

OPINION

Multidrug-resistance efflux pumps — not just for resistance

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Abstract | It is well established that multidrug-resistance efflux pumps encoded by bacteria can confer clinically relevant resistance to antibiotics. It is now understood that these efflux pumps also have a physiological role(s). They can confer resistance to natural substances produced by the host, including bile, hormones and host-defence molecules. In addition, some efflux pumps of the resistance nodulation division (RND) family have been shown to have a role in the colonization and the persistence of bacteria in the host. Here, I present the accumulating evidence that multidrug-resistance efflux pumps have roles in bacterial pathogenicity and propose that these pumps therefore have greater clinical relevance than is usually attributed to them.

In the past decade, the number of antibiotics to which bacteria have developed resistance has increased considerably. As a consequence, some agents are no longer useful for the treatment of infections. It is also of concern that an increasing number of bacterial species are becoming resistant to more than one antibiotic. Bacteria can resist the action of antibiotics through several mechanisms. These include preventing an antibiotic from entering the bacterial cell and actively exporting an antibiotic from the bacterial cell (that is, efflux of the antibiotic). The pumps that effect such efflux typically export several unrelated substances, including molecules produced by the host organism (such as bile), indicating that these systems might have a role in allowing bacteria to survive in their ecological niche. It is possible that efflux-pump-mediated resistance to the antibiotics that are used in human and veterinary medicine is a by-product of the physiological role(s) of these pumps that, by chance, is beneficial to the bacterium.

Efflux-pump genes and proteins are present in all organisms. In bacteria, the genes that encode efflux pumps are located on the chromosome or on transmissible genetic elements, such as plasmids. It is well known that, in bacteria, efflux pumps can confer decreased susceptibility to antibiotics; however, such decreases do not always result in clinical levels of antibiotic resistance. Efflux pumps can be specific for one substrate or can transport a range of structurally dissimilar compounds (including antibiotics of different chemical classes). Those pumps that transport several compounds can be associated with multidrug (antibiotic)

resistance (MDR). Many of these efflux pumps are therefore of clinical relevance because they can render a bacterial infection untreatable by the agent(s) of choice.

There are several recent reviews of efflux pumps that confer resistance to drugs, and these articles describe the various classes of efflux pump, as well as their structures, substrates and functions^{1–7}. The regulation of efflux pumps that confer resistance to drugs has also been reviewed recently⁸. Therefore, this article focuses on two areas. First, I provide examples of chromosomally encoded efflux pumps that are involved in clinically relevant resistance to antimicrobial agents used to treat human infections. Second, I put forward my opinion that efflux pumps that confer antibiotic resistance have greater clinical relevance than has previously been thought, because there is now accumulating evidence that certain classes of efflux pump not only confer resistance to drugs used in therapy but also have a role in bacterial pathogenicity: that is, in the colonization and the survival of bacteria in the host.

Classes of MDR efflux pump

There are five families of efflux-pump proteins that are associated with MDR: the ATP-binding cassette (ABC) superfamily, the major facilitator superfamily (MFS), the multidrug and toxic-compound extrusion (MATE) family, the small multidrug resistance (SMR) family and the resistance nodulation division (RND) family (FIG. 1). MDR efflux pumps are classified as belonging to these families on the basis of the number of components that the pump has (single or multiple), the number of transmembrane-spanning regions that

the transporter protein has, the energy source that the pump uses and the types of substrate that the pump exports. A single organism can express MDR efflux pumps from more than one family and/or more than one type of efflux pump belonging to the same family. For example, *Pseudomonas aeruginosa* can express more than one type of Mex efflux pump, and *Escherichia coli* can express more than one type of Acr efflux pump (both of which are pumps belonging to the RND family).

