	NorM	NF, PM	171
Escherichia coli	ABC	MacAB-TolC	ML	25
	MFS	EmrAB-TolC	CCCP, EB, TL	29,89
	MFS	EmrKY-TolC	DC	172
	MFS	EmrD	CCCP	173
	MFS	Dep	BM, CM, TC	174
	MFS	MdfA/Cmr/CmlA	CM, EB, IPTG, PU, RD, RF, TC, TPP	90,91
	MATE	YdhE	AC, FQ, TPP	30
	SMR	EmrE	AC, EB, MV, QAC	175
	SMR	SugE	QAC	106
	SMR	TehAB	DL, EB, KT, MV, PF	176
Pseudomonas aeruginosa	SMR	EmrE	AC, AG, EB	177
Salmonella enterica serovar Typhimurium	MFS	SmvA-OmpD	MV	178,179
	MFS	YddG-OmpD	MV	179
Vibrio cholerae	MFS	VceAB	CCCP, DC, NA, PCP, PMA	167
	MATE	VcmA	AC, DA, DO, EB, FQ, KM, SM	166
V. parahaemolyticus	MATE	NorM	EB, FQ, KM, SM	30

ABC = adenosine triphosphate-binding cassette superfamily; **AC** = acriflavine; **BM** = bicyclomycin; **CCCP** = carbonyl cyanide m-chlorophenylhydrazone; **CM** = chloramphenicol; **DA** = daunorubicin; **DC** = deoxylchloate; **DL** = dequalinium; **DO** = doxorubicin; **EB** = ethidum bromide; **FQ** = fluoroquinolones; **IPTG** = isopropyl-β-D-thiogalactopyranoside; **KM** = kanamycin; **KT** = potassium tellurite; **MATE** = multidrug and toxic compound extrusion; **MFS** = major facilitator superfamily; **ML** = macrolides; **MV** = methyl viologen; **NA** = nalidixic acid; **NF** = norfloxacin; **PCP** = pentachlorophenol; **PF** = proflavine; **PM** = polymyxins; **PMA** = phenylmercuric acetate; **PU** = puromycin; **QAC** = quaternary ammonium compounds; **RD** = rhodamine; **RF** = rifampicin; **SM** = streptomycin; **SMR** = small multidrug resistance; **TC** = tetracyclines; **TL** = thiolactomycin; **TPP** = tetraphenylphosphonium.

3. Drug Efflux in Gram-Positive Bacteria

Antibacterial resistance has become a major issue in Gram-positive bacteria, particularly with the emergence of methicillin-resistant *S. aureus* (MRSA), vancomycin-intermediate *S. aureus* (VISA), vancomycin-resistant enterococci (VRE) and penicillin-resistant streptococci.[180,181] Resistance is caused by multiple mechanisms (table I). But the contribution of efflux, especially to fluoroquinolone and macrolide resistance, has clearly undermined the use of these agents against Gram-positive bacteria. Compared with the cell envelope of Gram-negative bacteria, the cell envelope of Gram-positive bacteria has a relatively simple structure that contains a CM and a usually thick layer of peptidoglycan. Therefore, the efflux pumps of Gram-positive bacteria are simpler in organisation and have only one component located in the CM (figure 1; table IV). Both drug-specific and multidrug efflux pumps have been described in Gram-positive bacteria as detailed in this section.

3.1 *Staphylococcus aureus*

Staphylococcal infections account for a significant proportion of hospital-acquired infections.[208] Efflux pumps of *S. aureus* were first reported to be encoded on several *S. aureus* multidrug-resistant plasmids that code for multidrug efflux transporters, including QacA (an MFS member)[209,210] and Smr (an SMR member).[33] These are some of the earliest multidrug pumps studied. They are responsible for antiseptic and disinfectant resistance,[33,196] a feature important in *S. aureus* as a nosocomial pathogen. A survey of 98 clinical isolates of MRSA revealed that 70% of the strains were antiseptic-resistant. One-third of the antiseptic-resistant strains carried *qacA* and/or *smr* genes usually on plasmids,[211] highlighting the clinical relevance of the efflux mechanism.

Table IV. Drug efflux pumps of Gram-positive bacteria and Mycobacteria

Organism	Family	Efflux system	Substrate(s)	References
Gram-positive bacteria				
Bacillus subtilis	MFS	Blt	AD, EB, DO, FQ, RD, TPP	182,183
	MFS	Bmr	AD, EB, DO, FQ, RD, SD, TPP	182-184
	SMR	EbrAB	AC, EB, PY, SO	185
Enterococcus faecalis	ABC	ABC7	DA, DO, EB, OF	186
	ABC	ABC 11	CH, PT	186
	ABC	ABC 16	AZ, CR, EM	186
	ABC	ABC 23	QD, VM	186
	ABC	Lsa	CL, QD	187
	MFS	EmeA	AC, CL, EB, EM, FQ, NO	188
	?	?	CM, NF, TC	189
Lactococcus lactis	ABC	LmrA	DA, DO, EB, OL, RD, VB, VC	190
	MFS	LmrP	CL, ML, PG, TC	191
	MFS	MdtA[a]	LA, ML, SG, TC	192
Listeria monocytogenes	MFS	MdrL	CX, EB, ML	193
Staphylococcus aureus	ABC	MsrA	ML	194
	MFS	NorA	FQ	28,195
	MFS	QacA[a]	AC, CH, CV, DD, EB, QAC	196
Streptococcus agalactiae	MFS	MreA	CL, ML	197
S. pneumoniae	MFS	PmrA	FQ	198
	MFS	MefE	ML	199
S. pyogenes	MFS	MefA	ML	200
Mycobacteria				
Mycobacterium fortuitum	MFS	Tap	AG, TC	201
M. smegmatis	MFS	LfrA	FQ, EB	202
	?	?	Isoniazid	203
M. tuberculosis	ABC	DrrAB	DA, DO, EB	204
	MFS	EfpA	?	205
	MFS	P55	AG, TC	206
	MFS	Tap	TC	201
	SMR	Mmr	AC, EB, EM, TPP	207

a The genes encoding these pumps are plasmid-borne.

ABC = adenosine triphosphate-binding cassette superfamily; **AC** = acriflavine; **AD** = acridine dyes; **AG** = aminoglycosides; **AZ** = azithromycin; **CH** = chlorhexidine; **CL** = clindamycin; **CM** = chloramphenicol; **CR** = clarithromycin; **CV** = crystal violet; **CX** = cefotaxime; **DA** = daunorubicin; **DD** = diamidines; **DO** = doxorubicin; **EB** = ethidum bromide; **EM** = erythromycin; **FQ** = fluoroquinolones; **LA** = lincosamides; **MFS** = major facilitator superfamily; **ML** = macrolides; **NF** = norfloxacin; **NO** = novobiocin; **OF** = ofloxacin; **OL** = olchicine; **PG** = ptogramins; **PT** = pentamidine; **PY** = pyronine Y; **QAC** = quaternary ammonium compounds; **QD** = quinupristin-dalfopristin; **RD** = rhodamine; **SD** = spermidine; **SG** = streptogramins; **SMR** = small multidrug resistance; **SO** = safranin O; **TC** = tetracyclines; **TPP** = tetraphenyphosphonium; **VB** = vinblastine; **VC** = vincristine; **VM** = virginiamycin; **?** = unknown.

A chromosomally-encoded multidrug efflux pump, NorA, was identified in *S. aureus* in 1990.[28] This pump, an MFS member, extrudes quinolone compounds and contributes to low-level quinolone resistance.[28,212,213] In many studies (for example, see Ng et al. [213]), NorA was shown to produce resistance only to more hydrophilic fluoroquinolones and was totally ineffective against lipophilic compounds such as sparfloxacin (efflux of quinolone compounds by both Gram-positive and Gram-negative bacteria was reviewed by Piddock[214] and Poole[215,216]). Purified NorA was recently reconstituted into proteoliposomes in which NorA-mediated proton-dependent drug transport was demonstrated.[217] Because *norA* is weakly expressed in wild-type cells, the NorA-mediated resistance is depen-

dent on the induced or mutational up-regulation of the *norA* expression.[218] Indeed, mutations in *norA* promoter confer fluoroquinolone resistance.[219,220]

Expression of *norA* is also affected by a two-component system.[195] Besides NorA, at least ten putative proteins that share sequence homology with NorA have been identified within the *S. aureus* genome.[221] One such transporter provides resistance to fluoroquinolones and monocationic organic compounds such as acriflavine, ethidium and tetraphenylphosphonium bromide, and the pump expression is also induced by its substrates.[221] Non-NorA-meditated efflux mechanism was also reported in clinical isolates.[222] Together with other resistance mechanisms such as target alterations, the NorA and other efflux pumps can produce high level fluoroquinolone resistance in *S. aureus*.[222-224]

Resistance to macrolides and streptogramin B involves an ABC transporter system, which includes MsrA that corresponds to two ATP-binding domains.[194] Originally identified in an *S. epidermidis* plasmid,[225] MsrA has been found in other staphylococcal species, including *S. aureus*.[194] Although the transmembrane component of the MsrA-containing efflux system has not been identified, this pump clearly provides resistance to 14-membered (clarithromycin, dirithromycin, erythromycin and roxithromycin) and 15-membered (azithromycin) macrolides and streptogramin B.[194,225,226] The resistance is inducible by these macrolide substrates but not streptogramin B. Clindamycin is neither a substrate nor an inducer, and thus the MsrA strains are fully susceptible to this antibacterial.[227] In a recent European survey, the *msrA*-related resistance occurred in 13% of *S. aureus* isolates.[228]

3.2 Streptococci

A leading cause of bacterial respiratory infections, *Streptococcus pneumoniae* has shown increasing resistance to β-lactams, macrolides, quinolones and tetracyclines.[180,229] Although resistance in *S. pneumoniae* is often related to target alterations, such as in penicillin-binding proteins (PBPs) [for penicillin resistance] or DNA gyrase/topoisomerase IV (for quinolone resistance), efflux pumps clearly make an important contribution.[229-231] For example, 45% of 273 ciprofloxacin-resistant clinical isolates owed their resistance largely to efflux.[232] Ethidium-bromide-resistant *S. pneumoniae* mutants selected in the laboratory display cross-resistance to fluoroquinolones and this resistance can be reversed by the efflux pump inhibitor (EPI) reserpine (see section 10.2), again indicating the involvement of a multidrug transporter in this organism.[233] Active efflux of ciprofloxacin was documented in both wild-type and resistant strains of *S. pneumoniae*.[234-236] Initially identified in 1999,[198] the PmrA pump is a homologue of NorA of *S. aureus* (24% identity) and produces resistance to fluoroquinolones and dyes.[198,237,238] Disruption of *pmrA* in wild-type strains does not alter drug susceptibility, suggesting that the gene is not likely to be expressed in wild-type cells.[198] As with NorA, PmrA-mediated efflux could be inhibited by reserpine. Studies also suggest the presence of additional multidrug transporters in *S. pneumoniae*.[238,239]

Efflux pumps also play an important role in macrolide resistance in *S. pneumoniae*. In the genus *Streptococcus*, *mef(A)* genes encode an MFS efflux pump that can be found in clinical isolates of *S. pneumoniae*, *S. pyogenes* and in other species of streptococci (oral streptococci, group C and G streptococci, and *S. agalactiae*).[227] The MefA pump was originally identified in *S. pyogenes*,[200] while its homologue, MefE, was reported later in *S. pneumoniae*.[199] The two pumps are substrate-specific pumps mediating resistance to 14- and 15-membered macrolides but not to 16-membered macrolides, lincosamides or analogues of streptogramin B.[199,200,240] Thus the *mef*-mediated efflux systems produce a characteristic resistance pattern that can be readily distinguished by susceptibility data.[241,242]

3.3 Enterococci

Enterococci are the constituent of normal human flora, typically colonising the intestinal tract and skin. However, they are capable of causing diseases as opportunistic pathogens. Over the past decade, MDR enterococci have emerged and they constitute

a serious threat to public health.[243,244] Resistance in enterococci often seems to be the result of target alterations, as with vancomycin and fluoroquinolones.[245] Still, enterococci are inherently far more resistant to numerous antibacterials, including fluoroquinolones, than most Gram-positive bacteria, suggesting the presence of efflux mechanism. Efflux of fluoroquinolones and chloramphenicol was, in fact, demonstrated in wild-type cells of *E. faecalis* and *E. faecium*.[189] Also, 34 potential drug-efflux genes were identified in the *E. faecalis* genome.[186,188] Among them, EmeA, a NorA homologue, was recently shown to provide resistance to norfloxacin and ethidium bromide, and the efflux could be reversed by known efflux inhibitors such as reserpine, lansoprazole and verapamil.[188] Genome-wide inactivation of the putative efflux pumps yields evidence that several pumps have different but somewhat overlapping broad substrate profiles and contribute to intrinsic resistance of this organism[186] (table IV).

3.4 *Bacillus subtilis*

Although a non-pathogenic organism, *Bacillus subtilis* is an excellent model for studying resistance in Gram-positive bacteria. Two multidrug pumps, Bmr and Blt, have been well studied, including their mechanisms, substrate profiles,[246] putative physiological function[184] and regulation.[182,183,247,248] The knowledge on these pumps has helped us greatly to understand drug efflux pumps in general. Bmr and Blt are homologous to NorA, and thus belong to MFS.[249] Bmr is constitutively expressed in wild-type cells to mediate intrinsic resistance, but Blt expression requires mutational or physiological upregulation. Substrates for these two pumps include fluoroquinolones and non-antibiotic compounds such as ethidium bromide and energy inhibitors.

3.5 *Lactococcus lactis*

L. lactis is important in the food industry. The studies on the *L. lactis* multidrug efflux pumps LmrA and LmrP[250,251] provide us with excellent examples for understanding the molecular mechanisms of drug efflux. LmrA was the first multidrug ABC transporter identified in bacteria.[252] It has a putative topology of six α-helical transmembrane segments in the N-terminal domain, followed by a large hydrophilic domain containing the ATP-binding site. LmrA functions as a homodimer that resembles human P-glycoprotein in topology and in which the two membrane domains form the solute translocation path.[253,254] Interestingly, LmrA was successfully expressed in human lung fibroblast cells and complemented the P-glycoprotein defect.[255] LmrA captures amphiphilic substrates from the inner leaflet of CM and extrudes them into the external medium, a mechanism sometimes referred to as 'hydrophobic vacuum cleaner'.[190,256] Substrates of LmrA include anticancer drugs, DNA intercalators, peptides and many other lipophilic compounds[250] (table IV). When overexpressed in a drug-hypersusceptible *E. coli*, LmrA shows increased resistance to 17 of 21 clinically used antibacterials, including aminoglycosides, lincosamides, macrolides, quinolones, streptogramins and tetracyclines[257] (table IV).

In addition to LmrA, *L. lactis* possesses an MFS efflux pump, LmrP.[24,191] It shows a broad substrate specificity, including not only lipophilic cations (such as daunomycin, ethidium bromide, rhodamine 123 and tetraphenyphosphonium)[258] but also clinically relevant antibacterials (such as 14- and 15-membered ring macrolides, lincosamides, streptogramins and tetracyclines).[259] Like LmrA, LmrP was shown to function as a 'hydrophobic vacuum cleaner'.[256]

4. Drug Efflux in Mycobacteria

Mycobacterium tuberculosis causes infections in one-third of world population.[260] Mycobacteria are Gram-positive bacteria that are intrinsically highly resistant to a variety of antibacterials,[261] and this property is attributed, at least in part, to the unique cell wall structure. The cell wall of mycobacteria is rich in unusual lipids, including long chain (C60-C90) my*colic* acids, which are covalently linked to the peptidoglycan-associated polysaccharide, arabinogalactan. Moreover, mycobacterial porins, the water-filled channel proteins which form

the hydrophilic permeation pathways, are sparse.[262] A major porin of *M. smegmatis*, MspA, was recently reported to form a tetrameric complex with a single central pore.[263] The density of the MspA pores in *M. smegmatis* envelope was 50-fold lower than that of porins in *E. coli* OM.[263] Thus mycobacterial cell wall functions as a permeation barrier that is probably more effective than the OM of Gram-negative bacteria.[264]

The first mycobacterial MDR efflux pump, LfrA, was identified in 1996 in *M. smegmatis*.[202] Since then, several other mycobacterial drug efflux pumps have been reported but most are still not well characterised. LfrA is an MFS member, homologous to QacA of *S. aureus*, and when expressed on a plasmid it mediates low-level resistance to fluoroquinolones and other compounds, including ethidium bromide.[202,265] Inactivation of the chromosomal *lfrA* gene makes the mutant more susceptible to norfloxacin, ciprofloxacin and ethidium bromide (2- to 16-fold decrease in MIC values) [unpublished observations].[266] *M. smegmatis* carrying the cloned *lfrA* gene on plasmids seems to generate high-level resistant mutants more readily. Thus, short-term resistance mediated by *lfrA* may aid the development of permanent, high-level fluoroquinolone resistance.[202]

In *M. tuberculosis*, a gene encoding an MFS efflux pump, EfpA, was reported, and the *efpA*-homologous genes appear to be widely distributed in mycobacteria.[205] Still, the role of EfpA in drug resistance as well as its substrate specificity has not been established. Interestingly, genome-wide microarray analysis of the *M. tuberculosis* genes revealed that the *efpA* expression was increased in the presence of isoniazid, one of front-line antituberculosis drugs.[267] In this connection, efflux of isoniazid was observed in wild-type[203] and in a *katG* mutant (unpublished observations) of *M. smegmatis*, and efflux may provide explanation for some isoniazid-resistant *M. tuberculosis* isolates that apparently lack mutations in *katG*, *inhA* or *kasA*, which encode isoniazid-activating enzyme or target proteins. Interestingly, a recent study[268] reported that successive subculturing of *M. tuberculosis* in isoniazid-containing media produced genetically unstable high-level resistance, which was greatly decreased in the presence of efflux inhibitor reserpine. Efflux of pyrazinoic acid, the active derivative of pyrazinamide, now offers the explanation for the high-level intrinsic resistance of *M. smegmatis* (and perhaps many other mycobacteria) to pyrazinamide.[269] (The EmrAB-TolC efflux pump was found to contribute to pyrazinoic acid resistance in *E. coli*.[270])

Rifampicin is another front-line antituberculosis agent. Drug accumulation by intact cells in the presence and the absence of EPIs revealed small but reproducible difference in *M. tuberculosis*, *M. aurum* and *M. smegmatis*.[271] In this connection, early studies demonstrated lower accumulation of rifamycins in rifamycin-resistant mycobacteria including *M. tuberculosis,*[272,273] which might be reinterpreted as a contribution of drug efflux. Tap and P55 are two other MFS pumps reported in several mycobacteria (table IV), and when overexpressed from plasmids these pumps produce modest increase in resistance to aminoglycosides and tetracyclines.[201,206]

The genes encoding ABC transporters occupy 2.5% of the *M. tuberculosis* genome.[274] In fact, an ABC transporter, homologous to the DrrAB doxorubicin transporter known in the related organism *Streptomyces peuceticus*,[275,276] was recently shown to mediate low-level resistance to doxorubicin and several other antibacterials in *M. smegmatis* and *E. coli*.[204] The resistance phenotype could be abolished by reserpine and verapamil. Overexpression of an ABC transporter, the phosphate-importer (Pst), was reported to cause increased resistance to ciprofloxacin and decreased accumulation of the drug.[277] Inactivation of the *pstB* gene (encoding the ATPase subunit of Pst) promoted the fluoroquinolone hypersusceptibility as well as loss of high affinity phosphate uptake.[278] Bhatt et al.[278] claim that the Pst importer is also responsible for fluoroquinolone efflux. However, they have not ruled out the possibility that the expression of Pst affects the expression of other efflux transporters. In fact, ABC transporters catalysing export (such as DrrAB) are

quite different in their sequence from ABC importers[279] and Pst sequence clearly shows that it is an importer, not an exporter.

Mycobacteria also contain SMR transporters. Mmr is an SMR pump of *M. tuberculosis*, and is homologous to EmrE of *E. coli*.[207] Although it is not clear if Mmr mediates intrinsic drug resistance, introduction of the cloned *mmr* gene into *M. smegmatis* yielded increased resistance to erythromycin and dyes (table IV).[207] The purified Mmr protein was also demonstrated *in vitro* to function as proton/drug antiporter.[280]

Examination of genome sequences of *M. tuberculosis* and *M. smegmatis* suggests the presence of at least a dozen putative drug-efflux transporters in each organism. An open question is how big a role these transporters play in producing intrinsic or acquired resistance. Currently, multidrug-resistant clinical isolates of *M. tuberculosis* have been shown to carry several specific mutations that mediate resistance to individual antimicrobials.[281] The extent of drug efflux in these strains is simply unknown.

5. Recognition of Substrates by Multidrug Efflux Transporters

Multidrug transporters often pump out a wide range of substrates, with little or no common features in their structure; an extreme example is the AcrB of *E. coli*.[18] How would a protein recognise such an array of diverse compounds? One study that shed light on this question was the work on BmrR.[282] This repressor of the Bmr multidrug transporter binds various compounds that act as inducers, and the range of inducer compounds is more or less similar to that of the substrates. Zheleznova et al.[282] crystallised BmrR, a soluble protein, in the presence and absence of an inducer (and a substrate) tetraphosphonium. High-resolution X-ray crystallographic structures indicate that the drug binds mostly: (i) via stacking and van der Waals interactions; and (ii) via electrostatic interaction. The involvement of various residues in binding was further ascertained by site-directed mutagenesis.[283] A similar situation was also found for the binding of inducers to QacR, a repressor of the *qac* antiseptic efflux gene.[284]

Although BmrR and QacR are regulatory proteins, transporters can reasonably be assumed to bind their substrates by a similar mechanism. In both cases, the drug-binding pockets are large and flexible, and contain unusually large numbers of aromatic residues and one or two negatively charged residues that would neutralise the cationic charge of the inducers of these pumps.[285] The implications of this finding are profound and are presented persuasively by Neyfakh.[286] His arguments can be summarised as follows. We usually think that the ligand-binding pocket of a protein must present a very tight 'fit' to the ligand but this is because most examples we know about come from the binding of hydrophilic ligands. To remove these ligands out of the stable, hydrogen-bond-stabilised environment of water and to bring them to the generally lipophilic protein interior requires much energy, so the fit has to be tight in order to counteract this energy requirement. However, for lipophilic ligands that multidrug efflux pumps usually deal with, we do not need expenditure of much energy to take them out of water and, thus, a loose-fitting binding pocket will suffice, as long as there are weak van der Waals and stacking interactions and neutralisation of charge. In the examples Neyfakh discusses,[286] uncharged lipophilic ligands were shown to become bound to the same binding pocket of porcine odourant-binding protein in several different orientations,[287] and this shows clearly that the binding of lipophilic ligands is quite different from what we have learned with the binding of hydrophilic substrates to soluble enzymes.

6. Components of the Tripartite Drug Transporters and Efflux Mechanisms

Regardless of energy source, drug transporters in Gram-negative bacteria are often organised as multicomponent pumps, typically composed of an IM-associated periplasmic MFP, an IM transporter and an OM channel. Examples of such systems such as MacAB(ABC)-TolC, EmrAB(MFS)-TolC and AcrAB(RND)-TolC of *E. coli* have individually been described in section 2. These multicomponent

pumps provide the structural and functional basis for the direct drug efflux into the external medium, bypassing the periplasm. While the IM transporter and the MFP are typically encoded by the same operon, the location of the gene for OM channel is variable – it may be in the same operon or in a remote region of the chromosome. To date, the best-studied tripartite transporters are AcrAB-TolC of *E. coli* and MexAB-OprM of *P. aeruginosa*, which both contain RND family transporters (see section 2). The binding of drugs to the RND transporter AcrB is described in section 6.1.

6.1 Inner Membrane Efflux Transporters

The crystal structure of AcrB transporter was recently determined at 3.5 Å resolution,[288] representing the first high-resolution structure of a multidrug transporter. Three AcrB protomers are assembled as a homotrimer in the shape of a jellyfish. Each protomer is composed of a 70 Å high, periplasmic headpiece and a transmembrane region (50 Å thick) containing 12 transmembrane helices,[288] which agree with the prediction.[39,289] Given the high homology among RND transporters, it is expected that many other RND transporters would adopt a structure similar to AcrB. The folding topology of the MexB and MexD pumps of *P. aeruginosa* was experimentally tested,[290,291] and is similar to that of AcrB, containing 12 TMS with very large periplasmic loops of about 300 amino acids between TMS1 and 2, as well as TMS 7 and 8.

The IM transporters are responsible for drug recognition, as shown by combining components of different tripartite systems. For example, in *P. aeruginosa*, the hybrid MexAB-OprJ complex, but not the MexCD-OprM complex, confers the β-lactam resistance, a characteristic of the MexB pump.[124,292] Several recent studies further demonstrated that the two large periplasmic loops of the RND transporters, in fact, determined the substrate specificity of the transporters.[59,293,294] Similarly, amino acid alterations in the large loops of MexB were responsible for its loss of function to extrude carbenicillin (unpublished observations). These results are in agreement with the crystal structure of AcrB, where 'vestibules' between the periplasmic domains of neighbouring protomers were suggested to correspond to the site of entry, and perhaps of recognition, of substrates.[288]

Indeed, biochemical data suggested earlier that most substrates may be captured either from the periplasm or from the outer leaflet of IM. Firstly, the substrates of this system usually contained lipophilic domains; this suggested that the substrates become concentrated in IM by spontaneous partitioning.[18,86] Secondly, the diverse ionic nature of the substrates, which include non-ionic, anionic, cationic and zwitterionic compounds, was unusual. If these substrates were pumped out from the cytoplasm, the alterations in membrane potential must be adjusted by complex compensatory mechanisms. Finally, the system exported substrates that do not get into the cytoplasm.[86] These results suggested that the complex prefers to take up substrates from periplasm, or perhaps from the outer leaflet of the cytoplasmic membrane where the amphiphilic substrates become concentrated.[18] This concept is in agreement with the crystal structure of AcrB.[288] Furthermore, this mechanism may explain how some RND pumps, such as MexY or AcrD, can extrude aminoglycosides, which are polycationic drugs that do not reach the cytoplasm readily by spontaneous diffusion.

Recently, the X-ray crystallographic structures of AcrB with ligands (ethidium, rhodamine 6G, dequalinium and ciprofloxacin) have been obtained.[295] The drug molecules are bound to the walls of the large internal cavity formed by the three transmembrane domains of AcrB trimer, and are located approximately at the level corresponding to the outer limit of the membrane bilayer, suggesting that the drugs diffuse laterally through the 'vestibules' between the AcrB monomers,[288] while the drugs are associated with the outer leaflet of the lipid bilayer. Most interestingly, there are no anionic amino acid residues nearby that could neutralise the cationic charges of these substrates, an observation leading to the hypothesis that the positive charges of the substrates are neutralised by the anionic head groups of the phospholipids in the cavity.[295,296]

According to this view, the substrate-binding sites in AcrB are composite sites involving both the phospholipids and protein side-chains, and the flexibility and mobility of the phospholipids are probably important in creating an unprecedented wide substrate range to efflux pumps of this class.

It seems reasonable to assume that RND pumps such as AcrB also catalysed the efflux of drugs from the cytosol or at least from the inner leaflet of IM.[45,86] However, at present there is no experimental evidence that this occurs. The structure of AcrB also does not suggest a pathway for entry of drug molecules from cytosol and this remains a topic for future study.

Sequence alignments identify the highly conserved amino acid residues of the RND transporters.[297-299] These include several membrane-embedded charged residues in the transmembrane domain, for example, Asp407-Asp408 in TMS4, and Lys940 (AcrB) or Lys939 (MexB) in TMS10. Substitution of any of these residues completely abolishes the transport function of the pumps and, therefore, they are functionally required for the transporters. AcrB structure reveals that these three residues form ion pairs,[288] which may function in proton translocation, as was suggested earlier from the study of CzcA RND pump that extrudes toxic metal cations.[297]

6.2 Membrane Fusion Proteins

The IM-associated, periplasmic MFPs constitute a protein family that is involved in the transport of large and small molecules across the OM of Gram-negative bacteria.[21] Examples of the MFP members involved in drug resistance are given in table II. No functional MFP family member has been identified in Gram-positive bacteria, archaea or eukaryotes, although one homologue was found in *B. subtilis*.[20] The MFPs are of fairly uniform size (ca. 380 to 480 residues).

MFPs are essential for drug efflux in intact cells.[39] Using a lipid-deficient derivative of AcrA, Zgurskaya and Nikaido[300] demonstrated that AcrA is a highly asymmetric protein with the length of 10–20nm. This is compatible with the notion that the MFP coordinates the association and operation of the IM and OM efflux components. Purified AcrA accelerated the efflux reaction catalysed by AcrB in reconstituted proteoliposomes.[49] This was provisionally interpreted as a result of bridging between liposome vesicles by AcrA but we cannot rule out the possibility that AcrA simply activates AcrB by binding to it. AcrA can be cross-linked to other AcrA molecules as well as to AcrB in intact cells.[301] Low resolution crystal structure of AcrA shows that it is indeed an elongated protein.[302]

Many MFPs such as AcrA and MexA contain the lipoprotein consensus sequence,[303] suggesting that they are lipid-modified at their N-terminal conserved cysteine residue, after the cleavage of signal sequence. AcrE of the *E. coli* AcrEF pump was experimentally shown to be a lipoprotein.[304] Lipoprotein MFPs are likely to be associated with the IM via this lipid group. However, the N-terminal lipid modification may not be always necessary because AcrA[300] and MexA,[305] with a substitution or removal of the N-terminal cysteine, still remained fully functional. The N-terminal half of most MFPs contains a conserved, interrupted coiled-coil sequence, which is flanked on both sides with sequences homologous to lipoic acid-binding sites of various enzymes.[20] This discovery led to the idea that MFP may fold upon itself, using the coiled coil sequence, to bring the two membranes together;[306] however, this hypothesis remains to be tested. C-terminal regions of MFPs may be important for their function. For instance, mutations in the highly conserved C-terminal domain of CvaA, MFP of the CvaAB-TolC protein exporter in *E. coli*, render it inactive.[307,308] Similarly, in HlyD (the MFP component of another protein exporter, the *E. coli* HlyBD-TolC haemolysin translocator),[12] the C-terminus of HlyD seems to be important for its stability and function.[309]

6.3 Outer Membrane (OM) Channels

A family of OM proteins, called Outer Membrane Factor (OMF) family,[12,19] are required, in addition to the MFPs and the IM transporters, to form functional multiprotein efflux complexes that

export proteins, carbohydrates, heavy metals or drugs. These OM proteins exhibit fairly uniform size (ca. 400–500 residues), and the examples involved in drug resistance are listed in table II.

TolC, the OM component of many multicomponent transporters in *E. coli*, is so far the best-studied OM channel.[310,311] The TolC crystal structure consists of three protomers, which are assembled into a remarkable α-helical trans-periplasmic cylinder (tunnel) about 10nm long, which is connected to a contiguous β-barrel (channel) embedded in the OM. The periplasmic end of the TolC tunnel is sealed by a set of coiled helices, which could be untwisted to open the tunnel. This 'channel-tunnel' structure is long enough to span, not only the OM, but also much of the periplasm.[310,311] When the end of TolC tunnel contacts the top of the periplasmic domain of AcrB,[288] a 17nm long conduit is created, a length sufficient to span the entire depth of periplasm. Other OM channels of the OMF family are likely to adopt a topology similar to TolC. Although TolC and its homologue OprM of *P. aeruginosa* share only 20% identity at the amino acid level, mutational analysis of OprM supports the idea that OprM protomer exists as a largely periplasmic protein with four OM-spanning regions.[312,313]

OprM and many other homologues (such as OprJ, OprN, SmeC, SrpC, OpcM) [table II], however, contain a N-terminal lipoprotein box.[303] Therefore, after the cleavage of signal peptides, these proteins are likely to be modified with covalently linked acyl and diglyceride residues at their N-terminal Cys residue. However, OprM with the substitution of Cys or the replacement of lipoprotein signal sequence with a non-lipoprotein sequence was still functional.[313,314]

Reconstitution of TolC and OprM into black lipid system or proteoliposomes showed only weak pore-forming activities, at best.[315-317] This is consistent with the closed end of the tunnel, observed in the crystal structure of TolC.[310] Interestingly, TonB, which is involved in the opening of gated OM receptor proteins,[318] is needed for the full efflux activity of *P. aeruginosa* MexAB-OprM,[319] although it is not needed for that of *E. coli* AcrAB-TolC. Recent studies also confirmed the involvement of TonB in drug efflux in *P. putida*[320] and *N. gonorrhoeae*.[321] It is not known how TonB performs its function or why *E. coli* TolC behaves differently from other proteins.

6.4 Tripartite Transporter Complex and Efflux Mechanisms

A wealth of genetic evidence indicates that all of the tripartite components are required for drug efflux. Interaction between the components may be needed also for their stability and assembly. For example, in the MexEF-OprN pump of *P. aeruginosa*, absence of native MexF was found to affect proper processing of MexE and to lead to degradation of OprN.[322] Also with the *E. coli* HlyBD-TolC exporter, the absence of HlyB and/or TolC made HylD unstable.[309]

Models of the AcrAB-TolC complex have been proposed, particularly on the basis of the three-dimensional structures.[288,310] It is tempting to assume that the periplasmic tip of AcrB will fit the end of TolC tunnel because of their similar dimensions. Nevertheless, the precise location of AcrA in the complex remains unknown and it is not known if AcrA directly contacts TolC.

7. Regulation of Drug Efflux Pump Expression

The broad substrate specificity of some multidrug pumps suggests that they may even pump out normal metabolites, a possibility recently borne out by experiments with AcrAB.[323] In fact, the overexpression of AcrAB was found to be toxic for *E. coli* already in the first cloning experiments of the corresponding genes.[39] Perhaps this toxicity is one of the reasons why the expression of many pumps is controlled by an elaborate mechanism. Substantial progress has been made in understanding regulation of bacterial multidrug pumps. While expression of most RND and MFS transporters is known to be regulated at the transcription level, no evidence exists for such regulation of SMR pumps. Regulation of drug efflux pumps has recently been reviewed.[324]

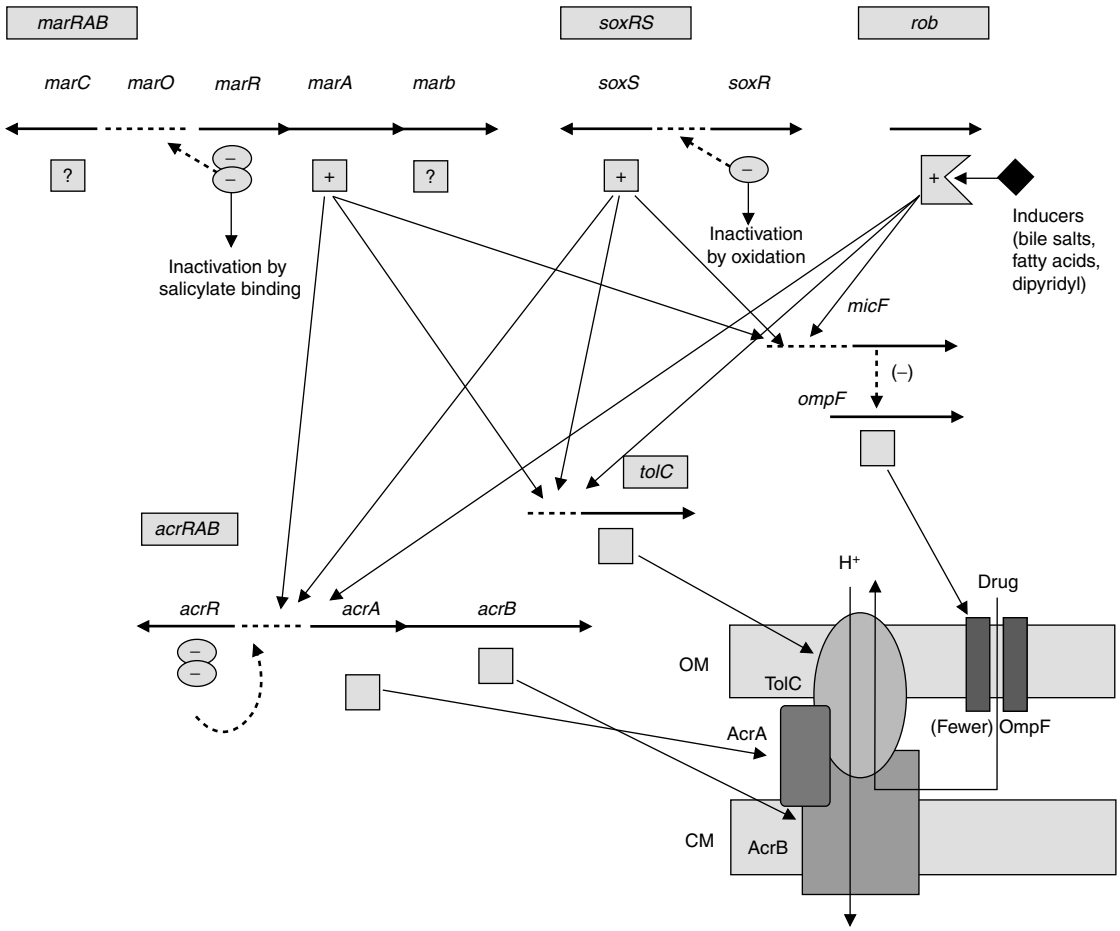

Fig. 2. Regulation of AcrAB-TolC expression. The *acrAB* operon is negatively regulated by the AcrR repressor (bottom left). In addition, *acrAB* and *tolC* are positively regulated by the three global activators, depicted on top, MarA, SoxS and Rob. The activators are shown by squares and a polygon with plus signs (+) in them; the repressors are shown by ellipses with minus (–) signs in them. The levels of MarA and SoxS are regulated by repressors MarR and SoxR, and the activity of Rob is apparently regulated by small ligands such as bile salts. These three activators also up-regulate *micF*, whose transcript inhibits the translation of *ompF* porin mRNA. Thus, all three activators increase the expression of efflux complex AcrAB-TolC, at the same time decreasing the influx of drugs through decreased expression of OmpF. For clarity, we have omitted the depiction of cross-regulation (e.g. SoxS and Rob both increase the production of MarA), as well as minor regulators such as MppA and SdiA. Please see sections 7.1 and 7.3 for these features.

7.1 Local Repressors and Activators

The expression of many multidrug pumps is controlled by local regulators, mostly repressors (table II). For example, in *E. coli*, *acrR*, which is divergently transcribed from the *acrAB* genes, encodes a repressor that belongs to the TetR repressor family[325] (figure 2). AcrR binds to promoters of *acrAB* and *acrR*, and thus represses *acrAB* transcription and its own transcription. Repression of *acrAB* by AcrR perhaps serves merely to prevent the unwanted overexpression of AcrAB. The loose repression allows the constitutive expression of AcrAB. As is discussed in section 7.3, the AcrAB pump is predominately regulated by global regulators. Expression of AcrEF, another RND transporter of *E. coli*, is likely to be repressed by AcrS, another member of TetR family, that is encoded by a divergently tran-

scribed *acrS* gene.[60] In fact, many known local repressors of multidrug pumps (such as MepR, MexL, MexZ, MtrR and SmeT; see table II) belong to the TetR family.

TetR[326,327] binds as a homodimer to its target operator DNA of *tetR* and *tetA*, and precludes the production of TetA, a tetracycline-specific efflux pump, which on the other hand makes the cells more vulnerable to certain toxic compounds.[328] Repression by TetR persists until tetracycline binds to TetR.[326] Given the inducible feature of TetA expression via ligand-repressor interaction, it would not be surprising to see the inducible expression of some pumps that are repressed by TetR-like repressors. MexXY expression are indeed induced by antibacterial agents that they pump out[329] and the organic solvent pump TtgABC is induced by toluene,[330] both apparently as a consequence of interaction between the local repressor protein and the inducer. Similarly, the *E. coli* multidrug exporter genes *emrAB* are repressed by the local repressor EmrR (of the MarR family), and this repression is relieved by the binding of inducers to the repressor protein.[331] This local regulator participates also in the regulation of an additional system, the *mcb-ABCDEFG* operon responsible for microcin B17 production.[332] Compounds that induce the *emrAB* operon repress the *mcb* operon.[332]

Each of the RND multidrug efflux systems of *P. aeruginosa* is regulated by the linked regulatory gene encoding repressor or activator (table II). The gene *mexR*, located upstream of *mexAB-oprM* and transcribed divergently, encodes a transcriptional repressor of MarR family. Inactivation of the *mexR* gene resulted in overexpression of MexAB-OprM and an MDR phenotype.[69] Originally discovered amongst mutants resistant to nalidixic acid,[333] '*nalB*' strains overproducing MexAB-OprM often carry mutations in *mexR*.[119,127,334-336] Purified MexR binds to the promoter regions of *mexR* and *mexAB-oprMin vitro*.[337] The crystal structure of MexR recently became available.[338] Four MexR dimers in the asymmetric unit were observed in multiple conformations and this high degree of flexibility is distinct from the rather rigid MarR protein, suggesting possibly different mechanism of repression.[339] Ligands for MexR, which could act as inducers, remain unknown.[338] Intriguingly, some *nalB*-type mutants expressed moderate levels of MexAB-OprM and did not show any mutations in either *mexR* or its promoter region, suggesting a second gene, dubbed *nalC*, has been mutated in these mutants.[119] A recent study indicated that mutations in a gene encoding a putative TetR family repressor (PA3721) of a two-gene operon (PA3720-3719) are involved in the NalC phenotype.[340]

Regulation of MexCD-OprJ is by the NfxB repressor, which is encoded by a gene located upstream of *mexCD-oprJ* operon.[70] NfxB shows similarity to several proteins of the LacI-GalR repressor family[341] and exhibits a conserved N-terminal helix-turn-helix motif characteristic of this family.[70] Purified NfxB binds upstream of the *nfxB* coding region, indicating it negatively autoregulates the expression of *nfxB* itself.[342] Expression of the *mexXY* operon is controlled by the TetR-like repressor MexZ, which is encoded by *mexZ* that lies upstream of the *mexXY* genes and is transcribed in the opposite direction.[72] That the cloned *mexZ-mexXY* gene cluster produced little resistance, while the cloned *mexXY* genes provided resistance, supports the notion that MexZ represses MexXY expression.[72]

The *mexEF-oprN* operon is positively regulated by a transcriptional activator, MexT,[343] which belongs to the LysR family of transcriptional regulators,[344] in contrast to the *P. aeruginosa* RND operons described so far in this section, which are all negatively regulated. The *mexT* gene is located upstream of *mexEF-oprN* and transcribed in the same direction as the efflux genes.[343] When overexpressed, MexT induces expression of the *mexEF-oprN* operon and decreases expression of OprD.[146] Thus, MexT negatively regulates OprD expression and this explains carbapenem resistance of *nfxC* mutants. Intriguingly, the *mexT* gene is not altered in the *nfxC* mutants that overproduce MexEF-OprN; thus it is likely that the *nfxC* gene is located far away from the *mexT*/*mexEF-oprN* complex.[343]

In Gram-positive bacteria, BmrR of *B. subtilis* is a local activator of MerR family and activates tran-

scription of *bmr*. The activity of BmrR depends on the binding of cationic lipophilic ligands; other MerR regulators also have, in addition to the homologous N-terminal DNA-binding domains, different C-terminal domains that enable them to bind specific ligand molecules.[248] Similarly, the regulator QacR (a TetR repressor) of *S. aureus* can bind to toxic compounds and promote the expression of transporter gene *qacA*.[345]

7.2 Two-Component Regulatory Systems

These systems are associated with expression of some multidrug pumps (table II and table IV). Two-component regulatory systems are widely present in bacteria and allow them to monitor their environment.[346] In response to stimuli, the sensor histidine kinases phosphorylate the cognate response regulators, which then activate or repress target genes.[346] Two-component systems that regulate drug efflux pumps include AlrSR (for the MFS transporter NorA) of *S. aureus*,[195] AdeSR (for ABC transporter AdeABC) of *Acinetobacter baumanii*,[50] BaeSR (for RND transporter MdtABC)[61,62] and EvgAS (for RND transporter YhiUV)[63] of *E. coli*, SmeSR (for RND transporter SmeABC) of *S. maltophilia*,[82] and SrpSR (for RND transporter SrpABC) of *P. putida*[347] (table II). In these systems, the genes encoding two-component sensors and regulators are all located upstream or downstream from the efflux gene operons, except *alrSR*. These response regulators all function as activators. When overexpressed on plasmids, they can usually activate the transcription of target genes on its own, without the simultaneous overexpression of the related sensor kinases.[61-63,82] A two-component regulatory system often regulates a range of genes. For instance, elevated SmeSR expression was also associated with overproduction of a class A L2 β-lactamase in *S. maltophilia*.[82]

7.3 Global Regulators

Control of AcrAB-TolC of *E. coli* is the best-studied example for the global regulatory mechanisms of multidrug pumps. There are at least four global transcriptional activators, MarA, SoxS, Rob and SdiA. The first three are shown in figure 2. The *mar* (multiple antibiotic resistance) locus consists of two divergent transcriptional units (*marRAB* and *marC*) expressed from a central operator/promoter region (*marO*).[348] While MarR and MarA are repressor and activator, respectively, the functions of MarB and MarC remain unclear. The MarR repressor forms a dimer, with each protomer containing a winged-helix DNA binding motif[339] and negatively controls *marRAB* expression, thus determining the cellular levels of MarA. Binding of MarR to *marO* DNA was inhibited by some inducers such as salicylate[349] and plumbagin.[350] The detailed mechanism has been provided by the crystal structure of MarR with or without the presence of its ligands.[339] MarA is a member of the XylS/AraC family of transcription activators[351] but is unusually small (129 amino acids), containing only the DNA-binding domain. Thus, regulation via the *mar* system must operate exclusively by the regulation of MarA *levels*. MarA is now known to control differential expression of over 60 chromosomal genes.[352] MarA also activates transcription of *marRAB* operon, resulting in autocatalytic activation of the system. Importantly, MarA activates the transcription of *acrAB* operon, increasing the drug efflux. In fact, the involvement of *mar* system in antibacterial resistance, often referred as Mar phenotype, is mostly explained by the increased expression of AcrAB.[353] Interestingly, *E. coli* strains carrying a null mutation in *mppA*, a gene encoding a periplasmic murein peptide-binding protein, overproduced MarA, and thus, displayed a Mar phenotype.[354]

In addition, another small MarA homologue, SoxS, also increases the transcription of *acrAB* and, thus, mediates elevated MDR, as was reported by Ma et al.,[355] and subsequently confirmed.[356,357] SoxS is the effector of the global superoxide response regulon SoxSR. Again, because SoxS contains only the DNA-binding domain, regulation via *sox* system takes place only by the alteration of the steady-state levels of SoxS in the cell and increases in SoxS occurs by oxidation and inactivation of SoxR repressor (containing FeS centers) caused by the presence of superoxide in the environment.[358]

This mechanism allows *E. coli* to increase its antibacterial resistance in the presence of superoxide radicals; for example, in tissues with the migration of polymorphonuclear leucocytes.