Efflux pumps can consist of either a single component or multiple components. Examples of efflux pumps from each family are shown in FIG. 1. Efflux pumps of the RND family, which are expressed by Gram-negative bacteria and are associated with clinically significant MDR, are organized as tripartite systems (FIG. 1). These efflux pumps comprise the following: a transporter (efflux) protein (for example, AcrB), which is located in the inner (cytoplasmic) membrane of the bacterium; an accessory protein (also known as a membrane-fusion protein) (for example, AcrA), which is located in the periplasmic space; and an outer-membrane protein (also known as an outer-membrane protein channel) (for example, TolC)⁹, which is located in the outer membrane of the bacterium. Using the AcrAB–TolC system (that is, the efflux pump comprising AcrA, AcrB and TolC) as an example, it is thought that the transporter protein AcrB, similar to AcrD¹⁰, captures its substrates either from within the phospholipid bilayer of the inner membrane or from the cytoplasm, and then transports them to the extracellular medium through TolC, which forms a channel in the outer membrane¹¹. The cooperation between AcrB and TolC is mediated by the periplasmic accessory protein AcrA. Efflux through RND-family pumps is driven by the proton motive force¹¹, an electrochemical gradient in which the movement of hydrogen ions drives transport of the substrate. In Enterobacteriaceae, TolC can function as the protein channel for different RND-family efflux pumps, and it can also interact with MFS transporters (for example, EmrAB of *E. coli*) and ABC-superfamily transporters (for example, MacAB of *E. coli*). Similarly, the RND-family outer-membrane protein OprM of *P. aeruginosa* can interact with several RND-family proteins.

Efflux pumps and antibiotic resistance

Intrinsic antibiotic resistance. Certain antibacterial agents cannot be used for the treatment of an infection with some species of Gram-negative bacteria, because these

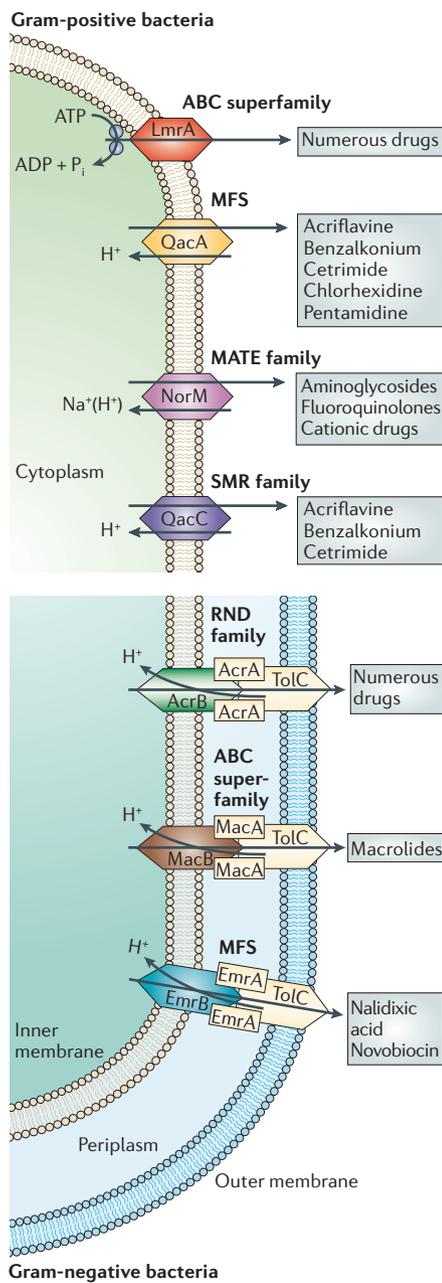


Figure 1 | Multidrug-resistance efflux pumps. There are five families of multidrug-resistance efflux pumps: the ATP-binding cassette (ABC) superfamily, the major facilitator superfamily (MFS), the multidrug and toxic-compound extrusion (MATE) family, the small multidrug resistance (SMR) family and the resistance nodulation division (RND) family. A diagrammatic representation of the structure and membrane location of efflux pumps from each of these families is shown. Common examples of the individual proteins that form each class of efflux pump are indicated. Antibiotic substrates and examples of other substrates are also listed for each class of efflux pump. Multidrug-resistance efflux pumps expressed by Gram-negative bacteria usually have several components, and the outer-membrane protein is typically TolC. P_i, inorganic phosphate.

bacteria have ‘intrinsic resistance’ to these agents. Such resistance was initially attributed to poor permeability of the bacterial membrane to the drug. In 1994, however, Li and colleagues¹² reported data indicating that the intrinsic resistance of *P. aeruginosa* to several antibiotics was due to efflux. The previous year, Poole and co-workers¹³ had described an operon encoding an efflux system, MexAB–OprM, in wild-type *P. aeruginosa* and showed that deletion of genes encoding components of this system conferred hypersusceptibility to a variety of antimicrobial agents. It has subsequently been shown that some classes of agent have poor activity against Gram-negative bacteria as a result of efflux. These include oxazolidinones and deformylase inhibitors^{14–16}. Similarly, there are examples of some, but not all, members of a class of drug having little or no activity as a result of efflux. For example, older macrolides such as erythromycin have little or no activity against *E. coli* and *Haemophilus influenzae*^{17–19}, and the new glycolcycline known as tigecycline has little or no activity against *P. aeruginosa* and *Proteus mirabilis*^{20,21}.