In contrast to MarA and SoxS, the third AraC/XylS family regulator, Rob, is twice as large and contains an additional domain in addition to the DNA-binding domain.[359] Rob was found to be involved in the regulation of *acrAB* operon because overproduction of plasmid-coded Rob resulted in resistance to organic solvents,[357,360,361] and was shown to activate the transcription of genes of *mar-sox* regulon *in vitro*.[362] However, Rob appears to be synthesised constitutively, unlike MarA and SoxS. Recently, the transcriptional activation of *mar-sox* regulon genes (including *acrAB*) by Rob was shown to occur through the binding of inducers such as dipyridyl,[362] medium-chain fatty acids and some bile acids.[363] In the case of both fatty acids and bile acids, the lipophilic inhibitors that are present in the normal environment of *E. coli* were indeed shown to increase the MIC of amphiphilic antibacterials only in those cells producing the intact Rob protein.

A putative *mar/sox/rob* box has been identified upstream of the *tolC* gene and TolC expression was indeed increased upon overproduction of MarA, SoxS or Rob.[364] Moreover, overexpression of MarA and SoxR decreases the synthesis of the porin OmpF of *E. coli* by induction of *micF*, whose antisense RNA product interacts with *ompF* mRNA to prevent OmpF translation.[365,366] Since antibacterials prefer the OmpF porin with its larger channel,[367,368] the decreased synthesis of OmpF,[365] synergistically with the increased expression of AcrAB,[355] prevents the entry of drugs into bacterial cells.

Recently, SdiA, an *E. coli* protein that is homologous to the receptor of acyl homoserine lactone quorum-sensing signal, was found to positively regulate the AcrAB expression.[369] Null mutants of *sdiA* show somewhat increased susceptibility to drugs. However, *E. coli* K-12 lacks the genes for the production of acyl homoserine lactones and it is not clear what signal SdiA is responding to in pure cultures of K-12.

MarA homologues are also present in several other bacteria including *M. smegmatis*.[348] MtrA is an AraC-like transcriptional activator[351] identified in *N. gonorrhoeae*, and it is required for inducible overexpression of the RND-type MtrCDE pump by inducers such as Triton X-100.[158] (MtrCDE is also negatively controlled by a local repressor, MtrR[66]). RamA, a MarA homologue, provides multiple resistance in *K. pneumoniae* when overproduced.[370] Bmr and Blt pumps of *B. subtilis* are positively regulated by a global regulator, Mta of the MerR family.[183,247] The presence of at least six RND pumps in *P. aeruginosa* suggests that the expression of these pumps may also be controlled by global regulators. Interestingly, Li et al.[143] showed an inverse relationship between expression of the MexAB-OprM pump and expression of the MexCD-OprJ and MexEF-OprN systems.

8. Development of Efflux-Mediated MDR Strains and Impact on Antibacterial Therapy

Bacteria exhibit a remarkable ability to develop defences against even the most sophisticated antibacterials. In many cases, drug efflux contributes to the final level of resistance. In terms of intrinsic resistance, it is well known that many antibacterials and chemotherapeutic agents are limited to Gram-positive bacteria in their efficacy. A 1993 survey of antibacterials that were new at the time of survey, showed that more than 90% of them lacked activity against a typical Gram-negative bacterium, *E. coli*.[371] This typical intrinsic resistance of Gram-negative bacteria is due to the combination of OM barrier and multidrug efflux pumps,[46] and inhibition or genetic inactivation of the major pump (for example AcrAB-TolC in *E. coli*) makes *E. coli* susceptible to most antibacterials as much as typical Gram-positive bacteria. For example, the genetic inactivation of this pump in *E. coli* K-12 decreased the MIC of oxacillin, a penicillin hitherto thought to be effective only against Gram-positive bacteria, from 256 μg/mL to only 0.5 μg/mL,[372] and a similar situation was also found with *Salmonella enterica* serovar typhimurium.[86]

The intrinsic resistance can be augmented by the overproduction of multidrug efflux pumps. For example, a vast majority of carbenicillin-resistant clinical isolates of *P. aeruginosa*, isolated in the British Isles in the late 1970s and early 1980s, owed their resistance entirely to the overproduction of MexAB-OprM efflux pump (see section 8.3). A survey of levofloxacin-resistant isolates of *P. aeruginosa* from Japan revealed that the increased activity of MexAB-OprM system plays a significant role in resistance in 96% of the strains.[373]

8.1 *In Vitro* Development of Efflux-Based Resistance

Spontaneous antibacterial-resistant mutants can often be selected *in vitro* on plates containing either a single antibacterial or multiple antibacterials. A single antibacterial (tetracycline or chloramphenicol) at concentrations slightly above the MIC readily selected resistant mutants of *E. coli* at a frequency of 10^{-6} to 10^{-7}, and these mutants displayed simultaneous cross-resistance, which is now known to be efflux-mediated, to other antibacterials including β-lactams, quinolones, rifampicin and puromycin.[374,375] Spontaneous MDR mutants of *P. aeruginosa* can arise at frequencies of 10^{-9} to 10^{-12}.[333] We and others have also selected various types of MDR mutants *in vitro* by exposure of wild-type *P. aeruginosa* strains to β-lactams, quinolones, aminoglycosides and/or chloramphenicol.[45,118] At least four types of MDR mutants of *P. aeruginosa* overexpressing efflux systems were selected *in vitro*, including the *nalB* or *nalC* type mutants overproducing MexAB-OprM, the *nfxB* type overproducing MexCD-OprJ, and the *nfxC* type overproducing MexEF-OprN.[118,119]

Importantly, MDR mutants can also be selected by antiseptics or other toxic chemicals. Such mutants of *P. aeruginosa* have also been selected by an organic solvent, *n*-hexane.[127] Similarly, efflux-based MDR mutants of *E. coli* and *P. aeruginosa* were selected by triclosan, a broad-spectrum antiseptic (a lipid biosynthesis inhibitor) used in soaps, toothpastes, plastics, and even included in a commercially available selective growth medium for *P. aeruginosa*.[76,356] Triclosan is a known substrate for the MDR pumps. The relationship between biocide usage and antibacterial resistance has already received some attention.[376]

Finally, antibacterial resistance of many bacteria is often transiently induced by salicylate, a compound existing naturally in plants. Salicylate and its derivatives are important components of drugs used clinically (such as aspirin). In *E. coli*, salicylate binds and inactivates MarR and, thus, increases MarA production culminating in the Mar phenotype,[339,377] while in *P. aeruginosa* it decreases expression of OprD, an OM channel responsible for imipenem influx and produces imipenem resistance.[118,146,378] Salicylate-inducible drug resistance is also documented in *B. cepacia*,[379] *K. pneumoniae*,[380] *M. tuberculosis*[381] and *S. aureus*.[382-384] When resistance is induced by physiologically relevant signals (see the case of superoxide and bile salts for *E. coli*, in section 7.3), this transient resistance will likely facilitate selection of resistant mutants.

8.2 *In Vivo* Development of Efflux-Based Resistance

Clinical isolates of MDR bacteria are often obtained during antimicrobial therapy. Reports in the early 1980s revealed that up to 15% of *P. aeruginosa* strains from British hospitals were resistant to carbenicillin, an antipseudomonal β-lactam widely used in the treatment of *P. aeruginosa* infections in the 1960s and 1970s.[385] Moreover, more than 80% of these carbenicillin-resistant strains displayed resistance to multiple antibacterials,[385] and they are now known to be MexAB-OprM overproducers,[44,45] as mentioned in section 2.2.1. In another study from France, about one-third of ticarcillin-resistant clinical isolates of *P. aeruginosa* presented a resistance profile characteristic of *nalB* type, MexAB-OprM overexpression mutants.[386] Of 21 pairs of *P. aeruginosa* isolates susceptible (pre-therapy) and resistant (post-therapy) to antipseudomonal β-lactams, 10 post-therapy isolates with β-lactam resistance overexpressed AmpC β-lactamase and the other 11 isolates had increased resistance to both β-lactams and non-β-lactams as a result of MexAB-

OprM overexpression.[120] Similarly, clinical isolates of the *nfxB*[336,387] and *nfxC*[388] types have been described. All 12 isolates of *P. aeruginosa* from animal sources were reported to express significant levels of the MexAB-OprM pump, whereas two isolates additionally expressed the MexEF-OprN or MexXY systems.[389] These studies clearly suggest that *in vivo* exposure of *P. aeruginosa* to antibacterials selected the mutants with increased expression of multidrug pumps. The high frequency of MDR in *P. aeruginosa* may also be related to the often persistent nature of *P. aeruginosa* infections (e.g. those in patients of cystic fibrosis), that require repeated exposures to antibacterials.[390]

Clinical MDR strains, at least in part due to increased efflux, have also been reported in many other Gram-negative bacteria, including *Campylobacter jejuni*,[391] *E. coli*,[392,393] *Enterobacter cloacae*,[394,395] *K. pneumoniae*,[396] *Morganella morganii*,[397] *Proteus vulgaris*,[398,399] *Serratia marcescens*[85] and *Shigella dysenteriae*[400]

In Gram-positive bacteria, efflux-based resistance is also seen in clinical isolates, particularly among fluoroquinolone- or macrolide-resistant isolates. A national survey carried out in Spain reported that erythromycin and ciprofloxacin resistance occurred in 35% and 7%, respectively, of 1684 clinical isolates of *S. pneumoniae*, and in 20% and 3.5%, respectively, of 2039 isolates of *S. pyogenes*.[401] Of the erythromycin resistant-strains, efflux accounted for 5% of the *S. pneumoniae* isolates and 90% of *S. pyogenes*.[401]

Active efflux is also a common mechanism for biocide resistance.[402] Indeed, the relationship between the emergence of MDR mutants and the use of non-antibiotic biocides in clinical settings have recently attracted much attention.[403-405] Two early studies revealed that chlorhexidine- or benzethonium chloride-resistant isolates of *P. aeruginosa* were isolated with incidence rates of 81% and 52%, respectively.[406,407] Current data continue to support the association of biocide usage with antibacterial resistance. A statistically significant inverse correlation was shown between intensity of chlorhexidine use and the overall susceptibility of several nosocomial pathogens including *S. aureus*, coagulase-negative staphylococci, *K. pneumoniae*, *P. aeruginosa*, *A. baumanii* and *C. albicans*.[408] The abuse of biocides clearly should not be ignored.[409] In some cases, the contribution of biocide usage could be indirect. Many plasmids in *S. aureus* containing antibacterial resistance genes also contain classical biocide (quaternary amine) efflux genes *qacA* or *qacB*.[410,411] Use of antiseptics in hospitals has, in this case, selected R plasmid-containing strains, which happened to contain genes causing antibacterial resistance by mechanisms not usually involving efflux.

The high percentage of the efflux mutants from clinical settings highlights the significance of the multidrug efflux systems as the clinically relevant mechanism of antibacterial resistance.

8.3 Which Drugs Are Important in the Development of Efflux-Based MDR?

As described in section 8.1, drugs of various classes can select for efflux-based MDR mutants *in vitro*. However, if non-efflux-based resistant strains are already available in the local population of the pathogens, they are likely to become prevalent as they impose less 'cost' on the bacteria than a broad specificity efflux does. This is why β-lactamase-producing strains usually emerge after treatment with older generations of β-lactams and aminoglycoside-modifying enzymes tend to be responsible for aminoglycoside-resistant strains. However, pharmaceutical companies have been successful in developing derivatives that resist these conventional mechanisms of drug inactivation. Thus, paradoxically, what typically selects for efflux-based resistance in the clinical setting is the most recently developed, sophisticated class of agents that cannot easily be made useless by the conventional resistance mechanisms.

Thus among β-lactams, carbenicillin was very effective in selecting for MexAB-OprM overproducers in *P. aeruginosa*, at the time when carbenicillin-hydrolysing β-lactamases were rare[385] (see section 8.2). It is probably the third- (or even fourth-) generation cephalosporins that selected for such mu-

tants in the more recent study in Besançon, France.[120]

Of all the classes of antibacterials, quinolones are perhaps the most studied group as regards selection of antibacterial-resistant mutants.[181,412-415] Fluoroquinolones were first introduced into clinical use in the 1980s and exert their antibacterial action by interfering with type II topoisomerases (i.e. DNA gyrase and topoisomerase IV).[414] Fluoroquinolones were not expected to generate resistant mutants readily, because these compounds were totally synthetic and because their targets are essential. Although they turned out to produce resistance caused by mutations in a small, limited domain of target (quinolone-resistance-determining region; QRDR),[414] this occurs with low frequency and produces only modest resistance levels. These factors, which give advantages to fluoroquinolones as antimicrobial agents, paradoxically made these compounds prime selective agents for efflux pump overproducers because efflux was the only readily available mechanism for resistance. It has also been speculated that the mutagenic ability of quinolones to damage bacterial DNA and trigger the error-prone SOS DNA repair system[416,417] may help in the generation of mutants.

All three MDR pump overproducers of *P. aeruginosa* – MexAB-OprM, MexCD-OprJ, and MexEF-OprN – were originally isolated in the laboratory after the use of quinolone drugs such as nalidixic acid (*nal*) and norfloxacin (*nfx*) for selection, as their names (*nalB*,[333] *nfxB*[387] and *nfx*[388]) imply. Mutants of *P. aeruginosa* PAO1 selected for resistance to one of the 12 different quinolone compounds were predominantly efflux-based MDR types, with the newer fluoroquinolones selecting the MexCD-OprJ overproducers while older quinolones exclusively selecting the MexEF-OprN or MexAB-OprM overproducers.[413] This differential selection was further demonstrated *in vivo* using a rat model, where trovafloxacin and ciprofloxacin preferentially selected overproducers of MexCD-OprJ and MexEF-OprN, respectively.[412] A 4-day therapy with ciprofloxacin resulted in the emergence of a double mutant carrying MexAB-OprM overexpression and a *gyr*B mutation.[418] Serial exposure of *P. aeruginosa* strains to fluoroquinolones yielded multiple antibacterial-resistant mutants with alterations simultaneously in both DNA gyrase and efflux systems.[419] Clinical isolates of *E. coli* showing high-level fluoroquinolone resistance are AcrAB pump overproducers (although they also contain target mutations), and this is likely to be due to *acrR* mutations that constitute additional genetic basis for quinolone resistance.[392,393] As well, fluoroquinolones also select the NorA and PmrA overproducing mutants of Gram-positive bacteria as described in section 3.

9. Interplay Between Drug Efflux and Other Resistance Mechanisms

In response to the widespread use of antibacterial agents, bacteria have developed diverse mechanisms of resistance (table I). Various mechanisms interact with each other usually positively (either additively or multiplicatively).

9.1 Interplay Between Efflux Pumps

Given the presence of multiple efflux pumps in a single bacterial cell and the overlapping substrate specificity of many pumps, it is obvious that the simultaneous presence of pumps with common substrates would increase the efflux capability to produce a higher resistance level. Indeed, genetic inactivation of multiple efflux genes of *E. coli* and *P. aeruginosa* renders the strains more susceptible to antibacterials than a single pump inactivation.[88,177,420] Lee et al.[421] contributed an important study in Gram-negative bacteria, which showed two kinds of positive interactions are possible. If the two pumps have the same type of mechanism (either efflux into the periplasm via a simple transporter located in CM, or efflux into the medium via a multiprotein complex containing MFP and OM channel in addition to the transporter), then the two pumps work in parallel and the augmentation effect should be additive. But if one simple transporter pumps out the drug into periplasm, and the second one, a tripartite pump, captures the drug from the periplasm (as described in section 6.1) and extrudes

it into the medium, then the two pumps work in series, and the augmentation should be multiplicative. This theory was validated with various combinations of pumps.

It is at first surprising that simple pumps like the Tet pump can create strong resistance in Gram-negative bacteria when we consider that they extrude lipophilic drugs only into periplasm[108] and that the drugs can diffuse back into the cytoplasm readily. However, tripartite pumps of wide specificity, such as AcrAB-TolC and MexAB-OprM, are usually constitutively expressed, and perhaps synergy with these pumps is in part responsible for making single-component pumps in CM more effective than first appears possible.

9.2 Efflux Pumps and the OM Permeability Barrier

The OM of Gram-negative bacteria is an asymmetric bilayer containing lipopolysaacharide (LPS), which greatly retards the entry of amphiphilic and hydrophobic compounds.[422,423] Disruption of LPS barrier (e.g. due to inactivation of *waaP* gene in *Salmonella* and *E. coli*[424,425] or by the addition of OM perturbant polymyxin B nonapeptide[426]) makes bacteria hypersusceptible to multiple antibacterial agents. Mutations in LPS and TolC together enhance the drug hypersusceptibility in *E. coli*.[427] MtrCDE-mediated MDR is also dependent on the lipooligosaccharide structures in *N. gonorrhoeae*.[428]

Since the OM barrier and the efflux by the tripartite systems act in series on drug molecules, the considerations similar to those described in section 9.1 predict that these two factors augment each other in a multiplicative fashion. Small hydrophilic agents readily penetrate the OM via water-filled porin channels.[114] Thus, a decrease or loss of porins reduces antibacterial uptake and contributes to antibacterial resistance.[429] Nevertheless, this contribution of the OM barrier is not seen clearly unless its effect is multiplicatively amplified by an additional (intrinsic) resistance mechanism, such as drug efflux or drug inactivation following their influx.[18,430] On the other hand, the synergistic interplay between the OM permeability barrier and the MexAB-OprM pump is seen very clearly in *P. aeruginosa*[431] because this organism has low OM permeability to both hydrophilic and hydrophobic compounds.[432] Either the inactivation of MexAB-OprM system or the permeabilisation of OM with polymyxin nonapeptide had very similar, strong effect to make the organism hypersusceptible,[44-46] showing the importance of OM barrier in the efflux-mediated resistance. For example, tetracycline MIC of 16 μg/mL in the wild type PAO1 decreases to 0.5 μg/mL on inactivation of the tripartite pump and to 1 μg/mL on OM permeabilisation.[431] One might expect that the assay of intracellular accumulation of fluorescent dye[433] may allow us to see if the two mechanisms interact truly multiplicatively, but the data cannot be interpreted quantitatively because we do not know the degree of OM permeabilisation or the exact activity of the pump system.

9.3 Efflux Pumps and Antibacterial-Inactivating Enzymes

P. aeruginosa and most species of the Enterobacteriaceae produce chromosomally encoded inducible (constitutive in *E. coli*) β-lactamases that hydrolyse many β-lactams.[434] Mutational derepression of the enzymes produces resistance. This resistance should be augmented, in an additive manner, by efflux catalysed by tripartite pumps, as both mechanisms act in parallel to lower the periplasmic drug concentrations. A theoretical analysis was attempted but it was possible only with the assumption of 'maximal' efflux.[435] Comparisons of MICs in *E. coli* strains lacking either *acrAB* or the constitutive *ampC* β-lactamase[372] are roughly compatible with this notion, although quantitative treatment is not possible because the kinetic constants of the efflux system are not known. As expected, efflux was a predominant contributor for the intrinsic resistance of *E. coli* to lipophilic penicillins (cloxacillin, oxacillin, etc.), and enzymatic hydrolysis was the major factor for hydrophilic cephalosporins of earlier generations (e.g. cephalothin, cephaloridine and cefamandole).

For many β-lactams, the interplay between the MexAB-OprM-catalysed efflux and the AmpC β-lactamase-catalysed hydrolysis in *P. aeruginosa* PAO1 was similar.[436-438] For example, the enzymatic hydrolysis plays a predominant role in resistance to amoxicillin, whereas efflux plays a major role in resistance to carbenicillin, piperacillin, aztreonam and cefsulodin.[437] However, unexpected data were obtained for some compounds. For example, the cefuroxime MIC of 800 μg/mL in the wild type decreases only 2-fold to 400 μg/mL on deletion of MexAB-OprM, and remains unchanged at 400 μg/mL on inactivation of the *ampC* gene. But the deletion of both of these resistance genes results in a precipitous fall of MIC to 0.2 μg/mL.[437] This effect is certainly not additive and is far more than multiplicative. Currently we cannot explain these data. In *S. maltophilia*, an aminoglycoside-modifying acetyltransferase (6')-*Iz* and an MDR efflux pump augment each other to enhance aminoglycoside resistance.[439]

9.4 Efflux Pumps and Antibacterial Target Alterations

The increased expression of NorA in *S. aureus* and PmrA in *S. pneumoniae* may occur together with *gyrA/parC* target mutations to provide high-level quinolone resistance.[231,237,238,440] In nine out of ten clinical isolates of *E. coli* showing high-level resistance (MIC ≥3 μg/mL) to ciprofloxacin, AcrAB was overexpressed in addtion to the mutations in the targets.[392] Strains of *P. aeruginosa* overexpressing both an efflux pump (MexAB-OprM or MexCD-OprJ) and DNA gyrase mutations were significantly more resistant to quinolones than those carrying only one of these resistance mechanisms.[420,441] Deletion of MexAB-OprM in a PBP mutant also modestly compromised β-lactam resistance of a *P. aeruginosa* mutant,[438] suggesting some involvement of efflux mechanisms in PBP-mediated β-lactam resistance. In *N. gonorrhoeae*, loci designated *penA, penB* and *mtr* contribute additively to penicillin resistance[163] and the target-altered penicillin resistance also requires the MtrCDE pump overexpression.[442]

10. Overcoming Efflux Activity: The Development of Efflux Pump Inhibitors and Antibacterials that Bypass Efflux Pumps

We can consider efflux pumps as potentially effective antibacterial targets. Currently used antibacterials target a surprisingly small number of vital cellular functions (table I), and instances of resistance to these antibacterials are widespread, in many cases caused or exacerbated by efflux. Exploring EPIs resembles the application of β-lactamase inhibitors to combat β-lactamase-mediated resistance in bacteria.[443] The genetic studies of efflux genes (for example, those coding for the RND multidrug pumps of *P. aeruginosa*)[42] have shown that their inactivation renders the strains markedly hypersusceptible to a wide variety of antibacterials. Inhibition of efflux pumps by an EPI would restore the activity of an agent subject to efflux. An alternative approach is to develop antibacterials that would bypass the action of efflux pumps.

10.1 Inhibitors for Drug-Specific Pumps

Inhibition of Tet efflux pumps has been studied for many years,[444-447] with a focus on tetracycline analogues. Of the inhibitors screened, an intact 4-membered naphthalene structure seemed necessary for maximal inhibition of efflux and 13-cyclopentylthio-5-OH tetracycline (13-CPTC) was the most potent inhibitor of TetA(B) pump with an IC_{50} value of 0.4 to 1 μmol/L. 13-CPTC alone is ineffective against *E. coli* harbouring Tet pumps but it potentiated markedly the activity of doxycycline against such strains. Intriguingly, 13-CPTC alone was active against *S. aureus* containing TetK and *E. faecalis* containing TetL with MIC values at 0.39–1.56 μg/mL.[445,448] 13-CPTC apparently binds to Tet pumps and, thus, blocks tetracycline efflux.

TetK and TetL proteins are efflux determinants of tetracycline resistance in Gram-positive bacteria. Low molecular weight inhibitors of these pumps were studied, and among these inhibitors there was an indan compound (RO 07-3149; 1,1-dimethyl-5-(1-hydroxypropyl)-4,5,6-trimethylindan) and its derivatives, which increased antibacterial activity of

tetracycline against TetK/L-containing *S. aureus*.[449,450] Additionally, some natural compounds such as ginsenosides (obtained from ginsen) and indole derivatives were identified as putative inhibitors for TetK or TetC pump.[448] Despite these efforts, none of the Tet pump inhibitors is yet in clinical use.

10.2 Inhibitors for Multidrug Efflux Pumps

With the increasing role of multidrug pumps in resistance, inhibitors for these pumps have been looked for.[451-453] Several compounds inhibit multidrug pumps of bacteria, parasites or mammalian cells. For instance, verapamil, a calcium channel antagonist, is an inhibitor for MDR pumps of mammalian cancer cells[454] and a parasite (*P. falciparum*).[455] It also enhances the antibacterial activity of tobramycin against *B. cepacia* but not *P. aeruginosa*.[456] Reserpine, a plant indole alkaloid, is an inhibitor of mammalian MDR pumps as well as Gram-positive bacterial MDR pumps such as Bmr and NorA.[249] In fact, reserpine is now routinely used to determine if resistance is caused by multidrug efflux in Gram-positive bacteria,[232,249,457-461] as it largely reverses, at 10 μg/mL, NorA/PmrA-mediated resistance to fluoroquinolones. Unfortunately reserpine is toxic to humans at the concentrations required for pump inhibition.