Efflux as a mechanism of antibiotic resistance. Intrinsic resistance to certain antimicrobial agents is conferred by basal levels of efflux. By contrast, in bacteria that are usually susceptible to a certain agent, resistance to antimicrobial agents — reflected by an increased minimum inhibitory concentration (MIC; that is, the lowest concentration of an antibiotic that inhibits growth of the organism) — results from a constitutive increase in expression of the efflux-pump protein (the transporter protein). Permanent upregulation of efflux-pump expression can occur by several mechanisms: mutation in the local repressor gene; mutation in a global regulatory gene (that is, a transcriptional activator); mutation in the promoter region of the efflux-pump (transporter protein) gene.

Efflux is suspected to be the mechanism of antibiotic resistance when there is a simultaneous increase in the MICs of three or more antibiotics for a particular bacterium compared with the MICs of these antibiotics for the parent strain. In the case of a clinical isolate from a patient (or animal), the MICs of the antibiotics are compared with those for a pre-therapy isolate or, more commonly, for a standard antibiotic-susceptible strain of the same species. The MICs of these antimicrobial agents for ‘efflux mutants’ are typically 2–8-fold higher than the MICs

of these agents for the susceptible strain. Although such resistance is considered to be broad spectrum (that is, across antimicrobial classes), such an increase in MIC is less than the >100-fold increase that is usually associated either with expression of enzymes that inactivate antimicrobial agents or with mutations in genes that encode the target proteins of antimicrobial agents. Nonetheless, there are clear examples in which increases in MIC as a result of efflux are sufficient to render the bacterium resistant to the recommended breakpoint concentration (that is, the concentration of an antibiotic above which a particular bacterium is considered to be resistant) of the drugs that are used to treat infection. This phenomenon has most frequently been described for fluoroquinolones.

The drug substrate profile of bacteria that overexpress an efflux pump typically includes a range of structurally diverse molecules: antibiotics such as chloramphenicol, quinolones (for example, nalidixic acid, ciprofloxacin and norfloxacin) and tetracycline; dyes such as acriflavine and ethidium bromide; detergents such as sodium dodecyl sulphate (SDS) and Triton X-100; and biocides such as cetrimide and triclosan. The agents that are thought to be substrates of each efflux pump vary depending on the pump and the bacterial species. The substrates of specific bacterial MDR efflux pumps have been detailed by Poole^{4,5}.

Clinically relevant Gram-negative bacteria and MDR efflux pumps. For Gram-negative bacteria, the efflux pumps that are associated with clinically significant resistance to drugs are those of the RND family, whereas for Gram-positive bacteria (discussed in the next section), the clinically significant efflux pumps are members of the MFS (TABLE 1). As more efflux pumps are identified and characterized, it can be anticipated, however, that pumps of other families might be found to confer clinically relevant antibiotic resistance. In this section, examples of clinically relevant Gram-negative bacteria that express MDR efflux pumps are discussed, including *P. aeruginosa*, *E. coli*, *Salmonella enterica* serovar Typhimurium (*S. typhimurium*), *Campylobacter jejuni* and *Neisseria gonorrhoeae*.

In humans, *P. aeruginosa* can cause various opportunistic infections, including skin and soft-tissue infections in patients with burns, and pneumonia in individuals with cystic fibrosis. The *P. aeruginosa* RND-family pumps MexAB–OprM, MexXY–OprM, MexCD–OprJ and MexEF–OprN can each export several types of antibiotic,

Table 1 | Antibiotic susceptibility of strains lacking or overexpressing multidrug-resistance efflux pumps