Structurally diverse inhibitors were identified among synthetic chemical libraries using NorA of *S. aureus* as target and some of these compounds were more potent than reserpine.[460] These potent inhibitors also decrease the frequency of emergence of ciprofloxacin-resistant mutants by >50-fold.[460] More recently, Stermitz et al.[462] identified a NorA inhibitor from the extracts of the leaves of *Berberis fremontii* as 5′-methoxyhydnocarpine (5′-MHC), a flavonoid. 5′-MHC enhanced the antimicrobial activity of another alkaloid present in the same plant (berberine) by inhibiting NorA-catalysed efflux of the latter. The fact that a plant simultaneously contains both an efflux substrate and an efflux inhibitor may indicate that plants have developed this combination to ward off bacterial invasion.

As described in section 2, RND transporters play a particularly important role in drug resistance of Gram-negative bacteria, for example, *P. aeruginosa*. A series of inhibitors have been developed to target specifically the RND transporters in order to potentiate the activity of fluoroquinolones, for which efflux is a crucial resistance determinant. The first broad-spectrum inhibitor reported for RND pumps, the compound MC-207,110 (phenylalanyl-arginyl-β-naphthylamide) virtually lacked antibacterial activity on its own, but at 10 μg/mL potentiated the activity of levofloxacin 8-fold against wild-type *P. aeruginosa*.[463] The potentiation of levofloxacin activity (measured by MIC) even reached 64-fold in MexAB-overexpressing *P. aeruginosa*.[463] Further chemical modifications of MC-207,110 led to other peptides that showed potentiation activity for *P. aeruginosa* strains producing the three best characterised Mex pumps and *E. coli* strains with AcrAB-TolC pump.[464,465] These inhibitors also dramatically reduced the frequency with which resistant bacteria emerged spontaneously.[465]

Effectiveness of efflux inhibitors was also confirmed in animal models of *P. aeruginosa* infection.[466] Thus, the EPIs worked to potentiate activities of fluoroquinolones, macrolides and florfenicol against a number of other Gram-negative bacteria, including *K. pneumoniae*, *H. influenzae*, *E. coli* and *S. typhimurium*, all of which possess RND efflux pumps (table II). More recently, peptidomimetics of the lead compound were prepared to achieve biological stability against proteases.[467] In addition, benastatins isolated from fermentation of an actinomycete were active against *P. aeruginosa* MexAB-OprM, reducing MIC of levofloxacin 4-fold at a concentration less than 1 μg/mL.[468] Altogether, these studies clearly indicate the feasibility of the combination of an efflux inhibitor with an antibacterial to restore the antibacterial activity against resistant organisms *in vitro* and *in vivo*. Nevertheless, the development of these inhibitors is still complicated by the need to combine them with antibacterial drugs of similar pharmacokinetic characteristics.

10.3 Bypassing Efflux Mechanisms

Some newly developed fluoroquinolones such as clindafloxacin, gatifloxacin, premafloxacin and trovafloxacin appear to be less affected by the presence of NorA and PmrA pumps of Gram-positive bacteria.[198,469-471] However, it has not been convincingly shown that this difference is due to their resistance to efflux, rather than to their higher affinity for the target. A new class of macrolides, ketolides, are often thought not to be pumped out efficiently, because their MIC values remain low even in MefA-overexpressing strains.[472] However, these compounds are active against wild-type strains at much lower concentrations than erythromycin and a more detailed study is needed to show that the difference is not simply due to the higher affinity to the target.

A different type of semisynthetic macrolide, CP544372, in which a carbamate substituent is added to 4′-position of cladiose moiety, shows only very minor change in MIC upon the overexpression of MefA or MsrA pump,[473,474] and this compound may indeed bypass the pumps. The new class of tetracyclines, glycylcyclines, indeed appear to be transported inefficiently by the TetA pump.[475] Nevertheless, a variety of newer fluoroquinolones as well as a glycylcycline (i.e. tigecycline) are substrates for the RND pumps of Gram-negative bacteria, including those of *E. coli*, *P. aeruginosa*, *B. cepacia* and *S. maltophilia*.[83,152]

11. Conclusions

Antibacterial resistance in bacteria is known to occur usually by the 'classical' mechanisms such as drug inactivation. In many cases, the origin of resistance genes has been traced to the producing organisms; for example, genes coding for aminoglycoside-inactivating enzymes.[476-478] As another example, vancomycin resistance gene complex of the resistant isolates from clinical sources is strikingly similar to that in the producing organisms.[479] Although this concept may not apply to the origin of β-lactamases, the production of β-lactams is a widespread trait among many diverse bacteria and fungi, and it is very likely that bacteria, especially soil bacteria, developed the capability to produce these enzymes in response to the presence of β-lactam-producing organisms in their environment.

The approach of the pharmaceutical industry in the last several decades has been to develop compounds that withstand the prevailing mechanisms of resistance. One approach advocated was to develop totally synthetic chemicals to which bacteria were not exposed during their evolution. Fluoroquinolones are good examples of this approach. Even for these compounds, mutational modification of the target is possible. However, if the target is an essential protein which requires precise changes in critical residues to develop resistance and yet to maintain the physiological function, such mutations should occur rarely, a condition satisfied by fluoroquinolones.

It was an unexpected outcome that these 'advanced' compounds would be affected most by the up-regulation mutants of multidrug efflux pumps. In retrospect, this is not surprising because bacteria have few other ways to develop resistance to these compounds. Up-regulation of broad-spectrum pumps, however, often produces selective disadvantages for the bacteria and this may limit the emergence of this resistance mechanism to some extent. On the other hand, selection, in one step, of resistance to many or most of the available antibacterial agents is a major danger presented by the multidrug efflux mechanisms. Furthermore, selection of such mutants by widely used biocides is a major concern.

The evolutionary origin of multidrug efflux pumps has been debated. Some scientists feel that they must function in the efflux of endogenous compounds, as exemplified by the notion that RND pumps are involved in the secretion of quorum-sensing signals in *P. aeruginosa*. However, there is little evidence to support this idea. In 1997, the fact that the B. *subtilis blt* gene is a part of the polyamine metabolism gene complex[184] was thought to implicate Blt in the metabolism of these 'natural' compounds, the polyamines. However, the force of this argument is now weakened somewhat because of the discovery of a global regulator, Mta.[183] (In this connection, it is most interesting that Krulwich and

coworkers[480] discovered that the tetracycline efflux transporters of *B. subtilis*, Tet(K) and Tet(L), also function in the transport of monovalent cations. Although inactivation of Tet(L) produces mutants that have difficulty in growing in K^+-limiting or alkaline media [in the absence of tetracycline],[481] it seems likely that the tetracycline efflux function is a more recently acquired additional capacity of Na^+ or K^+ transporters.[480])

Bacteria, in their evolution, had to survive in the presence of many lipophilic toxic chemicals and we feel that the broad-spectrum multidrug efflux pumps probably evolved to prevent the influx of such compounds. In fact, the observation that *E. coli* AcrAB-TolC has properties optimised for the efflux of bile salts,[49] the major inhibitors in its natural environment, fits this idea. We should note also that RND pumps are of ancient origin and exist in all three kingdoms,[35] consistent with the notion that organisms had to move lipophilic toxic chemicals all the time. In any case, this ancient origin of multidrug efflux pumps casts a shadow on future development of new antimicrobials. Restoring or increasing antibacterial activity through efflux pump inhibitors and developing compounds that are not pumped out now appear to be attractive approaches for pharmaceutical industry.

Acknowledgements

Research in the authors' laboratory was supported by a grant from the US Public Health Service (AI-09644). A portion of the review is based on the PhD thesis of X.-Z. Li, who thanks Keith Poole for his encouragement during the graduate study in his laboratory. Neither author has any disclosable interest relevant to the content of the review.

References

1. Levy SB. Antibiotic resistance: an ecological imbalance. Ciba Found Symp 1997; 207: 1-9
2. Normark BH, Normark S. Evolution and spread of antibiotic resistance. J Intern Med 2002 Aug; 252 (2): 91-106
3. Ball PR, Chopra I, Eccles SJ. Accumulation of tetracyclines by *Escherichia coli* K-12. Biochem Biophys Res Commun 1977 Aug 22; 77 (4): 1500-7
4. Ball PR, Shales SW, Chopra I. Plasmid-mediated tetracycline resistance in *Escherichia coli* involves increased efflux of the antibiotic. Biochem Biophys Res Commun 1980 Mar 13; 93 (1): 74-81
5. Levy SB, McMurry L. Plasmid-determined tetracycline resistance involves new transport systems for tetracycline. Nature 1978 Nov 2; 276 (5683): 90-2
6. McMurry L, Petrucci Jr RE, Levy SB. Active efflux of tetracycline encoded by four genetically different tetracycline resistance determinants in *Escherichia coli*. Proc Natl Acad Sci U S A 1980 Jul; 77 (7): 3974-7
7. Juliano RL, Ling V. A surface glycoprotein modulating drug permeability in Chinese hamster ovary cell mutants. Biochim Biophys Acta 1976 Nov 11; 455 (1): 152-62
8. Institute of Genome Research (TIGR) microbial database [online]. Available from URL: http://www.tigr.org/tdb/mdb/mdbcomplete.html [Accessed 2003 Nov 11]
9. Lomovskaya O, Warren MS, Lee V. Efflux mechanisms: molecular and clinical aspects. In: Hughes D, Andersson DI, editors. Antibiotic development and resistance. London: Taylor and Francis, 2001: 65-90
10. Poole K. Outer membranes and efflux: the path to multidrug resistance in Gram-negative bacteria. Curr Pharm Biotechnol 2002 Jun; 3 (2): 77-98
11. Paulsen IT, Lewis K. Microbial multidrug efflux. Wynmondham: Horizon Press, 2002
12. Fath MJ, Kolter R. ABC transporters: bacterial exporters. Microbiol Rev 1993 Dec; 57 (4): 995-1017
13. Higgins CF. ABC transporters: physiology, structure and mechanism: an overview. Res Microbiol 2001 Apr-May; 152 (3-4): 205-10
14. Pao SS, Paulsen IT, Saier Jr MH. Major facilitator superfamily. Microbiol Mol Biol Rev 1998 Mar; 62 (1): 1-34
15. Brown MH, Paulsen IT, Skurray RA. The multidrug efflux protein NorM is a prototype of a new family of transporters. Mol Microbiol 1999 Jan; 31 (1): 394-5
16. Paulsen IT, Skurray RA, Tam R, et al. The SMR family: a novel family of multidrug efflux proteins involved with the efflux of lipophilic drugs. Mol Microbiol 1996 Mar; 19 (6): 1167-75
17. Saier Jr MH, Tam R, Reizer A, et al. Two novel families of bacterial membrane proteins concerned with nodulation, cell division and transport. Mol Microbiol 1994 Mar; 11 (5): 841-7
18. Nikaido H. Multidrug efflux pumps of gram-negative bacteria. J Bacteriol 1996 Oct; 178 (20): 5853-9
19. Paulsen IT, Park JH, Choi PS, et al. A family of gram-negative bacterial outer membrane factors that function in the export of proteins, carbohydrates, drugs and heavy metals from gram-negative bacteria. FEMS Microbiol Lett 1997 Nov 1; 156 (1): 1-8
20. Johnson JM, Church GM. Alignment and structure prediction of divergent protein families: periplasmic and outer membrane proteins of bacterial efflux pumps. J Mol Biol 1999 Apr 2; 287 (3): 695-715
21. Dinh T, Paulsen IT, Saier Jr MH. A family of extracytoplasmic proteins that allow transport of large molecules across the outer membranes of gram-negative bacteria. J Bacteriol 1994 Jul; 176 (13): 3825-31
22. Saier Jr MH, Paulsen IT, Sliwinski MK, et al. Evolutionary origins of multidrug and drug-specific efflux pumps in bacteria. FASEB J 1998 Mar; 12 (3): 265-74
23. Higgins CF. ABC transporters: from microorganisms to man. Annu Rev Cell Biol 1992; 8: 67-113
24. Bolhuis H, van Veen HW, Brands JR, et al. Energetics and mechanism of drug transport mediated by the lactococcal multidrug transporter LmrP. J Biol Chem 1996 Sep 27; 271 (39): 24123-8

25. Kobayashi N, Nishino K, Yamaguchi A. Novel macrolide-specific ABC-type efflux transporter in *Escherichia coli*. J Bacteriol 2001 Oct; 183 (19): 5639-44
26. Marger MD, Saier Jr MH. A major superfamily of transmembrane facilitators that catalyse uniport, symport and antiport. Trends Biochem Sci 1993 Jan; 18 (1): 13-20
27. Saier Jr MH, Beatty JT, Goffeau A, et al. The major facilitator superfamily. J Mol Microbiol Biotechnol 1999 Nov; 1 (2): 257-79
28. Yoshida H, Bogaki M, Nakamura S, et al. Nucleotide sequence and characterization of the *Staphylococcus aureus norA* gene, which confers resistance to quinolones. J Bacteriol 1990 Dec; 172 (12): 6942-9
29. Lomovskaya O, Lewis K. Emr, an *Escherichia coli* locus for multidrug resistance. Proc Natl Acad Sci U S A 1992 Oct 1; 89 (19): 8938-42
30. Morita Y, Kodama K, Shiota S, et al. *NorM*, a putative multidrug efflux protein, of Vibrio parahaemolyticus and its homolog in *Escherichia coli*. Antimicrob Agents Chemother 1998 Jul; 42 (7): 1778-82
31. Chung YJ, Saier Jr MH. SMR-type multidrug resistance pumps. Curr Opin Drug Discov Devel 2001 Mar; 4 (2): 237-45
32. Paulsen IT, Brown MH, Skurray RA. Proton-dependent multidrug efflux systems. Microbiol Rev 1996 Dec; 60 (4): 575-608
33. Grinius L, Dreguniene G, Goldberg EB, et al. A staphylococcal multidrug resistance gene product is a member of a new protein family. Plasmid 1992 Mar; 27 (2): 119-29
34. Schuldiner S, Lebendiker M, Yerushalmi H. EmrE, the smallest ion-coupled transporter, provides a unique paradigm for structure-function studies. J Exp Biol 1997 Jan; 200 (Pt 2): 335-41
35. Tseng TT, Gratwick KS, Kollman J, et al. The RND permease superfamily: an ancient, ubiquitous and diverse family that includes human disease and development proteins. J Mol Microbiol Biotechnol 1999 Aug; 1 (1): 107-25
36. Grosse C, Grass G, Anton A, et al. Transcriptional organization of the *czc* heavy-metal homeostasis determinant from *Alcaligenes eutrophus*. J Bacteriol 1999 Apr; 181 (8): 2385-93
37. Droge M, Puhler A, Selbitschka W. Phenotypic and molecular characterization of conjugative antibiotic resistance plasmids isolated from bacterial communities of activated sludge. Mol Gen Genet 2000 Apr; 263 (3): 471-82
38. Nikaido H. Antibiotic resistance caused by gram-negative multidrug efflux pumps. Clin Infect Dis 1998 Aug; 27 Suppl. 1: S32-41
39. Ma D, Cook DN, Alberti M, et al. Molecular cloning and characterization of *acrA* and *acrE* genes of *Escherichia coli*. J Bacteriol 1993 Oct; 175 (19): 6299-313
40. Fralick JA. Evidence that *tolC* is required for functioning of the *mar/acrAB* efflux pump of *Escherichia coli*. J Bacteriol 1996 Oct; 178 (19): 5803-5
41. Poole K, Krebes K, McNally C, et al. Multiple antibiotic resistance in *Pseudomonas aeruginosa*: evidence for involvement of an efflux operon. J Bacteriol 1993 Nov; 175 (22): 7363-72
42. Li XZ, Nikaido H, Poole K. Role of MexA-MexB-OprM in antibiotic efflux in *Pseudomonas aeruginosa*. Antimicrob Agents Chemother 1995 Sep; 39 (9): 1948-53
43. Nikaido H. Outer membrane barrier as a mechanism of antimicrobial resistance. Antimicrob Agents Chemother 1989 Nov; 33 (11): 1831-6
44. Li XZ, Livermore DM, Nikaido H. Role of efflux pump (s) in intrinsic resistance of *Pseudomonas aeruginosa*: resistance to tetracycline, chloramphenicol, and norfloxacin. Antimicrob Agents Chemother 1994 Aug; 38 (8): 1732-41
45. Li XZ, Ma D, Livermore DM, et al. Role of efflux pump (s) in intrinsic resistance of *Pseudomonas aeruginosa*: active efflux as a contributing factor to β-lactam resistance. Antimicrob Agents Chemother 1994 Aug; 38 (8): 1742-52
46. Nikaido H. The role of outer membrane and efflux pumps in the resistance of gram-negative bacteria: can we improve drug access? Drug Resist Updat 1998; 1: 93-8
47. Nishino K, Yamaguchi A. Analysis of a complete library of putative drug transporter genes in *Escherichia coli*. J Bacteriol 2001 Oct; 183 (20): 5803-12
48. Nakamura H. Gene-controlled resistance to acriflavine and other basic dyes in *Escherichia coli*. J Bacteriol 1965 Jul; 90 (1): 8-14
49. Zgurskaya HI, Nikaido H. Bypassing the periplasm: reconstitution of the AcrAB multidrug efflux pump of *Escherichia coli*. Proc Natl Acad Sci U S A 1999 Jun 22; 96 (13): 7190-5
50. Magnet S, Courvalin P, Lambert T. Resistance-nodulation-cell division-type efflux pump involved in aminoglycoside resistance in *Acinetobacter baumannii* strain BM4454. Antimicrob Agents Chemother 2001 Dec; 45 (12): 3375-80
51. Palumbo JD, Kado CI, Phillips DA. An isoflavonoid-inducible efflux pump in *Agrobacterium tumefaciens* is involved in competitive colonization of roots. J Bacteriol 1998 Jun; 180 (12): 3107-13
52. Peng WT, Nester EW. Characterization of a putative RND-type efflux system in *Agrobacterium tumefaciens*. Gene 2001 May 30; 270 (1-2): 245-52
53. Krummenacher P, Narberhaus F. Two genes encoding a putative multidrug efflux pump of the RND/MFP family are cotranscribed with an *rpoH* gene in *Bradyrhizobium japonicum*. Gene 2000 Jan 11; 241 (2): 247-54
54. Burns JL, Wadsworth CD, Barry JJ, et al. Nucleotide sequence analysis of a gene from *Burkholderia* (*Pseudomonas*) *cepacia* encoding an outer membrane lipoprotein involved in multiple antibiotic resistance. Antimicrob Agents Chemother 1996 Feb; 40 (2): 307-13
55. Moore RA, DeShazer D, Reckseidler S, et al. Efflux-mediated aminoglycoside and macrolide resistance in *Burkholderia pseudomallei*. Antimicrob Agents Chemother 1999 Mar; 43 (3): 465-70
56. Lin J, Michel LO, Zhang Q. CmeABC functions as a multidrug efflux system in *Campylobacter jejuni*. Antimicrob Agents Chemother 2002 Jul; 46 (7): 2124-31
57. Pradel E, Pages JM. The AcrAB-TolC efflux pump contributes to multidrug resistance in the nosocomial pathogen *Enterobacter aerogenes*. Antimicrob Agents Chemother 2002 Aug; 46 (8): 2640-3
58. Rosenberg EY, Ma D, Nikaido H. AcrD of *Escherichia coli* is an aminoglycoside efflux pump. J Bacteriol 2000 Mar; 182 (6): 1754-6
59. Elkins CA, Nikaido H. Substrate specificity of the RND-type multidrug efflux pumps AcrB and AcrD of *Escherichia coli* is determined predominantly by two large periplasmic loops. J Bacteriol 2002 Dec; 184 (23): 6490-8
60. Ma D, Cook DN, Hearst JE, et al. Efflux pumps and drug resistance in gram-negative bacteria. Trends Microbiol 1994 Dec; 2 (12): 489-93
61. Baranova N, Nikaido H. The *baeSR* two-component regulatory system activates transcription of the *yegMNOB* (*mdtABCD*) transporter gene cluster in *Escherichia coli* and increases its

resistance to novobiocin and deoxycholate. J Bacteriol 2002 Aug; 184 (15): 4168-76
62. Nagakubo S, Nishino K, Hirata T, et al. The putative response regulator BaeR stimulates multidrug resistance of *Escherichia coli* via a novel multidrug exporter system, *MdtABC*. J Bacteriol 2002 Aug; 184 (15): 4161-7
63. Nishino K, Yamaguchi A. *EvgA* of the two-component signal transduction system modulates production of the *yhiUV* multidrug transporter in *Escherichia coli*. J Bacteriol 2002 Apr; 184 (8): 2319-23
64. Sanchez L, Pan W, Vinas M, et al. The *acrAB* homolog of *Haemophilus influenzae* codes for a functional multidrug efflux pump. J Bacteriol 1997 Nov; 179 (21): 6855-7
65. Hagman KE, Pan W, Spratt BG, et al. Resistance of *Neisseria gonorrhoeae* to antimicrobial hydrophobic agents is modulated by the *mtrRCDE* efflux system. Microbiology 1995 Mar; 141 (Pt 3): 611-22
66. Lucas CE, Balthazar JT, Hagman KE, et al. The MtrR repressor binds the DNA sequence between the *mtrR* and *mtrC* genes of *Neisseria gonorrhoeae*. J Bacteriol 1997 Jul; 179 (13): 4123-8
67. Lee EH, Shafer WM. The *farAB*-encoded efflux pump mediates resistance of gonococci to long-chained antibacterial fatty acids. Mol Microbiol 1999 Aug; 33 (4): 839-45
68. Ikeda T, Yoshimura F. A resistance-nodulation-cell division family xenobiotic efflux pump in an obligate anaerobe, *Porphyromonas gingivalis*. Antimicrob Agents Chemother 2002 Oct; 46 (10): 3257-60
69. Poole K, Tetro K, Zhao Q, et al. Expression of the multidrug resistance operon MexA-MexB-OprM in *Pseudomonas aeruginosa*: MexR encodes a regulator of operon expression. Antimicrob Agents Chemother 1996 Sep; 40 (9): 2021-8
70. Poole K, Gotoh N, Tsujimoto H, et al. Overexpression of the MexC-MexD-OprJ efflux operon in *nfxB*-type multidrug-resistant strains of *Pseudomonas aeruginosa*. Mol Microbiol 1996 Aug; 21 (4): 713-24
71. Kohler T, Michea-Hamzehpour M, Henze U, et al. Characterization of MexE-MexF-OprN, a positively regulated multidrug efflux system of *Pseudomonas aeruginosa*. Mol Microbiol 1997 Jan; 23 (2): 345-54
72. Aires JR, Kohler T, Nikaido H, et al. Involvement of an active efflux system in the natural resistance of *Pseudomonas aeruginosa* to aminoglycosides. Antimicrob Agents Chemother 1999 Nov; 43 (11): 2624-8
73. Mine T, Morita Y, Kataoka A, et al. Expression in *Escherichia coli* of a new multidrug efflux pump, MexXY, from *Pseudomonas aeruginosa*. Antimicrob Agents Chemother 1999 Feb; 43 (2): 415-7
74. Westbrock-Wadman S, Sherman DR, Hickey MJ, et al. Characterization of a *Pseudomonas aeruginosa* efflux pump contributing to aminoglycoside impermeability. Antimicrob Agents Chemother 1999 Dec; 43 (12): 2975-83
75. Aendekerk S, Ghysels B, Cornelis P, et al. Characterization of a new efflux pump, MexGHI-OpmD, from *Pseudomonas aeruginosa* that confers resistance to vanadium. Microbiology 2002 Aug; 148 (Pt 8): 2371-81
76. Chuanchuen R, Narasaki CT, Schweizer HP. The *MexJK* efflux pump of *Pseudomonas aeruginosa* requires OprM for antibiotic efflux but not for efflux of triclosan. J Bacteriol 2002 Sep; 184 (18): 5036-44
77. Kieboom J, Dennis JJ, de Bont JA, et al. Identification and molecular characterization of an efflux pump involved in *Pseudomonas putida* S12 solvent tolerance. J Biol Chem 1998 Jan 2; 273 (1): 85-91
78. Ramos JL, Duque E, Godoy P, et al. Efflux pumps involved in toluene tolerance in *Pseudomonas putida* DOT-T1E. J Bacteriol 1998 Jul; 180 (13): 3323-9
79. Fukumori F, Hirayama H, Takami H, et al. Isolation and transposon mutagenesis of a *Pseudomonas putida* KT2442 toluene-resistant variant: involvement of an efflux system in solvent resistance. Extremophiles 1998 Nov; 2 (4): 395-400
80. Mosqueda G, Ramos JL. A set of genes encoding a second toluene efflux system in *Pseudomonas putida* DOT-T1E is linked to the tod genes for toluene metabolism. J Bacteriol 2000 Feb; 182 (4): 937-43
81. Rojas A, Duque E, Mosqueda G, et al. Three efflux pumps are required to provide efficient tolerance to toluene in *Pseudomonas putida* DOT-T1E. J Bacteriol 2001 Jul; 183 (13): 3967-73
82. Li XZ, Zhang L, Poole K. SmeC, an outer membrane multidrug efflux protein of *Stenotrophomonas maltophilia*. Antimicrob Agents Chemother 2002 Feb; 46 (2): 333-43
83. Zhang L, Li XZ, Poole K. SmeDEF multidrug efflux pump contributes to intrinsic multidrug resistance in *Stenotrophomonas maltophilia*. Antimicrob Agents Chemother 2001 Dec; 45 (12): 3497-503
84. Alonso A, Martinez JL. Cloning and characterization of SmeDEF, a novel multidrug efflux pump from *Stenotrophomonas maltophilia*. Antimicrob Agents Chemother 2000 Nov; 44 (11): 3079-86
85. Kumar A, Worobec EA. Fluoroquinolone resistance of *Serratia marcescens*: involvement of a proton gradient-dependent efflux pump. J Antimicrob Chemother 2002 Oct; 50 (4): 593-6
86. Nikaido H, Basina M, Nguyen V, et al. Multidrug efflux pump AcrAB of *Salmonella typhimurium* excretes only those β-lactam antibiotics containing lipophilic side chains. J Bacteriol 1998 Sep; 180 (17): 4686-92
87. Lacroix FJ, Cloeckaert A, Grepinet O, et al. *Salmonella typhimurium acrB*-like gene: identification and role in resistance to biliary salts and detergents and in murine infection. FEMS Microbiol Lett 1996 Jan 15; 135 (2-3): 161-7
88. Sulavik MC, Houseweart C, Cramer C, et al. Antibiotic susceptibility profiles of *Escherichia coli* strains lacking multidrug efflux pump genes. Antimicrob Agents Chemother 2001 Apr; 45 (4): 1126-36
89. Furukawa H, Tsay JT, Jackowski S, et al. resistance in *Escherichia coli* is associated with the multidrug resistance efflux pump encoded by *emrAB*. J Bacteriol 1993 Jun; 175 (12): 3723-9
90. Bohn C, Bouloc P. The *Escherichia coli cmlA* gene encodes the multidrug efflux pump Cmr/MdfA and is responsible for isopropyl-β-D-thiogalactopyranoside exclusion and spectinomycin sensitivity. J Bacteriol 1998 Nov; 180 (22): 6072-5
91. Nilsen IW, Bakke I, Vader A, et al. Isolation of *cmr*, a novel *Escherichia coli* chloramphenicol resistance gene encoding a putative efflux pump. J Bacteriol 1996 Jun; 178 (11): 3188-93
92. Edgar R, Bibi E. A single membrane-embedded negative charge is critical for recognizing positively charged drugs by the *Escherichia coli* multidrug resistance protein MdfA. EMBO J 1999 Feb 15; 18 (4): 822-32
93. Mine T, Morita Y, Kataoka A, et al. Evidence for chloramphenicol/H^+ antiport in Cmr (MdfA) system of *Escherichia coli* and properties of the antiporter. J Biochem (Tokyo) 1998 Jul; 124 (1): 187-93
94. Dorman CJ, Foster TJ, Shaw WV. Nucleotide sequence of the R26 chloramphenicol resistance determinant and identification of its gene product. Gene 1986; 41 (2-3): 349-53

95. Ploy MC, Courvalin P, Lambert T. Characterization of In40 of *Enterobacter aerogenes* BM2688, a class 1 integron with two new gene cassettes, *cmlA2* and *qacF*. Antimicrob Agents Chemother 1998 Oct; 42 (10): 2557-63
96. Toro CS, Lobos SR, Calderon I, et al. Clinical isolate of a porinless *Salmonella typhi* resistant to high levels of chloramphenicol. Antimicrob Agents Chemother 1990 Sep; 34 (9): 1715-9
97. Bissonnette L, Champetier S, Buisson JP, et al. Characterization of the nonenzymatic chloramphenicol resistance (*cmlA*) gene of the In4 integron of Tn1696: similarity of the product to transmembrane transport proteins. J Bacteriol 1991 Jul; 173 (14): 4493-502
98. Kim E, Aoki T. Sequence analysis of the florfenicol resistance gene encoded in the transferable R-plasmid of a fish pathogen, *Pasteurella piscicida*. Microbiol Immunol 1996; 40 (9): 665-9
99. Bolton LF, Kelley LC, Lee MD, et al. Detection of multidrug-resistant *Salmonella enterica* serotype typhimurium DT104 based on a gene which confers cross-resistance to florfenicol and chloramphenicol. J Clin Microbiol 1999 May; 37 (5): 1348-51
100. Boyd D, Peters GA, Cloeckaert A, et al. Complete nucleotide sequence of a 43-kilobase genomic island associated with the multidrug resistance region of *Salmonella enterica* serovar Typhimurium DT104 and its identification in phage type DT120 and serovar Agona. J Bacteriol 2001 Oct; 183 (19): 5725-32
101. White DG, Hudson C, Maurer JJ, et al. Characterization of chloramphenicol and florfenicol resistance in *Escherichia coli* associated with bovine diarrhea. J Clin Microbiol 2000 Dec; 38 (12): 4593-8
102. Schuldiner S, Granot D, Steiner S, et al. Precious things come in little packages. J Mol Microbiol Biotechnol 2001 Apr; 3 (2): 155-62
103. Yerushalmi H, Schuldiner S. A model for coupling of H^+ and substrate fluxes based on 'time-sharing' of a common binding site. Biochemistry 2000 Dec 5; 39 (48): 14711-9
104. Yerushalmi H, Schuldiner S. A common binding site for substrates and protons in EmrE, an ion-coupled multidrug transporter. FEBS Lett 2000 Jun 30; 476 (1-2): 93-7
105. Yerushalmi H, Schuldiner S. An essential glutamyl residue in EmrE, a multidrug antiporter from *Escherichia coli*. J Biol Chem 2000 Feb 25; 275 (8): 5264-9
106. Chung YJ, Saier Jr MH. Overexpression of the *Escherichia colisugE* gene confers resistance to a narrow range of quaternary ammonium compounds. J Bacteriol 2002 May; 184 (9): 2543-5
107. Levy SB. Active efflux mechanisms for antimicrobial resistance. Antimicrob Agents Chemother 1992 Apr; 36 (4): 695-703
108. Thanassi DG, Suh GS, Nikaido H. Role of outer membrane barrier in efflux-mediated tetracycline resistance of *Escherichia coli*. J Bacteriol 1995 Feb; 177 (4): 998-1007
109. Yamaguchi A, Udagawa T, Sawai T. Transport of divalent cations with tetracycline as mediated by the transposon Tn10-encoded tetracycline resistance protein. J Biol Chem 1990 Mar 25; 265 (9): 4809-13
110. Linton KJ, Higgins CF. The *Escherichia coli* ATP-binding cassette (ABC) proteins. Mol Microbiol 1998 Apr; 28 (1): 5-13
111. Allikmets R, Gerrard B, Court D, et al. Cloning and organization of the *abc* and *mdl* genes of *Escherichia coli*: relationship to eukaryotic multidrug resistance. Gene 1993 Dec 22; 136 (1-2): 231-6
112. Nikaido H. Prevention of drug access to bacterial targets: permeability barriers and active efflux. Science 1994 Apr 15; 264 (5157): 382-8
113. Poole K, Heinrichs DE, Neshat S. Cloning and sequence analysis of an EnvCD homologue in *Pseudomonas aeruginosa*: regulation by iron and possible involvement in the secretion of the siderophore pyoverdine. Mol Microbiol 1993 Nov; 10 (3): 529-44
114. Nikaido H. Preventing drug access to targets: cell surface permeability barriers and active efflux in bacteria. Semin Cell Dev Biol 2001 Jun; 12 (3): 215-23
115. Poole K. Multidrug efflux pumps and antimicrobial resistance in *Pseudomonas aeruginosa* and related organisms. J Mol Microbiol Biotechnol 2001 Apr; 3 (2): 255-64
116. Stover CK, Pham XQ, Erwin AL, et al. Complete genome sequence of *Pseudomonas aeruginosa* PA01, an opportunistic pathogen. Nature 2000 Aug 31; 406 (6799): 959-64
117. Masuda N, Ohya S. Cross-resistance to meropenem, cephems, and quinolones in *Pseudomonas aeruginosa*. Antimicrob Agents Chemother 1992 Sep; 36 (9): 1847-51
118. Masuda N, Sakagawa E, Ohya S. Outer membrane proteins responsible for multiple drug resistance in *Pseudomonas aeruginosa*. Antimicrob Agents Chemother 1995 Mar; 39 (3): 645-9
119. Srikumar R, Paul CJ, Poole K. Influence of mutations in the MexR repressor gene on expression of the MexA-MexB-OprM multidrug efflux system of *Pseudomonas aeruginosa*. J Bacteriol 2000 Mar; 182 (5): 1410-4
120. Ziha-Zarifi I, Llanes C, Köhler T, et al. *In vivo* emergence of multidrug-resistant mutants of *Pseudomonas aeruginosa* overexpressing the active efflux system MexA-MexB-OprM. Antimicrob Agents Chemother 1999 Feb; 43 (2): 287-91
121. Köhler T, Kok M, Michea-Hamzehpour M, et al. Multidrug efflux in intrinsic resistance to trimethoprim and sulfamethoxazole in *Pseudomonas aeruginosa*. Antimicrob Agents Chemother 1996 Oct; 40 (10): 2288-90
122. Li XZ, Zhang L, Srikumar R, et al. β-Lactamase inhibitors are substrates for the multidrug efflux pumps of *Pseudomonas aeruginosa*. Antimicrob Agents Chemother 1998 Feb; 42 (2): 399-403
123. Schweizer HP. Intrinsic resistance to inhibitors of fatty acid biosynthesis in *Pseudomonas aeruginosa* is due to efflux: application of a novel technique for generation of unmarked chromosomal mutations for the study of efflux systems. Antimicrob Agents Chemother 1998 Feb; 42 (2): 394-8
124. Srikumar R, Li XZ, Poole K. Inner membrane efflux components are responsible for β-lactam specificity of multidrug efflux pumps in *Pseudomonas aeruginosa*. J Bacteriol 1997 Dec; 179 (24): 7875-81
125. Li XZ, Zhang L, Poole K. Role of the multidrug efflux systems of *Pseudomonas aeruginosa* in organic solvent tolerance. J Bacteriol 1998 Jun; 180 (11): 2987-91
126. Srikumar R, Kon T, Gotoh N, et al. Expression of *Pseudomonas aeruginosa* multidrug efflux pumps MexA-MexB-OprM and MexC-MexD-OprJ in a multidrug-sensitive *Escherichia coli* strain. Antimicrob Agents Chemother 1998 Jan; 42 (1): 65-71
127. Li XZ, Poole K. Organic solvent-tolerant mutants of *Pseudomonas aeruginosa* display multiple antibiotic resistance. Can J Microbiol 1999 Jan; 45 (1): 18-22
128. 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 Dec; 44 (12): 3322-7

129. Trias J, Nikaido H. Outer membrane protein D2 catalyzes facilitated diffusion of carbapenems and penems through the outer membrane of *Pseudomonas aeruginosa*. Antimicrob Agents Chemother 1990 Jan; 34 (1): 52-7
130. Okamoto K, Gotoh N, Nishino T. *Pseudomonas aeruginosa* reveals high intrinsic resistance to penem antibiotics: penem resistance mechanisms and their interplay. Antimicrob Agents Chemother 2001 Jul; 45 (7): 1964-71
131. Okamoto K, Gotoh N, Nishino T. Extrusion of penem antibiotics by multicomponent efflux systems MexAB-OprM, MexCD-OprJ, and MexXY-OprM of *Pseudomonas aeruginosa*. Antimicrob Agents Chemother 2002 Aug; 46 (8): 2696-9
132. Zhao Q, Li XZ, Srikumar R, et al. Contribution of outer membrane efflux protein OprM to antibiotic resistance in *Pseudomonas aeruginosa* independent of MexAB. Antimicrob Agents Chemother 1998 Jul; 42 (7): 1682-8
133. Gotoh N, Tsujimoto H, Nomura A, et al. Functional replacement of OprJ by OprM in the MexCD-OprJ multidrug efflux system of *Pseudomonas aeruginosa*. FEMS Microbiol Lett 1998 Aug 1; 165 (1): 21-7
134. Maseda H, Yoneyama H, Nakae T. Assignment of the substrate-selective subunits of the MexEF-OprN multidrug efflux pump of *Pseudomonas aeruginosa*. Antimicrob Agents Chemother 2000 Mar; 44 (3): 658-64
135. Passador L, Cook JM, Gambello MJ, et al. Expression of *Pseudomonas aeruginosa* virulence genes requires cell-to-cell communication. Science 1993 May 21; 260 (5111): 1127-30
136. Hastings JW, Greenberg EP. Quorum sensing: the explanation of a curious phenomenon reveals a common characteristic of bacteria. J Bacteriol 1999 May; 181 (9): 2667-8
137. Pearson JP, Van Delden C, Iglewski BH. Active efflux and diffusion are involved in transport of *Pseudomonas aeruginosa* cell-to-cell signals. J Bacteriol 1999 Feb; 181 (4): 1203-10
138. Evans K, Passador L, Srikumar R, et al. Influence of the MexAB-OprM multidrug efflux system on quorum sensing in *Pseudomonas aeruginosa*. J Bacteriol 1998 Oct; 180 (20): 5443-7
139. Hirakata Y, Srikumar R, Poole K, et al. Multidrug efflux systems play an important role in the invasiveness of *Pseudomonas aeruginosa*. J Exp Med 2002 Jul 1; 196 (1): 109-18
140. Chuanchuen R, Beinlich K, Hoang TT, et al. Cross-resistance between triclosan and antibiotics in *Pseudomonas aeruginosa* is mediated by multidrug efflux pumps: exposure of a susceptible mutant strain to triclosan selects *nfxB* mutants overexpressing MexCD-OprJ. Antimicrob Agents Chemother 2001 Feb; 45 (2): 428-32
141. Morita Y, Komori Y, Mima T, et al. Construction of a series of mutants lacking all of the four major mex operons for multidrug efflux pumps or possessing each one of the operons from *Pseudomonas aeruginosa* PAO1: MexCD-OprJ is an inducible pump. FEMS Microbiol Lett 2001 Aug 7; 202 (1): 139-43
142. Masuda N, Gotoh N, Ohya S, et al. Quantitative correlation between susceptibility and OprJ production in NfxB mutants of *Pseudomonas aeruginosa*. Antimicrob Agents Chemother 1996 Apr; 40 (4): 909-13
143. Li XZ, Barre N, Poole K. Influence of the MexA-MexB-OprM multidrug efflux system on expression of the MexC-MexD-OprJ and MexE-MexF-OprN multidrug efflux systems in *Pseudomonas aeruginosa*. J Antimicrob Chemother 2000 Dec; 46 (6): 885-93
144. Gotoh N, Tsujimoto H, Tsuda M, et al. Characterization of the MexC-MexD-OprJ multidrug efflux system in ΔMexA-MexB-OprM mutants of *Pseudomonas aeruginosa*. Antimicrob Agents Chemother 1998 Aug; 42 (8): 1938-43
145. Masuda N, Sakagawa E, Ohya S, et al. Hypersusceptibility of the *Pseudomonas aeruginosa nfxB* mutant to β-lactams due to reduced expression of the *AmpC* β-lactamase. Antimicrob Agents Chemother 2001 Apr; 45 (4): 1284-6
146. Ochs MM, McCusker MP, Bains M, et al. Negative regulation of the *Pseudomonas aeruginosa* outer membrane porin OprD selective for imipenem and basic amino acids. Antimicrob Agents Chemother 1999 May; 43 (5): 1085-90
147. Köhler T, van Delden C, Curty LK, et al. Overexpression of the MexEF-OprN multidrug efflux system affects cell-to-cell signaling in *Pseudomonas aeruginosa*. J Bacteriol 2001 Sep; 183 (18): 5213-22
148. Alonso A, Martinez JL. Multiple antibiotic resistance in *Stenotrophomonas maltophilia*. Antimicrob Agents Chemother 1997 May; 41 (5): 1140-2
149. Zhang L, Li XZ, Poole K. Multiple antibiotic resistance in *Stenotrophomonas maltophilia*: involvement of a multidrug efflux system. Antimicrob Agents Chemother 2000 Feb; 44 (2): 287-93
150. Alonso A, Martinez JL. Expression of multidrug efflux pump SmeDEF by clinical isolates of *Stenotrophomonas maltophilia*. Antimicrob Agents Chemother 2001 Jun; 45 (6): 1879-81
151. Burns JL, Hedin LA, Lien DM. Chloramphenicol resistance in *Pseudomonas cepacia* because of decreased permeability. Antimicrob Agents Chemother 1989 Feb; 33 (2): 136-41
152. Zhang L, Li XZ, Poole K. Fluoroquinolone susceptibilities of efflux-mediated multidrug-resistant *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia* and *Burkholderia cepacia*. J Antimicrob Chemother 2001 Oct; 48 (4): 549-52
153. Kim K, Lee S, Lee K, et al. Isolation and characterization of toluene-sensitive mutants from the toluene-resistant bacterium *Pseudomonas putida* GM73. J Bacteriol 1998 Jul; 180 (14): 3692-6
154. Sparling PF, Sarubbi Jr FA, Blackman E. Inheritance of low-level resistance to penicillin, tetracycline, and chloramphenicol in *Neisseria gonorrhoeae*. J Bacteriol 1975 Nov; 124 (2): 740-9
155. Guymon LF, Sparling PF. Altered crystal violet permeability and lytic behavior in antibiotic-resistant and -sensitive mutants of *Neisseria gonorrhoeae*. J Bacteriol 1975 Nov; 124 (2): 757-63
156. Lysko PG, Morse SA. *Neisseria gonorrhoeae* cell envelope: permeability to hydrophobic molecules. J Bacteriol 1981 Feb; 145 (2): 946-52
157. Pan W, Spratt BG. Regulation of the permeability of the gonococcal cell envelope by the *mtr* system. Mol Microbiol 1994 Feb; 11 (4): 769-75
158. Rouquette C, Harmon JB, Shafer WM. Induction of the *mtrCDE*-encoded efflux pump system of *Neisseria gonorrhoeae* requires MtrA, an AraC-like protein. Mol Microbiol 1999 Aug; 33 (3): 651-8
159. Delahay RM, Robertson BD, Balthazar JT, et al. Involvement of the gonococcal MtrE protein in the resistance of *Neisseria gonorrhoeae* to toxic hydrophobic agents. Microbiology 1997 Jul; 143 (Pt 7): 2127-33
160. Hagman KE, Lucas CE, Balthazar JT, et al. The MtrD protein of *Neisseria gonorrhoeae* is a member of the resistance/nodulation/division protein family constituting part of an efflux system. Microbiology 1997 Jul; 143 (Pt 7): 2117-25