Bacterial strain or genotype*	Efflux-pump protein or system affected	Minimum inhibitory concentration of antibiotic ($\mu\text{g per ml}$) [†]				References
		Ciprofloxacin	Chloramphenicol	Tetracycline	β -Lactam antibiotic	
<i>Pseudomonas aeruginosa</i>						
PAO1	–	64	64	16	64 [§]	12,13,23
<i>mexAB–OprM::kan</i>	MexAB–OprM	0.03	2	2	0.5 [§]	12,13,23
<i>nalR</i> mutant	MexAB–OprM	256	256	64	256 [§]	12,13,23
<i>Escherichia coli</i>						
W3110	–	0.015	8	1	16	93
<i>tolC::kan</i>	TolC	0.0025	1	0.12	8	93
<i>acrAB::kan</i>	AcrB	0.0025	1	0.12	4	93
<i>Salmonella enterica</i> serovar Typhimurium						
SL1344	–	0.03	4	2	1	38
<i>tolC::kan</i>	TolC	0.015	1	0.5	0.25	68
<i>acrB::kan</i>	AcrB	0.015	0.5	1	0.25	38
<i>acrAB</i> overexpresser [¶]	AcrB	0.5	16	16	ND	38
<i>Campylobacter jejuni</i>						
11168	–	0.25	1	0.25	4	48
<i>cmeB::kan</i>	CmeB	0.06	0.5	0.06	0.06	48
<i>cmeR</i> mutant	CmeABC	8	16	8	32	48
<i>Neisseria gonorrhoeae</i>						
FA19	–	0.025	ND	0.25	0.015 [#]	51
<i>mtrR</i> mutant (<i>mtrCDE</i> overexpresser)	MtrCDE	0.025	ND	0.25	0.03 [#]	51
<i>mtrR</i> mutant (<i>mtrCDE</i> overexpresser) and <i>mtrD::kan</i>	MtrCDE	0.025	ND	0.25	0.015 [#]	51
<i>Staphylococcus aureus</i>						
SA1199 or RN4222	–	0.5	ND	0.12	0.5 [#] (0.25 ^{**})	94,95
Δ <i>norA</i>	NorA	0.25 ^{††}	ND	0.12	0.25 ^{**}	95
<i>norA</i> overexpresser [¶]	NorA	16	ND	ND	0.5 [#]	94
<i>Streptococcus pneumoniae</i>						
R6	–	0.5	4	ND	ND	61
<i>pmrA::cat</i>	PmrA	0.5	2	ND	ND	61
<i>pmrA</i> overexpresser [¶]	PmrA	2	32	ND	ND	61

*For each species, the first row indicates the strain tested, and the second and subsequent rows show data for mutants derived from that strain (unless otherwise noted). [†]For ease of comparison, all minimum inhibitory concentrations were converted to the nearest doubling-dilution value. The recommended breakpoint concentrations that define antimicrobial susceptibility or resistance vary depending on the infecting bacterial species. For further details, refer to the web sites of the Clinical and Laboratory Standards Institute (United States), the British Society for Antimicrobial Chemotherapy, and the European Committee on Antimicrobial Susceptibility Testing (see Further information). [§]Carbenicillin. ^{||}Ampicillin. [#]These strains are not laboratory mutants; all other listed strains are. [#]Penicillin. ^{**}Cephalothin. ^{††}Norfloxacin (which, similar to ciprofloxacin, is a fluoroquinolone). *cat*, chloramphenicol O-acetyltransferase-resistance cassette (in this case, disrupting the gene indicated); *cmeR*, regulates expression of the genes encoding the CmeABC efflux system; *kan*, kanamycin-resistance cassette (in this case, disrupting the gene indicated); *mtrR*, regulates expression of the genes encoding the MtrCDE efflux system; *nalR*, regulates expression of the genes encoding the MexAB–OprM efflux system; ND, not determined.

including chloramphenicol, fluoroquinolones and tetracycline, although not all of these Mex systems are constitutively expressed⁵. These four efflux pumps also export other substances, including acriflavine, ethidium bromide, SDS, triclosan and some organic solvents²². MexAB–OprM and MexXY–OprM are expressed at basal levels by wild-type cells, whereas the expression of MexCD–OprJ and MexEF–OprN is

induced by some of the substrates of these efflux pumps. The drug substrate profile of these four systems does differ, however. For example, the MexXY–OprM system exports aminoglycosides, whereas the MexAB–OprM system exports certain β -lactam antibiotics, including carbenicillin²³. Both MexAB–OprM and MexCD–OprJ export novobiocin and the third-generation cephalosporin cefsulodin. The list of antimicrobial agents that

are used to treat infection with *P. aeruginosa* and are substrates of Mex efflux pumps includes ciprofloxacin and (depending on the individual Mex pump) ceftazidime, gentamicin, imipenem, piperacillin–tazobactam, ticarcillin–clavulanate and tobramycin. Overexpression of Mex efflux pumps in association with clinically relevant levels of MDR has been shown for clinical isolates of *P. aeruginosa*^{24–26}.

E. coli is a commensal organism that is found in the human gut; however, it can also be pathogenic and often causes urinary-tract infections. In addition, enteropathogenic *E. coli* (EPEC) and enterotoxigenic *E. coli* (ETEC) cause diarrhoea. Depending on the type of infection, treatment usually involves administration of co-trimoxazole, nitrofurantoin or a fluoroquinolone. A third-generation cephalosporin (for example, ceftriaxone) can be used for invasive or life-threatening infections. The drug substrate profile of *E. coli* AcrAB–TolC (which is from the RND family) includes the antibiotics chloramphenicol, fluoroquinolones, fusidic acid, lipophilic β -lactam antibiotics, nalidixic acid, novobiocin, rifampin and tetracycline, as well as acriflavine, ethidium bromide, bile salts, short-chain fatty acids, SDS, Triton X-100 and triclosan^{27–30}. Although there are other Acr efflux pumps in *E. coli*^{31–33}, only AcrAB–TolC has been found to be overexpressed by clinical isolates^{34–36}. It should be noted that overexpression of AcrAB–TolC alone does not confer clinical levels of resistance; however, when a mutation(s) in a topoisomerase gene (the product of which is a target for the bactericidal activity of fluoroquinolones) occurs in the same bacterium as increased efflux, such isolates are resistant to the fluoroquinolone ciprofloxacin^{34–37}.

S. typhimurium and *Salmonella enterica* serovar *Enteritidis* (*S. enteritidis*) typically cause gastroenteritis, and invasive salmonellosis occurs in 1–4% of human infections. When treatment with antimicrobial agents is required, the antibiotics that are commonly administered are fluoroquinolones (for example, ciprofloxacin) or the cephalosporin ceftriaxone. The AcrAB–TolC system of *S. typhimurium* is similar to that of *E. coli*, and its substrates include the antibiotics chloramphenicol, quinolones and tetracycline, as well as acriflavine, ethidium bromide, bile salts, SDS, Triton X-100, cetrime and triclosan^{38–40}. My research group and others have shown that overexpression of AcrB has been associated with MDR in laboratory mutants of *S. typhimurium*, as well as in human and veterinary isolates of *S. typhimurium*^{39,41,42}. For such strains, the MICs of chloramphenicol, nalidixic acid and tetracycline were above the recommended breakpoint concentrations, and for ciprofloxacin, the MIC was at a level associated with failures in therapy^{43–45} (TABLE 1).

The most common cause of bacterial gastroenteritis in developed countries is infection with *C. jejuni*. The substrates of the *C. jejuni* RND-family efflux pump

CmeABC include ciprofloxacin and erythromycin, both of which are common first-line agents if antimicrobial treatment of human *C. jejuni* infection is warranted^{46,47}. My research group and others have shown that overexpression of CmeB confers resistance to ciprofloxacin, erythromycin, ampicillin, chloramphenicol and tetracycline, as well as decreased susceptibility to bile salts, SDS, Triton X-100 and triclosan^{48–50}. Both human and veterinary isolates of *C. jejuni* have been found to overexpress *cmeB*, to accumulate low concentrations of ciprofloxacin and to show MDR⁴⁸.

N. gonorrhoeae is the causative agent of gonorrhoea, and the substrates of its RND-family efflux pump MtrCDE include the β -lactam antibiotic penicillin⁵¹. Increased expression of this efflux pump alone, however, is insufficient to confer clinically relevant levels of antibiotic resistance. In penicillin-resistant strains of *N. gonorrhoeae*, overexpression of MtrCDE occurs concomitantly with other mechanisms of resistance to β -lactam antibiotics, including downregulation of the outer-membrane pore-forming proteins (such as that encoded by *penB*) and alterations in low-affinity penicillin-binding proteins⁵¹. However, clinically significant levels of resistance to penicillin (MIC >1 μ g per ml) depend on MtrCDE, because inactivation of this efflux pump gives rise to a strain that requires exposure to only 0.25 μ g of penicillin per ml for growth inhibition, despite the presence of other resistance mechanisms⁵¹. The strain of *N. gonorrhoeae* that has been extensively studied was originally isolated in Durham (North Carolina, United States), and the demise of penicillin therapy in the United States has been attributed to the clonal spread of this strain (W. Shafer, personal communication).

Clinically relevant Gram-positive bacteria and MDR efflux pumps.