161. Zarantonelli L, Borthagaray G, Lee EH, et al. Decreased susceptibility to azithromycin and erythromycin mediated by a novel *mtr(R)* promoter mutation in *Neisseria gonorrhoeae*. J Antimicrob Chemother 2001 May; 47 (5): 651-4
162. McFarland L, Mietzner TA, Knapp JS, et al. Gonococcal sensitivity to fecal lipids can be mediated by an Mtr-independent mechanism. J Clin Microbiol 1983 Jul; 18 (1): 121-7
163. Morse SA, Lysko PG, McFarland L, et al. Gonococcal strains from homosexual men have outer membranes with reduced permeability to hydrophobic molecules. Infect Immun 1982 Aug; 37 (2): 432-8
164. Shafer WM, Veal WL, Lee EH, et al. Genetic organization and regulation of antimicrobial efflux systems possessed by *Neisseria gonorrhoeae* and *N. meningitidis*. J Mol Microbiol Biotechnol 2001 Apr; 3 (2): 219-24
165. Garg P, Chakraborty S, Basu I, et al. Expanding multiple antibiotic resistance among clinical strains of *Vibrio cholerae* isolated from 1992-7 in Calcutta, India. Epidemiol Infect 2000 Jun; 124 (3): 393-9
166. Huda MN, Morita Y, Kuroda T, et al. Na^+-driven multidrug efflux pump VcmA from *Vibrio cholerae* non-O1, a non-halophilic bacterium. FEMS Microbiol Lett 2001 Sep 25; 203 (2): 235-9
167. Colmer JA, Fralick JA, Hamood AN. Isolation and characterization of a putative multidrug resistance pump from *Vibrio cholerae*. Mol Microbiol 1998 Jan; 27 (1): 63-72
168. Baranwal S, Dey K, Ramamurthy T, et al. Role of active efflux in association with target gene mutations in fluoroquinolone resistance in clinical isolates of *Vibrio cholerae*. Antimicrob Agents Chemother 2002 Aug; 46 (8): 2676-8
169. Miyamae S, Ueda O, Yoshimura F, et al. A MATE family multidrug efflux transporter pumps out fluoroquinolones in *Bacteroides thetaiotaomicron*. Antimicrob Agents Chemother 2001 Dec; 45 (12): 3341-6
170. Wigfield SM, Rigg GP, Kavari M, et al. Identification of an immunodominant drug efflux pump in *Burkholderia cepacia*. J Antimicrob Chemother 2002 Apr; 49 (4): 619-24
171. Miyamae CC, Valvano MA. Cloning and characterization of the *Burkholderia vietnamiensis norM* gene encoding a multi-drug efflux protein. FEMS Microbiol Lett 2002 Oct 8; 215 (2): 279-83
172. Nishino K, Yamaguchi A. Overexpression of the response regulator *evgA* of the two-component signal transduction system modulates multidrug resistance conferred by multidrug resistance transporters. J Bacteriol 2001 Feb; 183 (4): 1455-8
173. Naroditskaya V, Schlosser MJ, Fang NY, et al. An *E. coli* gene *emrD* is involved in adaptation to low energy shock. Biochem Biophys Res Commun 1993 Oct 29; 196 (2): 803-9
174. Phadtare S, Yamanaka K, Kato I, et al. Antibacterial activity of 4,5-dihydroxy-2-cyclopentan-1-one (DHCP) and cloning of a gene conferring DHCP resistance in *Escherichia coli*. J Mol Microbiol Biotechnol 2001 Jul; 3 (3): 461-5
175. Yerushalmi H, Lebendiker M, Schuldiner S. EmrE, an *Escherichia coli* 12-kDa multidrug transporter, exchanges toxic cations and H^+ and is soluble in organic solvents. J Biol Chem 1995 Mar 24; 270 (12): 6856-63
176. Turner RJ, Taylor DE, Weiner JH. Expression of *Escherichia coli* TehA gives resistance to antiseptics and disinfectants similar to that conferred by multidrug resistance efflux pumps. Antimicrob Agents Chemother 1997 Feb; 41 (2): 440-4
177. Li X-Z, Poole K, Nikaido H. Contributions of MexAB-OprM and an EmrE homolog to intrinsic resistance of *Pseudomonas aeruginosa* to aminoglycosides and dyes. Antimicrob Agents Chemother 2003; 47 (1): 27-33
178. Hongo E, Morimyo M, Mita K, et al. The methyl viologen-resistance-encoding gene *smvA* of *Salmonella typhimurium*. Gene 1994 Oct 11; 148 (1): 173-4
179. Santiviago CA, Fuentes JA, Bueno SM, et al. The *Salmonella enterica* sv. Typhimurium *smvA*, *yddG* and *ompD* (porin) genes are required for the efficient efflux of methyl viologen. Mol Microbiol 2002 Nov; 46 (3): 687-98
180. Baquero F. Gram-positive resistance: challenge for the development of new antibiotics. J Antimicrob Chemother 1997 May; 39 Suppl. A: 1-6
181. Hooper DC. Fluoroquinolone resistance among Gram-positive cocci. Lancet Infect Dis 2002 Sep; 2 (9): 530-8
182. Ahmed M, Lyass L, Markham PN, et al. Two highly similar multidrug transporters of *Bacillus subtilis* whose expression is differentially regulated. J Bacteriol 1995 Jul; 177 (14): 3904-10
183. Baranova NN, Danchin A, Neyfakh AA. Mta, a global MerR-type regulator of the *Bacillus subtilis* multidrug-efflux transporters. Mol Microbiol 1999 Mar; 31 (5): 1549-59
184. Woolridge DP, Vazquez-Laslop N, Markham PN, et al. Efflux of the natural polyamine spermidine facilitated by the *Bacillus subtilis* multidrug transporter Blt. J Biol Chem 1997 Apr 4; 272 (14): 8864-6
185. Masaoka Y, Ueno Y, Morita Y, et al. A two-component multidrug efflux pump, EbrAB, in *Bacillus subtilis*. J Bacteriol 2000 Apr; 182 (8): 2307-10
186. Davis DR, McAlpine JB, Pazoles CJ, et al. *Enterococcus faecalis* multi-drug resistance transporters: application for antibiotic discovery. J Mol Microbiol Biotechnol 2001 Apr; 3 (2): 179-84
187. Singh KV, Weinstock GM, Murray BE. An *Enterococcus faecalis* ABC homologue (Lsa) is required for the resistance of this species to clindamycin and quinupristin-dalfopristin. Antimicrob Agents Chemother 2002 Jun; 46 (6): 1845-50
188. Jonas BM, Murray BE, Weinstock GM. Characterization of *emeA*, a *norA* homolog and multidrug resistance efflux pump, in *Enterococcus faecalis*. Antimicrob Agents Chemother 2001 Dec; 45 (12): 3574-9
189. Lynch C, Courvalin P, Nikaido H. Active efflux of antimicrobial agents in wild-type strains of enterococci. Antimicrob Agents Chemother 1997 Apr; 41 (4): 869-71
190. Bolhuis H, van Veen HW, Molenaar D, et al. Multidrug resistance in *Lactococcus lactis*: evidence for ATP-dependent drug extrusion from the inner leaflet of the cytoplasmic membrane. EMBO J 1996 Aug 15; 15 (16): 4239-45
191. Bolhuis H, Poelarends G, van Veen HW, et al. The lactococcal *lmrP* gene encodes a proton motive force-dependent drug transporter. J Biol Chem 1995 Nov 3; 270 (44): 26092-8
192. Perreten V, Schwarz FV, Teuber M, et al. Mdt (A), a new efflux protein conferring multiple antibiotic resistance in *Lactococcus lactis* and *Escherichia coli*. Antimicrob Agents Chemother 2001 Apr; 45 (4): 1109-14
193. Mata MT, Baquero F, Perez-Diaz JC. A multidrug efflux transporter in *Listeria monocytogenes*. FEMS Microbiol Lett 2000 Jun 15; 187 (2): 185-8
194. Ross JI, Eady EA, Cove JH, et al. Identification of a chromosomally encoded ABC-transport system with which the staphylococcal erythromycin exporter MsrA may interact. Gene 1995 Feb 3; 153 (1): 93-8
195. Fournier B, Aras R, Hooper DC. Expression of the multidrug resistance transporter NorA from *Staphylococcus aureus* is modified by a two-component regulatory system. J Bacteriol 2000 Feb; 182 (3): 664-71

196. Littlejohn TG, Paulsen IT, Gillespie MT, et al. Substrate specificity and energetics of antiseptic and disinfectant resistance in *Staphylococcus aureus*. FEMS Microbiol Lett 1992 Aug 15; 74 (2-3): 259-65
197. Clancy J, Dib-Hajj F, Petitpas JW, et al. Cloning and characterization of a novel macrolide efflux gene, *mreA*, from *Streptococcus agalactiae*. Antimicrob Agents Chemother 1997 Dec; 41 (12): 2719-23
198. Gill MJ, Brenwald NP, Wise R. Identification of an efflux pump gene, *pmrA*, associated with fluoroquinolone resistance in *Streptococcus pneumoniae*. Antimicrob Agents Chemother 1999 Jan; 43 (1): 187-9
199. Tait-Kamradt A, Clancy J, Cronan M, et al. *mefE* is necessary for the erythromycin-resistant M phenotype in *Streptococcus pneumoniae*. Antimicrob Agents Chemother 1997 Oct; 41 (10): 2251-5
200. Clancy J, Petitpas J, Dib-Hajj F, et al. Molecular cloning and functional analysis of a novel macrolide-resistance determinant, *mefA*, from *Streptococcus pyogenes*. Mol Microbiol 1996 Dec; 22 (5): 867-79
201. Ainsa JA, Blokpoel MC, Otal I, et al. Molecular cloning and characterization of Tap, a putative multidrug efflux pump present in *Mycobacterium fortuitum* and *Mycobacterium tuberculosis*. J Bacteriol 1998 Nov; 180 (22): 5836-43
202. Takiff HE, Cimino M, Musso MC, et al. Efflux pump of the proton antiporter family confers low-level fluoroquinolone resistance in *Mycobacterium smegmatis*. Proc Natl Acad Sci U S A 1996 Jan 9; 93 (1): 362-6
203. Choudhuri BS, Sen S, Chakrabarti P. Isoniazid accumulation in *Mycobacterium smegmatis* is modulated by proton motive force-driven and ATP-dependent extrusion systems. Biochem Biophys Res Commun 1999 Mar 24; 256 (3): 682-4
204. Choudhuri BS, Bhakta S, Barik R, et al. Overexpression and functional characterization of an ABC (ATP-binding cassette) transporter encoded by the genes *drrA* and *drrB* of *Mycobacterium tuberculosis*. Biochem J 2002 Oct 1; 367 (Pt 1): 279-85
205. Doran JL, Pang Y, Mdluli KE, et al. *Mycobacterium tuberculosis efpA* encodes an efflux protein of the QacA transporter family. Clin Diagn Lab Immunol 1997 Jan; 4 (1): 23-32
206. Silva PE, Bigi F, de la Paz Santangelo M, et al. Characterization of P55, a multidrug efflux pump in *Mycobacterium bovis* and *Mycobacterium tuberculosis*. Antimicrob Agents Chemother 2001 Mar; 45 (3): 800-4
207. De Rossi E, Branzoni M, Cantoni R, et al. *mmr*, a *Mycobacterium tuberculosis* gene conferring resistance to small cationic dyes and inhibitors. J Bacteriol 1998 Nov; 180 (22): 6068-71
208. Carbon C. MRSA and MRSE: is there an answer? Clin Microbiol Infect 2000 Aug; 6 Suppl. 2: 17-22
209. Tennent JM, Lyon BR, Midgley M, et al. Physical and biochemical characterization of the *qacA* gene encoding antiseptic and disinfectant resistance in *Staphylococcus aureus*. J Gen Microbiol 1989 Jan; 135 (Pt 1): 1-10
210. Paulsen IT, Brown MH, Littlejohn TG, et al. Multidrug resistance proteins QacA and QacB from *Staphylococcus aureus*: membrane topology and identification of residues involved in substrate specificity. Proc Natl Acad Sci U S A 1996 Apr 16; 93 (8): 3630-5
211. Noguchi N, Hase M, Kitta M, et al. Antiseptic susceptibility and distribution of antiseptic-resistance genes in methicillin-resistant *Staphylococcus aureus*. FEMS Microbiol Lett 1999 Mar 15; 172 (2): 247-53
212. Kaatz GW, Seo SM, Ruble CA. Efflux-mediated fluoroquinolone resistance in *Staphylococcus aureus*. Antimicrob Agents Chemother 1993 May; 37 (5): 1086-94
213. Ng EY, Trucksis M, Hooper DC. Quinolone resistance mediated by *norA*: physiologic characterization and relationship to *flqB*, a quinolone resistance locus on the *Staphylococcus aureus* chromosome. Antimicrob Agents Chemother 1994 Jun; 38 (6): 1345-55
214. Piddock LJ. Mechanisms of fluoroquinolone resistance: an update 1994-1998. Drugs 1999; 58 Suppl. 2: 11-8
215. Poole K. Efflux-mediated resistance to fluoroquinolones in gram-negative bacteria. Antimicrob Agents Chemother 2000; 44 (9): 2233-41
216. Poole K. Efflux-mediated resistance to fluoroquinolones in gram-positive bacteria and the mycobacteria. Antimicrob Agents Chemother 2000; 44 (10): 2595-9
217. Yu JL, Grinius L, Hooper DC. NorA functions as a multidrug efflux protein in both cytoplasmic membrane vesicles and reconstituted proteoliposomes. J Bacteriol 2002 Mar; 184 (5): 1370-7
218. Kaatz GW, Seo SM. Inducible NorA-mediated multidrug resistance in *Staphylococcus aureus*. Antimicrob Agents Chemother 1995 Dec; 39 (12): 2650-5
219. Fournier B, Truong-Bolduc QC, Zhang X, et al. A mutation in the 5' untranslated region increases stability of *norA* mRNA, encoding a multidrug resistance transporter of *Staphylococcus aureus*. J Bacteriol 2001 Apr; 183 (7): 2367-71
220. Kaatz GW, Seo SM, Foster TJ. Introduction of a *norA* promoter region mutation into the chromosome of a fluoroquinolone-susceptible strain of *Staphylococcus aureus* using plasmid integration. Antimicrob Agents Chemother 1999 Sep; 43 (9): 2222-4
221. Kaatz GW, Seo SM, O'Brien L, et al. Evidence for the existence of a multidrug efflux transporter distinct from NorA in *Staphylococcus aureus*. Antimicrob Agents Chemother 2000 May; 44 (5): 1404-6
222. Munoz-Bellido JL, Alonzo Manzanares M, Martinez Andres JA, et al. Efflux pump-mediated quinolone resistance in *Staphylococcus aureus* strains wild type for *gyrA*, *gyrB*, *grlA*, and *norA*. Antimicrob Agents Chemother 1999 Feb; 43 (2): 354-6
223. Piddock LJ, Jin YF, Webber MA, et al. Novel ciprofloxacin-resistant, nalidixic acid-susceptible mutant of *Staphylococcus aureus*. Antimicrob Agents Chemother 2002 Jul; 46 (7): 2276-8
224. Noguchi N, Tamura M, Narui K, et al. Frequency and genetic characterization of multidrug-resistant mutants of *Staphylococcus aureus* after selection with individual antiseptics and fluoroquinolones. Biol Pharm Bull 2002 Sep; 25 (9): 1129-32
225. Ross JI, Eady EA, Cove JH, et al. Inducible erythromycin resistance in staphylococci is encoded by a member of the ATP-binding transport super-gene family. Mol Microbiol 1990 Jul; 4 (7): 1207-14
226. Ross JI, Eady EA, Cove JH, et al. Minimal functional system required for expression of erythromycin resistance by *msrA* in *Staphylococcus aureus* RN4220. Gene 1996 Dec 12; 183 (1-2): 143-8
227. Leclercq R. Mechanisms of resistance to macrolides and lincosamides: nature of the resistance elements and their clinical implications. Clin Infect Dis 2002 Feb 15; 34 (4): 482-92
228. Schmitz FJ, Sadurski R, Kray A, et al. Prevalence of macrolide-resistance genes in *Staphylococcus aureus* and *Enterococcus faecium* isolates from 24 European university hospitals. J Antimicrob Chemother 2000 Jun; 45 (6): 891-4
229. Schmitz FJ, Perdikouli M, Beeck A, et al. Molecular surveillance of macrolide, tetracycline and quinolone resistance mechanisms in 1191 clinical European *Streptococcus pneu-*

moniae isolates. Int J Antimicrob Agents 2001 Nov; 18 (5): 433-6

230. Broskey J, Coleman K, Gwynn MN, et al. Efflux and target mutations as quinolone resistance mechanisms in clinical isolates of *Streptococcus pneumoniae*. J Antimicrob Chemother 2000 Apr; 45 Suppl. 1: 95-9
231. Bast DJ, Low DE, Duncan CL, et al. Fluoroquinolone resistance in clinical isolates of *Streptococcus pneumoniae*: contributions of type II topoisomerase mutations and efflux to levels of resistance. Antimicrob Agents Chemother 2000 Nov; 44 (11): 3049-54
232. Brenwald NP, Gill MJ, Wise R. Prevalence of a putative efflux mechanism among fluoroquinolone-resistant clinical isolates of *Streptococcus pneumoniae*. Antimicrob Agents Chemother 1998 Aug; 42 (8): 2032-5
233. Baranova NN, Neyfakh AA. Apparent involvement of a multidrug transporter in the fluoroquinolone resistance of *Streptococcus pneumoniae*. Antimicrob Agents Chemother 1997 Jun; 41 (6): 1396-8
234. Zeller V, Janoir C, Kitzis MD, et al. Active efflux as a mechanism of resistance to ciprofloxacin in *Streptococcus pneumoniae*. Antimicrob Agents Chemother 1997 Sep; 41 (9): 1973-8
235. Marshall NJ, Piddock LJ. Antibacterial efflux systems. Microbiologia 1997 Sep; 13 (3): 285-300
236. Piddock LJ, Jin YF, Everett MJ. Non-*gyrA*-mediated ciprofloxacin resistance in laboratory mutants of *Streptococcus pneumoniae*. J Antimicrob Chemother 1997 May; 39 (5): 609-15
237. Piddock LJ, Johnson MM. Accumulation of 10 fluoroquinolones by wild-type or efflux mutant *Streptococcus pneumoniae*. Antimicrob Agents Chemother 2002 Mar; 46 (3): 813-20
238. Piddock LJ, Johnson MM, Simjee S, et al. Expression of efflux pump gene *pmrA* in fluoroquinolone-resistant and -susceptible clinical isolates of *Streptococcus pneumoniae*. Antimicrob Agents Chemother 2002 Mar; 46 (3): 808-12
239. Pestova E, Millichap JJ, Siddiqui F, et al. Non-PmrA-mediated multidrug resistance in *Streptococcus pneumoniae*. J Antimicrob Chemother 2002 Mar; 49 (3): 553-6
240. Kataja J, Seppala H, Skurnik M, et al. Different erythromycin resistance mechanisms in group C and group G streptococci. Antimicrob Agents Chemother 1998 Jun; 42 (6): 1493-4
241. Sutcliffe J, Tait-Kamradt A, Wondrack L. *Streptococcus pneumoniae* and *S. pyogenes* resistant to macrolides but sensitive to clindamycin: a common resistance pattern mediated by an efflux system. Antimicrob Agents Chemother 1996 Aug; 40 (8): 1817-24
242. Widdowson CA, Klugman KP. Molecular mechanisms of resistance to commonly used non-betalactam drugs in *Streptococcus pneumoniae*. Semin Respir Infect 1999 Sep; 14 (3): 255-68
243. French GL. Enterococci and vancomycin resistance. Clin Infect Dis 1998 Aug; 27 Suppl. 1: S75-83
244. Moellering Jr RC. Emergence of Enterococcus as a significant pathogen. Clin Infect Dis 1992 Jun; 14 (6): 1173-6
245. Hallgren A, Abednazari H, Ekdahl C, et al. Antimicrobial susceptibility patterns of enterococci in intensive care units in Sweden evaluated by different MIC breakpoint systems. J Antimicrob Chemother 2001 Jul; 48 (1): 53-62
246. Neyfakh AA. The ostensible paradox of multidrug recognition. J Mol Microbiol Biotechnol 2001 Apr; 3 (2): 151-4
247. Godsey MH, Baranova NN, Neyfakh AA, et al. Crystal structure of MtaN, a global multidrug transporter gene activator. J Biol Chem 2001 Dec 14; 276 (50): 47178-84
248. Heldwein EE, Brennan RG. Crystal structure of the transcription activator BmrR bound to DNA and a drug. Nature 2001 Jan 18; 409 (6818): 378-82
249. Neyfakh AA. The multidrug efflux transporter of *Bacillus subtilis* is a structural and functional homolog of the *Staphylococcus* NorA protein. Antimicrob Agents Chemother 1992 Feb; 36 (2): 484-5
250. Poelarends GJ, Mazurkiewicz P, Konings WN. Multidrug transporters and antibiotic resistance in *Lactococcus lactis*. Biochim Biophys Acta 2002 Sep 10; 1555 (1-3): 1-7
251. van Veen HW, Putman M, Margolles A, et al. Molecular pharmacological characterization of two multidrug transporters in *Lactococcus lactis*. Pharmacol Ther 2000 Mar; 85 (3): 245-9
252. van Veen HW, Venema K, Bolhuis H, et al. Multidrug resistance mediated by a bacterial homolog of the human multidrug transporter MDR1. Proc Natl Acad Sci U S A 1996 Oct 1; 93 (20): 10668-72
253. van Veen HW, Margolles A, Muller M, et al. The homodimeric ATP-binding cassette transporter LmrA mediates multidrug transport by an alternating two-site (two-cylinder engine) mechanism. EMBO J 2000 Jun 1; 19 (11): 2503-14
254. Poelarends GJ, Konings WN. The transmembrane domains of the ABC multidrug transporter LmrA form a cytoplasmic exposed, aqueous chamber within the membrane. J Biol Chem 2002 Nov 8; 277 (45): 42891-8
255. van Veen HW, Callaghan R, Soceneantu L, et al. A bacterial antibiotic-resistance gene that complements the human multidrug-resistance P-glycoprotein gene. Nature 1998 Jan 15; 391 (6664): 291-5
256. Hofmeyr JH, Rohwer JM, Snoep JL, et al. How to distinguish between the vacuum cleaner and flippase mechanisms of the LmrA multi-drug transporter in *Lactococcus lactis*. Mol Biol Rep 2002; 29 (1-2): 107-12
257. Putman M, Van Veen HW, Degener JE, et al. Antibiotic resistance: era of the multidrug pump. Mol Microbiol 2000 May; 36 (3): 772-3
258. Putman M, Koole LA, van Veen HW, et al. The secondary multidrug transporter LmrP contains multiple drug interaction sites. Biochemistry 1999 Oct 19; 38 (42): 13900-5
259. Putman M, van Veen HW, Degener JE, et al. The lactococcal secondary multidrug transporter LmrP confers resistance to lincosamides, macrolides, streptogramins and tetracyclines. Microbiology 2001 Oct; 147 (Pt 10): 2873-80
260. Dye C, Scheele S, Dolin P, et al. Consensus statement. Global burden of tuberculosis: estimated incidence, prevalence, and mortality by country. WHO global surveillance and monitoring project. JAMA 1999; 282 (7): 677-86
261. Jarlier V, Nikaido H. Mycobacterial cell wall: structure and role in natural resistance to antibiotics. FEMS Microbiol Lett 1994 Oct 15; 123 (1-2): 11-8
262. Trias J, Benz R. Permeability of the cell wall of *Mycobacterium smegmatis*. Mol Microbiol 1994 Oct; 14 (2): 283-90
263. Engelhardt H, Heinz C, Niederweis M. A tetrameric porin limits the cell wall permeability of *Mycobacterium smegmatis*. J Biol Chem 2002 Oct 4; 277 (40): 37567-72
264. Brennan PJ, Nikaido H. The envelope of mycobacteria. Annu Rev Biochem 1995; 64: 29-63
265. Liu J, Takiff HE, Nikaido H. Active efflux of fluoroquinolones in *Mycobacterium smegmatis* mediated by LfrA, a multidrug efflux pump. J Bacteriol 1996 Jul; 178 (13): 3791-5