Examples of clinically relevant Gram-positive bacteria that express MDR efflux pumps are *Staphylococcus aureus* and *Streptococcus pneumoniae*. *S. aureus* usually causes skin and soft-tissue infections. Overexpression of the *S. aureus* MFS efflux pump NorA confers resistance to chloramphenicol and fluoroquinolones, as well as to dyes and biocides such as cetrime^{52–56}. Of the antimicrobial agents that are used to treat methicillin (formerly methicillin)-susceptible *S. aureus* and methicillin-resistant *S. aureus* (MRSA), only the fluoroquinolone ciprofloxacin is a substrate of NorA; the MICs of nafcillin, vancomycin and linezolid are unaffected by

overexpression of NorA (G. Kaatz, personal communication). Several studies have found that clinical isolates that are resistant to the fluoroquinolone norfloxacin overexpress *norA*^{55,57,58}, whereas other studies have found little or no relationship between overexpression of *norA* and resistance to fluoroquinolones^{59,60}. These conflicting data might result from lack of transport by NorA of the particular fluoroquinolones that were investigated. Other efflux-pump genes are also present in the *S. aureus* genome, but the clinical relevance of these transporters is unknown.

S. pneumoniae causes bronchitis, pneumonia and meningitis, and such infections can be fatal in the young and the elderly. Depending on the country in which the patient resides and the severity of the infection, treatment typically involves administration of a β -lactam antibiotic, fluoroquinolone or macrolide. The *S. pneumoniae* MFS efflux pump PmrA exports the fluoroquinolones ciprofloxacin and norfloxacin, as well as the dyes acriflavine and ethidium bromide⁶¹. My research group determined the prevalence of *pmrA* overexpression in clinical isolates of *S. pneumoniae* from several geographically distinct areas, and we found that *pmrA* overexpression was not exclusively associated with norfloxacin-resistant *S. pneumoniae* and could be found in norfloxacin-susceptible isolates⁶². Recently, overexpression of an ABC-superfamily transporter has been associated with ciprofloxacin resistance⁶³, but whether this occurs in clinical isolates has yet to be determined. In addition, the MFS efflux pump Mef and the ABC-superfamily transporter Mel of *S. pneumoniae* each confer resistance to macrolides, an increasing problem worldwide⁶⁴. The *mef* and *mel* genes are located on a conjugative transposon-related element in the pneumococcal genome, indicating that, although they were not associated with a chromosomal location initially, these genes have been acquired and are now passed to daughter cells.

MDR efflux pumps and pathogenicity

Some bacterial efflux pumps export not only antibiotics and other substances, such as dyes and detergents, but also host-derived antimicrobial agents. This finding has led to the suggestion that the physiological role of these systems is evasion of such naturally produced molecules, thereby allowing the bacterium to survive in its ecological niche. In addition, there is accumulating evidence that efflux pumps that confer clinically

Table 2 | Multidrug-resistance efflux pumps that are involved in the survival of bacteria in their ecological niche

Efflux pump	Class of pump	Bacterial species	Properties associated with pump expression	Host-derived substrates of pump
AcrAB–TolC	RND	<i>Salmonella enterica</i> serovar Typhimurium	Adherence to, and invasion of, host cells ⁶⁸ ; colonization of, and persistence in, chickens ⁶⁸	Bile ^{66,68,88,89}
AcrAB–TolC	RND	<i>Escherichia coli</i>	ND	Bile ⁹ and mammalian steroids ⁹¹
AcrAB–TolC	RND	<i>Erwinia amylovora</i>	Colonization of plants ⁷¹	Plant isoflavonoids, isoprenoids and alkaloids ⁷¹
MexAB–OprM	RND	<i>Pseudomonas aeruginosa</i>	Invasion of host cells ⁶⁹ ; infection of rats and BALB/c mice ⁶⁹	ND
CmeABC	RND	<i>Campylobacter jejuni</i>	Colonization of, and persistence in, chickens ⁵⁰	Bile ^{48–50}
MtrCDE	RND	<i>Neisseria gonorrhoeae</i>	Colonization of genito-urinary tract of female mice ⁷⁰	Gonadal hormones ⁷⁰ , and porcine protegrin-1 and human LL-37 (REF. 92)

ND, not determined; RND, resistance nodulation division.

relevant antibiotic resistance are important for the pathogenicity of the bacterium. It is also possible that, in some species, efflux pumps that export antimicrobial agents also export virulence determinants, such as adhesins, toxins or other proteins that are important for the colonization and infection of human and animal cells. So far, evidence of a role for these pumps in pathogenicity has been obtained for members of the RND and MATE families. In this section, I discuss these additional activities of efflux pumps, focusing on transporters that are known to be involved in antimicrobial resistance. Transporters that are involved in pathogenicity but not antimicrobial resistance are not discussed.