266. Sander P, De Rossi E, Boddinghaus B, et al. Contribution of the multidrug efflux pump LfrA to innate mycobacterial drug resistance. FEMS Microbiol Lett 2000 Dec 1; 193 (1): 19-23
267. Wilson M, DeRisi J, Kristensen HH, et al. Exploring drug-induced alterations in gene expression in *Mycobacterium tuberculosis* by microarray hybridization. Proc Natl Acad Sci U S A 1999 Oct 26; 96 (22): 12833-8
268. Viveiros M, Portugal I, Bettencourt R, et al. Isoniazid-induced transient high-level resistance in *Mycobacterium tuberculosis*. Antimicrob Agents Chemother 2002 Sep; 46 (9): 2804-10
269. Zhang Y, Scorpio A, Nikaido H, et al. Role of acid pH and deficient efflux of pyrazinoic acid in unique susceptibility of *Mycobacterium tuberculosis* to pyrazinamide. J Bacteriol 1999 Apr; 181 (7): 2044-9
270. Schaller A, Guo M, Gisanrin O, et al. *Escherichia coli* genes involved in resistance to pyrazinoic acid, the active component of the tuberculosis drug pyrazinamide. FEMS Microbiol Lett 2002 Jun 4; 211 (2): 265-70
271. Piddock LJ, Williams KJ, Ricci V. Accumulation of rifampicin by *Mycobacterium aurum*, *Mycobacterium smegmatis* and *Mycobacterium tuberculosis*. J Antimicrob Chemother 2000 Feb; 45 (2): 159-65
272. Hui J, Gordon N, Kajioka R. Permeability barrier to rifampin in mycobacteria. Antimicrob Agents Chemother 1977 May; 11 (5): 773-9
273. Li XZ, Wang YS, He ZN. Alteration of permeability of bacterial envelope barrier in rifamdin-resistant *Mycobacterium tuberculosis* [in Chinese]. Hua Xi Yi Ke Da Xue Xue Bao 1988 Dec; 19 (4): 388-91
274. Braibant M, Gilot P, Content J. The ATP binding cassette (ABC) transport systems of *Mycobacterium tuberculosis*. FEMS Microbial Rev 2000; 24 (4): 449-67
275. Kaur P, Russell J. Biochemical coupling between the DrrA and DrrB proteins of the doxorubicin efflux pump of *Streptomyces peucetius*. J Biol Chem 1998 Jul 10; 273 (28): 17933-9
276. Guilfoile PG, Hutchinson CR. A bacterial analog of the *mdr* gene of mammalian tumor cells is present in *Streptomyces peucetius*, the producer of daunorubicin and doxorubicin. Proc Natl Acad Sci U S A 1991 Oct 1; 88 (19): 8553-7
277. Banerjee SK, Bhatt K, Misra P, et al. Involvement of a natural transport system in the process of efflux-mediated drug resistance in *Mycobacterium smegmatis*. Mol Gen Genet 2000 Jan; 262 (6): 949-56
278. Bhatt K, Banerjee SK, Chakraborti PK. Evidence that phosphate specific transporter is amplified in a fluoroquinolone resistant *Mycobacterium smegmatis*. Eur J Biochem 2000 Jul; 267 (13): 4028-32
279. Reizer J, Reizer A, Saier Jr MH. A new subfamily of bacterial ABC-type transport systems catalyzing export of drugs and carbohydrates. Protein Sci 1992 Oct; 1 (10): 1326-32
280. Ninio S, Rotem D, Schuldiner S. Functional analysis of novel multidrug transporters from human pathogens. J Biol Chem 2001 Dec 21; 276 (51): 48250-6
281. Ramaswamy S, Musser JM. Molecular genetic basis of antimicrobial agent resistance in *Mycobacterium tuberculosis*: 1998 update. Tuber Lung Dis 1998; 79 (1): 3-29
282. Zheleznova EE, Markham PN, Neyfakh AA, et al. Structural basis of multidrug recognition by BmrR, a transcription activator of a multidrug transporter. Cell 1999 Feb 5; 96 (3): 353-62
283. Vazquez-Laslop N, Markham PN, Neyfakh AA. Mechanism of ligand recognition by BmrR, the multidrug-responding transcriptional regulator: mutational analysis of the ligand-binding site. Biochemistry 1999 Dec 21; 38 (51): 16925-31
284. Schumacher MA, Miller MC, Grkovic S, et al. Structural mechanisms of QacR induction and multidrug recognition. Science 2001 Dec 7; 294 (5549): 2158-63
285. Schumacher MA, Brennan RG. Structural mechanisms of multidrug recognition and regulation by bacterial multidrug transcription factors. Mol Microbiol 2002 Aug; 45 (4): 885-93
286. Neyfakh AA. Mystery of multidrug transporters: the answer can be simple. Mol Microbiol 2002 Jun; 44 (5): 1123-30
287. Vincent F, Spinelli S, Ramoni R, et al. Complexes of porcine odorant binding protein with odorant molecules belonging to different chemical classes. J Mol Biol 2000 Jun 30; 300 (1): 127-39
288. Murakami S, Nakashima R, Yamashita E, et al. Crystal structure of bacterial multidrug efflux transporter AcrB. Nature 2002 Oct 10; 419 (6907): 587-93
289. Fujihira E, Tamura N, Yamaguchi A. Membrane topology of a multidrug efflux transporter, AcrB, in *Escherichia coli*. J Biochem (Tokyo) 2002 Jan; 131 (1): 145-51
290. Gotoh N, Kusumi T, Tsujimoto H, et al. Topological analysis of an RND family transporter, MexD of *Pseudomonas aeruginosa*. FEBS Lett 1999 Sep 10; 458 (1): 32-6
291. Guan L, Ehrmann M, Yoneyama H, et al. Membrane topology of the xenobiotic-exporting subunit, MexB, of the MexA,B-OprM extrusion pump in *Pseudomonas aeruginosa*. J Biol Chem 1999 Apr 9; 274 (15): 10517-22
292. Yoneyama H, Ocaktan A, Gotoh N, et al. Subunit swapping in the Mex-extrusion pumps in *Pseudomonas aeruginosa*. Biochem Biophys Res Commun 1998 Mar 27; 244 (3): 898-902
293. Tikhonova EB, Wang Q, Zgurskaya HI. Chimeric analysis of the multicomponent multidrug efflux transporters from gram-negative bacteria. J Bacteriol 2002 Dec; 184 (23): 6499-507
294. Mao W, Warren MS, Black DS, et al. On the mechanism of substrate specificity by resistance nodulation division (RND)-type multidrug resistance pumps: the large periplasmic loops of MexD from *Pseudomonas aeruginosa* are involved in substrate recognition. Mol Microbiol 2002 Nov; 46 (3): 889-901
295. Yu EW, McDermott G, Zgurskaya HI, et al. Structural basis of multiple drug-binding capacity of the AcrB multidrug efflux pump. Science 2003; 300 (5621): 976-80
296. Yu EW, Aires JR, Nikaido H. AcrB multidrug efflux pump of *Escherichia coli*: composite substrate-binding cavity of exceptional flexibility generates its extremely wide substrate specificity. J Bacteriol 2003; 185 (19): 5657-64
297. Goldberg M, Pribyl T, Juhnke S, et al. Energetics and topology of CzcA, a cation/proton antiporter of the resistance-nodulation-cell division protein family. J Biol Chem 1999 Sep 10; 274 (37): 26065-70
298. Guan L, Nakae T. Identification of essential charged residues in transmembrane segments of the multidrug transporter MexB of *Pseudomonas aeruginosa*. J Bacteriol 2001 Mar; 183 (5): 1734-9
299. Aires JR, Pechere JC, Van Delden C, et al. Amino acid residues essential for function of the MexF efflux pump protein of *Pseudomonas aeruginosa*. Antimicrob Agents Chemother 2002 Jul; 46 (7): 2169-73
300. Zgurskaya HI, Nikaido H. AcrA is a highly asymmetric protein capable of spanning the periplasm. J Mol Biol 1999 Jan 8; 285 (1): 409-20
301. Zgurskaya HI, Nikaido H. Cross-linked complex between oligomeric periplasmic lipoprotein AcrA and the inner-membrane-associated multidrug efflux pump AcrB from *Escherichia coli*. J Bacteriol 2000 Aug; 182 (15): 4264-7

302. Avila-Sakar AJ, Misaghi S, Wilson-Kubalek EM, et al. Lipid-layer crystallization and preliminary three-dimensional structural analysis of AcrA, the periplasmic component of a bacterial multidrug efflux pump. J Struct Biol 2001 Oct; 136 (1): 81-8
303. Hayashi S, Wu HC. Lipoproteins in bacteria. J Bioenerg Biomembr 1990 Jun; 22 (3): 451-71
304. Seiffer D, Klein JR, Plapp R. EnvC, a new lipoprotein of the cytoplasmic membrane of *Escherichia coli*. FEMS Microbiol Lett 1993 Mar 1; 107 (2-3): 175-8
305. Yoneyama H, Maseda H, Kamiguchi H, et al. Function of the membrane fusion protein, MexA, of the MexA,B-OprM efflux pump in *Pseudomonas aeruginosa* without an anchoring membrane. J Biol Chem 2000 Feb 18; 275 (7): 4628-34
306. Zgurskaya HI, Nikaido H. Multidrug resistance mechanisms: drug efflux across two membranes. Mol Microbiol 2000 Jul; 37 (2): 219-25
307. Hwang J, Tai PC. Mutational analysis of CvaA in the highly conserved domain of the membrane fusion protein family. Curr Microbiol 1999 Oct; 39 (4): 195-9
308. Hwang J, Zhong X, Tai PC. Interactions of dedicated export membrane proteins of the colicin V secretion system: CvaA, a member of the membrane fusion protein family, interacts with CvaB and TolC. J Bacteriol 1997 Oct; 179 (20): 6264-70
309. Pimenta AL, Young J, Holland IB, et al. Antibody analysis of the localisation, expression and stability of HlyD, the MFP component of the *E. coli* haemolysin translocator. Mol Gen Genet 1999 Feb; 261 (1): 122-32
310. 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 Jun 22; 405 (6789): 914-9
311. Andersen C, Hughes C, Koronakis V. Chunnel vision: export and efflux through bacterial channel-tunnels. EMBO Rep 2000 Oct; 1 (4): 313-8
312. Wong KK, Brinkman FS, Benz RS, et al. Evaluation of a structural model of *Pseudomonas aeruginosa* outer membrane protein OprM, an efflux component involved in intrinsic antibiotic resistance. J Bacteriol 2001 Jan; 183 (1): 367-74
313. Li XZ, Poole K. Mutational analysis of the OprM outer membrane component of the MexA-MexB-OprM multidrug efflux system of *Pseudomonas aeruginosa*. J Bacteriol 2001 Jan; 183 (1): 12-27
314. Nakajima A, Sugimoto Y, Yoneyama H, et al. Localization of the outer membrane subunit OprM of resistance-nodulation-cell division family multicomponent efflux pump in *Pseudomonas aeruginosa*. J Biol Chem 2000 Sep 29; 275 (39): 30064-8
315. Benz R, Maier E, Gentschev I. TolC of *Escherichia coli* functions as an outer membrane channel. Zentralbl Bakteriol 1993 Apr; 278 (2-3): 187-96
316. Wong KK, Hancock RE. Insertion mutagenesis and membrane topology model of the *Pseudomonas aeruginosa* outer membrane protein OprM. J Bacteriol 2000 May; 182 (9): 2402-10
317. Yoshihara E, Maseda H, Saito K. The outer membrane component of the multidrug efflux pump from *Pseudomonas aeruginosa* may be a gated channel. Eur J Biochem 2002 Oct; 269 (19): 4738-45
318. Postle K. TonB protein and energy transduction between membranes. J Bioenerg Biomembr 1993 Dec; 25 (6): 591-601
319. Zhao Q, Li XZ, Mistry A, et al. Influence of the TonB energy-coupling protein on efflux-mediated multidrug resistance in *Pseudomonas aeruginosa*. Antimicrob Agents Chemother 1998 Sep; 42 (9): 2225-31
320. Godoy P, Ramos-Gonzalez MI, Ramos JL. Involvement of the TonB system in tolerance to solvents and drugs in *Pseudomonas putida* DOT-T1E. J Bacteriol 2001 Sep; 183 (18): 5285-92
321. Rouquette-Loughlin C, Stojiljkovic I, Hrobowski T, et al. Inducible, but not constitutive, resistance of gonococci to hydrophobic agents due to the MtrC-MtrD-MtrE efflux pump requires TonB-ExbB-ExbD proteins. Antimicrob Agents Chemother 2002 Feb; 46 (2): 561-5
322. Maseda H, Kitao M, Eda S, et al. A novel assembly process of the multicomponent xenobiotic efflux pump in *Pseudomonas aeruginosa*. Mol Microbiol 2002 Nov; 46 (3): 677-86
323. Helling RB, Janes BK, Kimball H, et al. Toxic waste disposal in *Escherichia coli*. J Bacteriol 2002 Jul; 184 (13): 3699-703
324. Grkovic S, Brown MH, Skurray RA. Regulation of bacterial drug export systems. Microbiol Mol Biol Rev 2002 Dec; 66 (4): 671-701
325. Ma D, Alberti M, Lynch C, et al. The local repressor AcrR plays a modulating role in the regulation of *acrAB* genes of *Escherichia coli* by global stress signals. Mol Microbiol 1996 Jan; 19 (1): 101-12
326. Orth P, Schnappinger D, Hillen W, et al. Structural basis of gene regulation by the tetracycline inducible Tet repressor-operator system. Nat Struct Biol 2000 Mar; 7 (3): 215-9
327. Orth P, Cordes F, Schnappinger D, et al. Conformational changes of the Tet repressor induced by tetracycline trapping. J Mol Biol 1998 Jun 5; 279 (2): 439-47
328. Bochner BR, Huang HC, Schieven GL, et al. Positive selection for loss of tetracycline resistance. J Bacteriol 1980 Aug; 143 (2): 926-33
329. Masuda N, Sakagawa E, Ohya S, et al. Contribution of the MexX-MexY-OprM efflux system to intrinsic resistance in *Pseudomonas aeruginosa*. Antimicrob Agents Chemother 2000 Sep; 44 (9): 2242-6
330. Duque E, Segura A, Mosqueda G, et al. Global and cognate regulators control the expression of the organic solvent efflux pumps TtgABC and TtgDEF of *Pseudomonas putida*. Mol Microbiol 2001 Feb; 39 (4): 1100-6
331. Lomovskaya O, Lewis K, Matin A. EmrR is a negative regulator of the *Escherichia coli* multidrug resistance pump *EmrAB*. J Bacteriol 1995 May; 177 (9): 2328-34
332. Lomovskaya O, Kawai F, Matin A. Differential regulation of the *mcb* and *emr* operons of *Escherichia coli*: role of *mcb* in multidrug resistance. Antimicrob Agents Chemother 1996 Apr; 40 (4): 1050-2
333. Rella M, Haas D. Resistance of *Pseudomonas aeruginosa* PAO to nalidixic acid and low levels of β-lactam antibiotics: mapping of chromosomal genes. Antimicrob Agents Chemother 1982 Aug; 22 (2): 242-9
334. Adewoye L, Sutherland A, Srikumar R, et al. The MexR repressor of the MexAB-OprM multidrug efflux operon in *Pseudomonas aeruginosa*: characterization of mutations compromising activity. J Bacteriol 2002 Aug; 184 (15): 4308-12
335. Saito K, Yoneyama H, Nakae T. *nalB*-type mutations causing the overexpression of the MexAB-OprM efflux pump are located in the *mexR* gene of the *Pseudomonas aeruginosa* chromosome. FEMS Microbiol Lett 1999 Oct 1; 179 (1): 67-72
336. Jalal S, Wretlind B. Mechanisms of quinolone resistance in clinical strains of *Pseudomonas aeruginosa*. Microb Drug Resist 1998 Winter; 4 (4): 257-61

337. Evans K, Adewoye L, Poole K. MexR repressor of the MexAB-OprM multidrug efflux operon of *Pseudomonas aeruginosa*: identification of MexR binding sites in the MexA-MexR intergenic region. J Bacteriol 2001 Feb; 183 (3): 807-12
338. Lim D, Poole K, Strynadka NC. Crystal structure of the MexR repressor of the *mexRAB-oprM* multidrug efflux operon of *Pseudomonas aeruginosa*. J Biol Chem 2002 Aug 9; 277 (32): 29253-9
339. Alekshun MN, Levy SB, Mealy TR, et al. The crystal structure of MarR, a regulator of multiple antibiotic resistance, at 2.3 Å resolution. Nat Struct Biol 2001 Aug; 8 (8): 710-4
340. Cao L, Srikumar R, Poole K. Identification and characterization of *nalC* multidrug-resistant isolates of *Pseudomonas aeruginosa* [abstract no. C1-430]. Abstracts of the 42nd Interscience Conference on Antimicrobial Agents and Chemotherapy, American Society for Microbiology, Washington, DC; 2002 Sep 27-30, San Diego (CA), 29
341. Weickert MJ, Adhya S. A family of bacterial regulators homologous to Gal and Lac repressors. J Biol Chem 1992 Aug 5; 267 (22): 15869-74
342. Shiba T, Ishiguro K, Takemoto N, et al. Purification and characterization of the *Pseudomonas aeruginosa* NfxB protein, the negative regulator of the *nfxB* gene. J Bacteriol 1995 Oct; 177 (20): 5872-7
343. Köhler T, Epp SF, Curty LK, et al. Characterization of MexT, the regulator of the MexE-MexF-OprN multidrug efflux system of *Pseudomonas aeruginosa*. J Bacteriol 1999 Oct; 181 (20): 6300-5
344. Schell MA. Molecular biology of the LysR family of transcriptional regulators. Annu Rev Microbiol 1993; 47: 597-626
345. Grkovic S, Brown MH, Roberts NJ, et al. QacR is a repressor protein that regulates expression of the *Staphylococcus aureus* multidrug efflux pump QacA. J Biol Chem 1998 Jul 17; 273 (29): 18665-73
346. Stock AM, Robinson VL, Goudreau PN. Two-component signal transduction. Annu Rev Biochem 2000; 69: 183-215
347. Kieboom J, Dennis JJ, Zylstra GJ, et al. Active efflux of organic solvents by *Pseudomonas putida* S12 is induced by solvents. J Bacteriol 1998 Dec; 180 (24): 6769-72
348. Alekshun MN, Levy SB. Regulation of chromosomally mediated multiple antibiotic resistance: the *mar* regulon. Antimicrob Agents Chemother 1997 Oct; 41 (10): 2067-75
349. Martin RG, Rosner JL. Binding of purified multiple antibiotic-resistance repressor protein (MarR) to *mar* operator sequences. Proc Natl Acad Sci U S A 1995 Jun 6; 92 (12): 5456-60
350. Seoane AS, Levy SB. Characterization of MarR, the repressor of the multiple antibiotic resistance (*mar*) operon in *Escherichia coli*. J Bacteriol 1995 Jun; 177 (12): 3414-9
351. Gallegos MT, Michan C, Ramos JL. The XylS/AraC family of regulators. Nucleic Acids Res 1993 Feb 25; 21 (4): 807-10
352. Barbosa TM, Levy SB. Differential expression of over 60 chromosomal genes in *Escherichia coli* by constitutive expression of MarA. J Bacteriol 2000 Jun; 182 (12): 3467-74
353. Okusu H, Ma D, Nikaido H. AcrAB efflux pump plays a major role in the antibiotic resistance phenotype of *Escherichia coli* multiple-antibiotic-resistance (Mar) mutants. J Bacteriol 1996 Jan; 178 (1): 306-8
354. Li H, Park JT. The periplasmic murein peptide-binding protein MppA is a negative regulator of multiple antibiotic resistance in *Escherichia coli*. J Bacteriol 1999 Aug; 181 (16): 4842-7
355. Ma D, Cook DN, Alberti M, et al. Genes *acrA* and *acrB* encode a stress-induced efflux system of *Escherichia coli*. Mol Microbiol 1995 Apr; 16 (1): 45-55
356. McMurry LM, Oethinger M, Levy SB. Overexpression of *marA*, *soxS*, or *acrAB* produces resistance to triclosan in laboratory and clinical strains of *Escherichia coli*. FEMS Microbiol Lett 1998 Sep 15; 166 (2): 305-9
357. White DG, Goldman JD, Demple B, et al. Role of the *acrAB* locus in organic solvent tolerance mediated by expression of *marA*, *soxS*, or *robA* in *Escherichia coli*. J Bacteriol 1997 Oct; 179 (19): 6122-6
358. Hidalgo E, Ding H, Demple B. Redox signal transduction via iron-sulfur clusters in the SoxR transcription activator. Trends Biochem Sci 1997 Jun; 22 (6): 207-10
359. Kwon HJ, Bennik MH, Demple B, et al. Crystal structure of the *Escherichia coli* Rob transcription factor in complex with DNA. Nat Struct Biol 2000 May; 7 (5): 424-30
360. Nakajima H, Kobayashi K, Kobayashi M, et al. Overexpression of the *robA* gene increases organic solvent tolerance and multiple antibiotic and heavy metal ion resistance in *Escherichia coli*. Appl Environ Microbiol 1995 Jun; 61 (6): 2302-7
361. Ariza RR, Li Z, Ringstad N, et al. Activation of multiple antibiotic resistance and binding of stress-inducible promoters by *Escherichia coli* Rob protein. J Bacteriol 1995 Apr; 177 (7): 1655-61
362. Jair KW, Yu X, Skarstad K, et al. Transcriptional activation of promoters of the superoxide and multiple antibiotic resistance regulons by Rob, a binding protein of the *Escherichia coli* origin of chromosomal replication. J Bacteriol 1996 May; 178 (9): 2507-13
363. Rosenberg EY, Bertenthal D, Nilles ML, et al. Bile salts and fatty acids induce the expression of *Escherichia coli* AcrAB multidrug efflux pump through their interaction with Rob regulatory protein. Mol Microbiol 2003; 48 (6): 1609-19
364. Aono R, Tsukagoshi N, Yamamoto M. Involvement of outer membrane protein TolC, a possible member of the *mar-sox* regulon, in maintenance and improvement of organic solvent tolerance of *Escherichia coli* K-12. J Bacteriol 1998 Feb; 180 (4): 938-44
365. Cohen SP, McMurry LM, Levy SB. *marA* locus causes decreased expression of OmpF porin in multiple-antibiotic-resistant (Mar) mutants of *Escherichia coli*. J Bacteriol 1988 Dec; 170 (12): 5416-22
366. Rosner JL, Chai TJ, Foulds J. Regulation of *ompF* porin expression by salicylate in *Escherichia coli*. J Bacteriol 1991 Sep; 173 (18): 5631-8
367. Nikaido H, Rosenberg EY, Foulds J. Porin channels in *Escherichia coli*: studies with β-lactams in intact cells. J Bacteriol 1983 Jan; 153 (1): 232-40
368. Nikaido H, Rosenberg EY. Porin channels in *Escherichia coli*: studies with liposomes reconstituted from purified proteins. J Bacteriol 1983 Jan; 153 (1): 241-52
369. Rahmati S, Yang S, Davidson AL, et al. Control of the AcrAB multidrug efflux pump by quorum-sensing regulator SdiA. Mol Microbiol 2002 Feb; 43 (3): 677-85
370. George AM, Hall RM, Stokes HW. Multidrug resistance in *Klebsiella pneumoniae*: a novel gene, *ramA*, confers a multidrug resistance phenotype in *Escherichia coli*. Microbiology 1995 Aug; 141 (Pt 8): 1909-20
371. Vaara M. Antibiotic-supersusceptible mutants of *Escherichia coli* and *Salmonella typhimurium*. Antimicrob Agents Chemother 1993 Nov; 37 (11): 2255-60
372. Mazzariol A, Cornaglia G, Nikaido H. Contributions of the AmpC β-lactamase and the AcrAB multidrug efflux system in intrinsic resistance of *Escherichia coli* K-12 to β-lactams. Antimicrob Agents Chemother 2000 May; 44 (5): 1387-90

373. Cho D, Blais J, Tangen K, et al. Prevalence of efflux pumps among clinical isolates of fluoroquinolone-resistant *Pseudomonas aeruginosa* [abstract no. 1267]. Abstracts of the 39th Interscience Conference on Antimicrobial Agents and Chemotherapy. American Society for Microbiology, Washington, DC; 1999 Sep 26-29; San Francisco (CA), 327
374. George AM, Levy SB. Amplifiable resistance to tetracycline, chloramphenicol, and other antibiotics in *Escherichia coli*: involvement of a non-plasmid-determined efflux of tetracycline. J Bacteriol 1983 Aug; 155 (2): 531-40
375. George AM, Levy SB. Gene in the major cotransduction gap of the *Escherichia coli* K-12 linkage map required for the expression of chromosomal resistance to tetracycline and other antibiotics. J Bacteriol 1983 Aug; 155 (2): 541-8
376. Russell AD. Do biocides select for antibiotic resistance? J Pharm Pharmacol 2000 Feb; 52 (2): 227-33
377. Cohen SP, Levy SB, Foulds J, et al. Salicylate induction of antibiotic resistance in *Escherichia coli*: activation of the mar operon and a mar-independent pathway. J Bacteriol 1993 Dec; 175 (24): 7856-62
378. Sumita Y, Fukasawa M. Transient carbapenem resistance induced by salicylate in *Pseudomonas aeruginosa* associated with suppression of outer membrane protein D2 synthesis. Antimicrob Agents Chemother 1993 Dec; 37 (12): 2743-6
379. Burns JL, Clark DK. Salicylate-inducible antibiotic resistance in *Pseudomonas cepacia* associated with absence of a pore-forming outer membrane protein. Antimicrob Agents Chemother 1992 Oct; 36 (10): 2280-5
380. Domenico P, Hopkins T, Cunha BA. The effect of sodium salicylate on antibiotic susceptibility and synergy in *Klebsiella pneumoniae*. J Antimicrob Chemother 1990 Sep; 26 (3): 343-51
381. Schaller A, Sun Z, Yang Y, et al. Salicylate reduces susceptibility of *Mycobacterium tuberculosis* to multiple antituberculosis drugs. Antimicrob Agents Chemother 2002 Aug; 46 (8): 2636-9
382. Price CT, O'Brien FG, Shelton BP, et al. Effects of salicylate and related compounds on fusidic acid MICs in *Staphylococcus aureus*. J Antimicrob Chemother 1999 Jul; 44 (1): 57-64
383. Gustafson JE, Candelaria PV, Fisher SA, et al. Growth in the presence of salicylate increases fluoroquinolone resistance in *Staphylococcus aureus*. Antimicrob Agents Chemother 1999 Apr; 43 (4): 990-2
384. Price CT, Kaatz GW, Gustafson JE. The multidrug efflux pump NorA is not required for salicylate-induced reduction in drug accumulation by *Staphylococcus aureus*. Int J Antimicrob Agents 2002 Sep; 20 (3): 206-13
385. Williams RJ, Livermore DM, Lindridge MA, et al. Mechanisms of β-lactam resistance in British isolates of *Pseudomonas aeruginosa*. J Med Microbiol 1984 Jun; 17 (3): 283-93
386. Bert F, Lambert-Zechovsky N. Comparative distribution of resistance patterns and serotypes in *Pseudomonas aeruginosa* isolates from intensive care units and other wards. J Antimicrob Chemother 1996 Apr; 37 (4): 809-13
387. Jakics EB, Iyobe S, Hirai K, et al. Occurrence of the *nfxB* type mutation in clinical isolates of *Pseudomonas aeruginosa*. Antimicrob Agents Chemother 1992 Nov; 36 (11): 2562-5
388. Fukuda H, Hosaka M, Iyobe S, et al. *nfxC*-type quinolone resistance in a clinical isolate of *Pseudomonas aeruginosa*. Antimicrob Agents Chemother 1995 Mar; 39 (3): 790-2
389. Beinlich KL, Chuanchuen R, Schweizer HP. Contribution of multidrug efflux pumps to multiple antibiotic resistance in veterinary clinical isolates of *Pseudomonas aeruginosa*. FEMS Microbiol Lett 2001 May 1; 198 (2): 129-34
390. Hoiby N. New antimicrobials in the management of cystic fibrosis. J Antimicrob Chemother 2002 Feb; 49 (2): 235-8
391. Charvalos E, Tselentis Y, Hamzehpour MM, et al. Evidence for an efflux pump in multidrug-resistant *Campylobacter jejuni*. Antimicrob Agents Chemother 1995 Sep; 39 (9): 2019-22
392. Mazzariol A, Tokue Y, Kanegawa TM, et al. High-level fluoroquinolone-resistant clinical isolates of *Escherichia coli* overproduce multidrug efflux protein AcrA. Antimicrob Agents Chemother 2000 Dec; 44 (12): 3441-3
393. Wang H, Dzink-Fox JL, Chen M, et al. Genetic characterization of highly fluoroquinolone-resistant clinical *Escherichia coli* strains from China: role of *acrR* mutations. Antimicrob Agents Chemother 2001 May; 45 (5): 1515-21
394. George AM. Multidrug resistance in enteric and other gram-negative bacteria. FEMS Microbiol Lett 1996 May 15; 139 (1): 1-10
395. Linde HJ, Notka F, Irtenkauf C, et al. Increase in MICs of ciprofloxacin *in vivo* in two closely related clinical isolates of *Enterobacter cloacae*. J Antimicrob Chemother 2002 Apr; 49 (4): 625-30
396. Deguchi T, Kawamura T, Yasuda M, et al. *In vivo* selection of *Klebsiella pneumoniae* strains with enhanced quinolone resistance during fluoroquinolone treatment of urinary tract infections. Antimicrob Agents Chemother 1997 Jul; 41 (7): 1609-11
397. del Mar Tavio M, Vila J, Ruiz J, et al. Decreased permeability and enhanced proton-dependent active efflux in the development of resistance to quinolones in *Morganella morganii*. Int J Antimicrob Agents 2000 Mar; 14 (2): 157-60
398. Ishii H, Sato K, Hoshino K, et al. Active efflux of ofloxacin by a highly quinolone-resistant strain of *Proteus vulgaris*. J Antimicrob Chemother 1991 Dec; 28 (6): 827-36
399. Ishida H, Fuziwara H, Kaibori Y, et al. Cloning of multidrug resistance gene *pqrA* from *Proteus vulgaris*. Antimicrob Agents Chemother 1995 Feb; 39 (2): 453-7
400. Ghosh AS, Ahamed J, Chauhan KK, et al. Involvement of an efflux system in high-level fluoroquinolone resistance of *Shigella dysenteriae*. Biochem Biophys Res Commun 1998 Jan 6; 242 (1): 54-6
401. Perez-Trallero E, Fernandez-Mazarrasa C, Garcia-Rey C, et al. Antimicrobial susceptibilities of 1,684 *Streptococcus pneumoniae* and 2,039 *Streptococcus pyogenes* isolates and their ecological relationships: results of a 1-year (1998-1999) multicenter surveillance study in Spain. Antimicrob Agents Chemother 2001 Dec; 45 (12): 3334-40
402. Levy SB. Active efflux, a common mechanism for biocide and antibiotic resistance. J Appl Microbiol 2002; 92 Suppl.: 65S-71S
403. Lambert RJ, Joynson J, Forbes B. The relationships and susceptibilities of some industrial, laboratory and clinical isolates of *Pseudomonas aeruginosa* to some antibiotics and biocides. J Appl Microbiol 2001 Dec; 91 (6): 972-84
404. Stickler DJ. Susceptibility of antibiotic-resistant Gram-negative bacteria to biocides: a perspective from the study of catheter biofilms. J Appl Microbiol 2002; 92 Suppl.: 163S-70S
405. Higgins CS, Murtough SM, Williamson E, et al. Resistance to antibiotics and biocides among non-fermenting Gram-negative bacteria. Clin Microbiol Infect 2001 Jun; 7 (6): 308-15
406. Nakahara H, Asakawa M, Yonekura I, et al. Benzethonium chloride resistance in *Pseudomonas aeruginosa* isolated from clinical lesions. Zentralbl Bakteriol Mikrobiol Hyg [A] 1984 Aug; 257 (3): 409-13

407. Nakahara H, Kozukue H. Isolation of chlorhexidine-resistant *Pseudomonas aeruginosa* from clinical lesions. J Clin Microbiol 1982 Jan; 15 (1): 166-8
408. Block C, Furman M. Association between intensity of chlorhexidine use and micro-organisms of reduced susceptibility in a hospital environment. J Hosp Infect 2002 Jul; 51 (3): 201-6
409. Fraise AP. Biocide abuse and antimicrobial resistance: a cause for concern? J Antimicrob Chemother 2002 Jan; 49 (1): 11-2
410. Skurray RA, Rouch DA, Lyon BR, et al. Multiresistant *Staphylococcus aureus*: genetics and evolution of epidemic Australian strains. J Antimicrob Chemother 1988 Apr; 21 Suppl. C: 19-39
411. Lyon BR, Skurray R. Antimicrobial resistance of *Staphylococcus aureus*: genetic basis. Microbiol Rev 1987 Mar; 51 (1): 88-134
412. Join-Lambert OF, Michea-Hamzehpour M, Kohler T, et al. Differential selection of multidrug efflux mutants by trovafloxacin and ciprofloxacin in an experimental model of *Pseudomonas aeruginosa* acute pneumonia in rats. Antimicrob Agents Chemother 2001 Feb; 45 (2): 571-6
413. Köhler T, Michea-Hamzehpour M, Plesiat P, et al. Differential selection of multidrug efflux systems by quinolones in *Pseudomonas aeruginosa*. Antimicrob Agents Chemother 1997 Nov; 41 (11): 2540-3
414. Hooper DC. Mechanisms of action and resistance of older and newer fluoroquinolones. Clin Infect Dis 2000 Aug; 31 Suppl. 2: S24-8
415. Zhao X, Drlica K. Restricting the selection of antibiotic-resistant mutants: a general strategy derived from fluoroquinolone studies. Clin Infect Dis 2001 Sep 15; 33 Suppl. 3: S147-56
416. Mamber SW, Kolek B, Brookshire KW, et al. Activity of quinolones in the Ames *Salmonella* TA102 mutagenicity test and other bacterial genotoxicity assays. Antimicrob Agents Chemother 1993 Feb; 37 (2): 213-7
417. Ysern P, Clerch B, Castano M, et al. Induction of *SOS* genes in *Escherichia coli* and mutagenesis in *Salmonella typhimurium* by fluoroquinolones. Mutagenesis 1990 Jan; 5 (1): 63-6
418. Le Thomas I, Couetdic G, Clermont O, et al. *In vivo* selection of a target/efflux double mutant of *Pseudomonas aeruginosa* by ciprofloxacin therapy. J Antimicrob Chemother 2001 Oct; 48 (4): 553-5
419. Zhanel GG, Karlowsky JA, Saunders MH, et al. Development of multiple-antibiotic-resistant (Mar) mutants of *Pseudomonas aeruginosa* after serial exposure to fluoroquinolones. Antimicrob Agents Chemother 1995 Feb; 39 (2): 489-95
420. Lomovskaya O, Lee A, Hoshino K, et al. Use of a genetic approach to evaluate the consequences of inhibition of efflux pumps in *Pseudomonas aeruginosa*. Antimicrob Agents Chemother 1999 Jun; 43 (6): 1340-6
421. Lee A, Mao W, Warren MS, et al. Interplay between efflux pumps may provide either additive or multiplicative effects on drug resistance. J Bacteriol 2000 Jun; 182 (11): 3142-50
422. Plesiat P, Nikaido H. Outer membranes of gram-negative bacteria are permeable to steroid probes. Mol Microbiol 1992 May; 6 (10): 1323-33
423. Vaara M. The outer membrane as the penetration barrier against mupirocin in gram-negative enteric bacteria. J Antimicrob Chemother 1992 Feb; 29 (2): 221-2
424. Yethon JA, Gunn JS, Ernst RK, et al. *Salmonella enterica* serovar typhimurium *waaP* mutants show increased susceptibility to polymyxin and loss of virulence *in vivo*. Infect Immun 2000 Aug; 68 (8): 4485-91
425. Yethon JA, Heinrichs DE, Monteiro MA, et al. Involvement of *waaY*, *waaQ*, and *waaP* in the modification of *Escherichia coli* lipopolysaccharide and their role in the formation of a stable outer membrane. J Biol Chem 1998 Oct 9; 273 (41): 26310-6
426. Vaara M. Agents that increase the permeability of the outer membrane. Microbiol Rev 1992 Sep; 56 (3): 395-411
427. Fralick JA, Burns-Keliher LL. Additive effect of *tolC* and *rfa* mutations on the hydrophobic barrier of the outer membrane of *Escherichia coli* K-12. J Bacteriol 1994 Oct; 176 (20): 6404-6
428. Lucas CE, Hagman KE, Levin JC, et al. Importance of lipooligosaccharide structure in determining gonococcal resistance to hydrophobic antimicrobial agents resulting from the mtr efflux system. Mol Microbiol 1995 Jun; 16 (5): 1001-9
429. Li XZ, Nikaido H, Williams KE. Silver-resistant mutants of *Escherichia coli* display active efflux of Ag^+ and are deficient in porins. J Bacteriol 1997 Oct; 179 (19): 6127-32
430. Nikaido H, Normark S. Sensitivity of *Escherichia coli* to various β-lactams is determined by the interplay of outer membrane permeability and degradation by periplasmic β-lactamases: a quantitative predictive treatment. Mol Microbiol 1987 Jul; 1 (1): 29-36
431. Li XZ, Zhang L, Poole K. Interplay between the MexA-MexB-OprM multidrug efflux system and the outer membrane barrier in the multiple antibiotic resistance of *Pseudomonas aeruginosa*. J Antimicrob Chemother 2000 Apr; 45 (4): 433-6
432. Plesiat P, Aires JR, Godard C, et al. Use of steroids to monitor alterations in the outer membrane of *Pseudomonas aeruginosa*. J Bacteriol 1997 Nov; 179 (22): 7004-10
433. Germ M, Yoshihara E, Yoneyama H, et al. Interplay between the efflux pump and the outer membrane permeability barrier in fluorescent dye accumulation in *Pseudomonas aeruginosa*. Biochem Biophys Res Commun 1999 Aug 2; 261 (2): 452-5
434. Livermore DM. Multiple mechanisms of antimicrobial resistance in *Pseudomonas aeruginosa*: our worst nightmare? Clin Infect Dis 2002 Mar 1; 34 (5): 634-40
435. Lakaye B, Dubus A, Lepage S, et al. When drug inactivation renders the target irrelevant to antibiotic resistance: a case story with β-lactams. Mol Microbiol 1999 Jan; 31 (1): 89-101
436. Nakae T, Nakajima A, Ono T, et al. Resistance to β-lactam antibiotics in *Pseudomonas aeruginosa* due to interplay between the MexAB-OprM efflux pump and β-lactamase. Antimicrob Agents Chemother 1999 May; 43 (5): 1301-3
437. Masuda N, Gotoh N, Ishii C, et al. Interplay between chromosomal β-lactamase and the MexAB-OprM efflux system in intrinsic resistance to β-lactams in *Pseudomonas aeruginosa*. Antimicrob Agents Chemother 1999 Feb; 43 (2): 400-2
438. Srikumar R, Tsang E, Poole K. Contribution of the MexAB-OprM multidrug efflux system to the β-lactam resistance of penicillin-binding protein and β-lactamase-derepressed mutants of *Pseudomonas aeruginosa*. J Antimicrob Chemother 1999 Oct; 44 (4): 537-40
439. Li XZ, Zhamg L, McKay GA, et al. Role of the acetyltransferase AAC(6')-*Iz* modifying enzyme in aminoglycoside resistance in *Stenotrophomonas maltophilia*. J Antimicrob Chemother 2003; 51 (4): 803-11
440. Kaatz GW, Seo SM. Mechanisms of fluoroquinolone resistance in genetically related strains of *Staphylococcus aureus*. Antimicrob Agents Chemother 1997 Dec; 41 (12): 2733-7
441. Nakajima A, Sugimoto Y, Yoneyama H, et al. High-level fluoroquinolone resistance in *Pseudomonas aeruginosa* due to interplay of the MexAB-OprM efflux pump and the DNA gyrase mutation. Microbiol Immunol 2002; 46 (6): 391-5

442. Veal WL, Nicholas RA, Shafer WM. Overexpression of the MtrC-MtrD-MtrE efflux pump due to an *mtrR* mutation is required for chromosomally mediated penicillin resistance in *Neisseria gonorrhoeae*. J Bacteriol 2002 Oct; 184 (20): 5619-24
443. Maiti SN, Phillips OA, Micetich RG, et al. Beta-lactamase inhibitors: agents to overcome bacterial resistance. Curr Med Chem 1998 Dec; 5 (6): 441-56
444. Chopra I. New developments in tetracycline antibiotics: glycylcyclines and tetracycline efflux pump inhibitors. Drug Resist Updat 2002 Aug 6; 5 (3-4): 119
445. Nelson ML, Levy SB. Reversal of tetracycline resistance mediated by different bacterial tetracycline resistance determinants by an inhibitor of the Tet(B) antiport protein. Antimicrob Agents Chemother 1999 Jul; 43 (7): 1719-24
446. Nelson ML, Park BH, Levy SB. Molecular requirements for the inhibition of the tetracycline antiport protein and the effect of potent inhibitors on the growth of tetracycline-resistant bacteria. J Med Chem 1994 Apr 29; 37 (9): 1355-61
447. Rothstein DM, McGlynn M, Bernan V, et al. Detection of tetracyclines and efflux pump inhibitors. Antimicrob Agents Chemother 1993 Aug; 37 (8): 1624-9
448. Nelson ML. Modulation of antibiotic efflux in bacteria. Curr Med Chem-Anti-Infective Agents 2002; 1 (1): 35-54
449. Hirata T, Wakatabe R, Nielsen J, et al. A novel compound, 1,1-dimethyl-5 (1-hydroxypropyl)-4,6,7-trimethylindan, is an effective inhibitor of the *tet(K)* gene-encoded metal-tetracycline/H^+ antiporter of *Staphylococcus aureus*. FEBS Lett 1997 Jul 28; 412 (2): 337-40
450. Hirata T, Wakatabe R, Nielsen J, et al. Screening of an inhibitor of the tetracycline efflux pump in a tetracycline-resistant clinical-isolate of *Staphylococcus aureus* 743. Biol Pharm Bull 1998 Jul; 21 (7): 678-81
451. Barrett JF. MC-207110 Daiichi Seiyaku/Microcide Pharmaceuticals. Curr Opin Investig Drugs 2001 Feb; 2 (2): 212-5
452. Ryan BM, Dougherty TJ, Beaulieu D, et al. Efflux in bacteria: what do we really know about it? Expert Opin Investig Drugs 2001 Aug; 10 (8): 1409-22
453. Lewis K. In search of natural substrates and inhibitors of MDR pumps. J Mol Microbiol Biotechnol 2001 Apr; 3 (2): 247-54
454. Wigler PW, Patterson FK. Inhibition of the multidrug resistance efflux pump. Biochim Biophys Acta 1993 Oct 29; 1154 (2): 173-81
455. Martin SK, Oduola AM, Milhous WK. Reversal of chloroquine resistance in *Plasmodium falciparum* by verapamil. Science 1987 Feb 20; 235 (4791): 899-901
456. Cohn RC, Rudzienski L, Putnam RW. Verapamil-tobramycin synergy in *Pseudomonas cepacia* but not *Pseudomonas aeruginosa in vitro*. Chemotherapy 1995 Sep-Oct; 41 (5): 330-3
457. Brenwald NP, Gill MJ, Wise R. The effect of reserpine, an inhibitor of multi-drug efflux pumps, on the *in-vitro* susceptibilities of fluoroquinolone-resistant strains of *Streptococcus pneumoniae* to norfloxacin. J Antimicrob Chemother 1997 Sep; 40 (3): 458-60
458. Gibbons S, Udo EE. The effect of reserpine, a modulator of multidrug efflux pumps, on the *in vitro* activity of tetracycline against clinical isolates of methicillin resistant *Staphylococcus aureus* (MRSA) possessing the *tet(K)* determinant. Phytother Res 2000 Mar; 14 (2): 139-40
459. Beyer R, Pestova E, Millichap JJ, et al. A convenient assay for estimating the possible involvement of efflux of fluoroquinolones by *Streptococcus pneumoniae* and *Staphylococcus aureus*: evidence for diminished moxifloxacin, sparfloxacin, and trovafloxacin efflux. Antimicrob Agents Chemother 2000 Mar; 44 (3): 798-801
460. Markham PN, Westhaus E, Klyachko K, et al. Multiple novel inhibitors of the NorA multidrug transporter of *Staphylococcus aureus*. Antimicrob Agents Chemother 1999 Oct; 43 (10): 2404-8
461. Neyfakh AA, Borsch CM, Kaatz GW. Fluoroquinolone resistance protein NorA of *Staphylococcus aureus* is a multidrug efflux transporter. Antimicrob Agents Chemother 1993 Jan; 37 (1): 128-9
462. Stermitz FR, Lorenz P, Tawara JN, et al. Synergy in a medicinal plant: antimicrobial action of berberine potentiated by 5′-methoxyhydnocarpin, a multidrug pump inhibitor. Proc Natl Acad Sci U S A 2000 Feb 15; 97 (4): 1433-7
463. Renau TE, Leger R, Flamme EM, et al. Inhibitors of efflux pumps in *Pseudomonas aeruginosa* potentiate the activity of the fluoroquinolone antibacterial levofloxacin. J Med Chem 1999 Dec 2; 42 (24): 4928-31
464. Renau TE, Leger R, Flamme EM, et al. Addressing the stability of C-capped dipeptide efflux pump inhibitors that potentiate the activity of levofloxacin in *Pseudomonas aeruginosa*. Bioorg Med Chem Lett 2001 Mar 12; 11 (5): 663-7
465. Lomovskaya O, Warren MS, Lee A, et al. Identification and characterization of inhibitors of multidrug resistance efflux pumps in *Pseudomonas aeruginosa*: novel agents for combination therapy. Antimicrob Agents Chemother 2001 Jan; 45 (1): 105-16
466. Griffith D, Lomovskaya O, Lee V, et al. Potentiation of levofloxacin by a broad-spectrum efflux inhibitor (EPI) in mouse models of infection caused by *Pseudomonas aeruginosa* [abstract no. 1268]. Abstracts of the 39th Interscience conference on Antimicrobial Agents and Chemotherapy. American Society for Microbiology, Washington, DC; 1999 Sep 26-29; San Francisco (CA), 327
467. Renau TE, Leger R, Yen R, et al. Peptidomimetics of efflux pump inhibitors potentiate the activity of levofloxacin in *Pseudomonas aeruginosa*. Bioorg Med Chem Lett 2002 Mar 11; 12 (5): 763-6
468. Lee MD, Galazzo JL, Staley AL, et al. Microbial fermentation-derived inhibitors of efflux-pump-mediated drug resistance. Farmaco 2001 Jan-Feb; 56 (1-2): 81-5
469. Fukuda H, Hori S, Hiramatsu K. Antibacterial activity of gatifloxacin (AM-1155, CG5501, BMS-206584), a newly developed fluoroquinolone, against sequentially acquired quinolone-resistant mutants and the *norA* transformant of *Staphylococcus aureus*. Antimicrob Agents Chemother 1998 Aug; 42 (8): 1917-22
470. Gootz TD, Zaniewski RP, Haskell SL, et al. Activities of trovafloxacin compared with those of other fluoroquinolones against purified topoisomerases and *gyrA* and *grlA* mutants of *Staphylococcus aureus*. Antimicrob Agents Chemother 1999 Aug; 43 (8): 1845-55
471. Ince D, Hooper DC. Mechanisms and frequency of resistance to premafloxacin in *Staphylococcus aureus*: novel mutations suggest novel drug-target interactions. Antimicrob Agents Chemother 2000 Dec; 44 (12): 3344-50
472. Zhong P, Shortridge VD. The role of efflux in macrolide resistance. Drug Resist Updat 2000 Dec; 3 (6): 325-9
473. Chu DT. Recent progress in novel macrolides, quinolones, and 2-pyridones to overcome bacterial resistance. Med Res Rev 1999 Nov; 19 (6): 497-520
474. Brennan L, Duignan J, Petitpas J, et al. CP-544372: MIC90 studies and killing kinetics against key respiratory tract patho-

gens [abstract no. F-124]. Abstracts of the 38th Interscience Conference on Antimicrobial Agents and Chemotherapy. American Society for Microbiology, Washington, DC; 1998 Sep 24-27; San Diego (CA), 264

475. Someya Y, Yamaguchi A, Sawai T. A novel glycylcycline, 9-(N,N-dimethylglycylamido)-6-demethyl-6-deoxytetracycline, is neither transported nor recognized by the transposon Tn10-encoded metal-tetracycline/H^+ antiporter. Antimicrob Agents Chemother 1995 Jan; 39 (1): 247-9
476. Benveniste R, Davies J. Aminoglycoside antibiotic-inactivating enzymes in actinomycetes similar to those present in clinical isolates of antibiotic-resistant bacteria. Proc Natl Acad Sci U S A 1973 Aug; 70 (8): 2276-80
477. Davies J. Inactivation of antibiotics and the dissemination of resistance genes. Science 1994 Apr 15; 264 (5157): 375-82
478. Davies J. Another look at antibiotic resistance: 1991 Fred Griffith Review Lecture. J Gen Microbiol 1992 Aug; 138 (Pt 8): 1553-9
479. Marshall CG, Lessard IA, Park I, et al. Glycopeptide antibiotic resistance genes in glycopeptide-producing organisms. Antimicrob Agents Chemother 1998 Sep; 42 (9): 2215-20
480. Krulwich TA, Jin J, Guffanti AA, et al. Functions of tetracycline efflux proteins that do not involve tetracycline. J Mol Microbiol Biotechnol 2001 Apr; 3 (2): 237-46
481. Wang W, Guffanti AA, Wei Y, et al. Two types of *Bacillus subtilisTetA(L)* deletion strains reveal the physiological importance of *TetA(L)* in K^+ acquisition as well as in Na^+, alkali, and tetracycline resistance. J Bacteriol 2000 Apr; 182 (8): 2088-95

Correspondence and offprints: Dr *Hiroshi Nikaido*, Department of Molecular and Cell Biology, 426 Barker Hall, University of California, Berkeley, CA 94720-3202, USA.
E-mail: nhiroshi@uclink4.berkeley.edu