Several studies have shown that lack of efflux-pump expression by a Gram-negative bacterium has a deleterious effect on the ability of the bacterium to be pathogenic in animal models (TABLE 2). My research group and others have found that the AcrAB–TolC efflux pump is important for the ability of *S. typhimurium* to infect BALB/c mice^{40,65,66} and to colonize chickens^{67,68}. Although most studies have focused on *S. typhimurium* and the AcrAB–TolC system, several studies have also indicated that RND-family efflux pumps have a role in the pathogenicity of other bacterial species, including plant pathogens. For example, *P. aeruginosa* lacking the RND-family efflux pump MexAB–OprM could not kill mice that were deficient in leukocytes, whereas the parent strain of this mutant *P. aeruginosa* caused a fatal infection⁶⁹. In addition, Lin and colleagues⁵⁰ showed that the *C. jejuni* RND-family efflux pump CmeABC is essential for the colonization of 1-day-old chickens. And Jerse and co-workers⁷⁰ showed that the *N. gonorrhoeae* RND-family efflux pump MtrCDE is important for infection of the genito-urinary tract of female mice.

Immunity to host defences. Bacterial phytopathogens have MDR efflux pumps belonging to the same families as those present in bacteria that infect humans and animals. Burse and colleagues⁷¹ have shown that the genome of *Erwinia amylovora* (which causes the disease fire-blight in apple and pear trees) contains homologues of *E. coli* *acrA* and *acrB*. Disruption of *E. amylovora* *acrB* was found to cause a significant impairment in the survival of *E. amylovora* in apple plantlets and a marked reduction in its ability to cause fire-blight symptoms. This led Burse *et al.*⁷¹ to propose that the *E. amylovora* RND-family efflux pump AcrAB has an important role in resistance against plant-derived antibacterial toxins — such as flavonoids, isoprenoids and alkaloids (which are secondary metabolites that are produced by plants and are involved in their protection against infection) — and therefore in the colonization of the plant. They then showed that leaf-tissue extracts from apple plantlets contained antimicrobial secondary metabolites to which the *acrB*-disrupted mutant was susceptible but to which the parent strain was resistant⁷¹. Furthermore, the expression of *acrAB* was shown to increase twofold during growth of the bacteria in apple tissue, supporting the hypothesis that AcrAB has a role in the colonization of the host plant⁷¹. The *E. amylovora* MATE-family efflux pump NorM has also been implicated in facilitating colonization, because it confers tolerance to toxins that are produced by co-colonizing epiphytic bacteria⁷².

Export of virulence determinants.

For certain bacterial species, such as *P. aeruginosa* and *C. jejuni*, the outer-membrane proteins are usually specific to each efflux pump. By contrast, TolC is the outer-membrane protein for

many MDR efflux pumps in *E. coli* and other Enterobacteriaceae, including *S. typhimurium*⁹. TolC is promiscuous and can interact with a variety of transporters, only some of which transport antibiotics. Therefore, any evidence that MDR efflux pumps have a role in pathogenicity needs to be distinguished from the participation of their constituent outer-membrane proteins in other functions, such as in type I protein secretion. Bacteria use many mechanisms to establish infection in the host and to evade host defences, including the production of pore-forming toxins and degradative enzymes. Type I protein secretion involves the export of these proteins from the cell by ABC-superfamily transporters that are not involved in MDR^{73,74}. For example, *E. coli* TolC is essential for the secretion of haemolysin⁷⁵ and colicin V (REF. 76); similarly, secretion of RTX toxin (a member of the repeats-in-toxin family) by *Vibrio cholerae* requires TolC^{77,78}.

P. aeruginosa lacking efflux pumps was examined by Hirakata and colleagues⁶⁹ for the ability to invade epithelial cells (Madin–Darby canine kidney (MDCK) cells) growing as a monolayer. Hirakata *et al.*⁶⁹ found that mutants lacking MexAB–OprM could not invade these cells and that invasion could be restored by complementation with MexAB–OprM or by supplementation with culture supernatant obtained from MDCK cells infected with wild-type *P. aeruginosa*. These findings indicate that MexAB–OprM exports virulence determinants that allow *P. aeruginosa* to be invasive and cause infection. Further support for this hypothesis comes from an experimental model of *P. aeruginosa*-induced acute pneumonia in rats, in which, even in the absence of antibiotic treatment, isolates of *P. aeruginosa* that overexpress Mex efflux pumps were identified⁷⁹.

Recently, my research group showed that, compared with wild-type *S. typhimurium*, *S. typhimurium* in which *acrB* (of the AcrAB–TolC efflux pump) had been disrupted was less able to adhere to, invade and survive in mouse macrophages and less able to invade and survive in human embryonic intestinal cells⁶⁸. Furthermore, *S. typhimurium* in which *tolC* had been disrupted could not adhere to, invade or survive in either cell type. In addition to type I protein secretion, the ability of bacteria to cause an infection is associated with type III secretion systems, which are expressed by many pathogenic bacteria (for example, *S. typhimurium*) and export virulence factors (proteins) from the bacterium directly into the host cell, through a syringe-like structure⁸⁰. Using electron microscopy, it has been observed that, during infection with *S. typhimurium*, the actin cytoskeleton of eukaryotic cells is rearranged such that these cells appear to have ‘ruffles’⁸¹, and this reorganization is mediated by effector proteins delivered by the type III secretion system. The bacteria are located close to the membrane ruffles during infection, thereby promoting adherence and invasion by the bacteria. The mutants of *S. typhimurium* in which either *acrB* or *tolC* had been disrupted gave rise to fewer membrane ruffles than did wild-type *S. typhimurium*, suggesting that expression of these genes or the encoded proteins affects type III secretion. However, it is not yet known how the AcrAB–TolC system interacts with either the known mechanisms of bacterial pathogenicity or with the host cell.

Hirakata *et al.*⁶⁹ have noted that, although components of RND-family efflux pumps (but not those that export antibiotics) associate with proteins that constitute the type I secretion system in other bacteria (such as *E. coli*), the effector proteins that are secreted by type III secretion systems are too large to be exported by MexAB–OprM. So, as-yet-unidentified factors that might be exported by MexAB–OprM must be small enough to be accommodated by this MDR efflux system; this might also be the case for AcrAB–TolC. Hirakata and colleagues also proposed that contact of bacteria with epithelial cells might trigger the efflux of other virulence determinants. These virulence determinants have yet to be identified, but one is thought to be a small peptide (R. E. W. Hancock, personal communication).

Another situation in which the export of virulence determinants might be involved in determining pathogenicity involves quorum sensing (a bacterial cell–cell communication

system), although in this case, overexpression of efflux pumps might be counter-productive for the bacteria. For *P. aeruginosa*, the acylated homoserine lactones that are involved in quorum sensing⁸² are substrates of the MexAB–OprM system^{22,83}. So, the reduced virulence that has been shown for *P. aeruginosa* overexpressing Mex efflux pumps⁸⁴ might result from an increase in efflux of quorum-sensing signals, thereby reducing the expression of virulence determinants that are regulated by quorum-sensing molecules. This idea is supported by recent studies of *E. coli* showing that strains that overexpress the efflux-pump proteins AcrB and NorE grow to lower cell densities at stationary phase than do wild-type cells, and mutants in which *acrB* or *norE* has been disrupted grow to higher cell densities⁸⁵.

The findings discussed in this section therefore indicate that the expression of MDR efflux pumps might be important for pathogenicity; however, it should be noted that, as mentioned, overexpression of these pumps might be counter-productive. Another example of this is provided by Sanchez and colleagues⁸⁴, who showed that *P. aeruginosa* overexpressing the MexAB–OprM system or the MexCD–OprJ system is less able to survive in water or in the nematode (*Caenorhabditis elegans*) model of infection. In addition, overexpression of the MexCD–OprJ system or the MexEF–OprN system (but not the MexAB–OprM system or the MexXY–OprM system) by *P. aeruginosa* was recently shown to be associated with a reduction in the expression of genes that encode components of the type III secretion system and was proposed to give rise to a concomitant lack of fitness⁸⁶. This was associated with lack of expression of *exsA*, which encodes the master regulator of type III secretion in *P. aeruginosa*, but the connection between *exsA* and Mex efflux-pump expression is unknown.

Host-derived substrates

Before, and in parallel with, investigations into the role of efflux pumps in pathogenicity, studies to identify the natural substrates of efflux pumps were underway. In this section, I summarize our current knowledge of the natural substrates of RND-family MDR efflux pumps.

At first, many of the bacteria under investigation were Enterobacteriaceae, and initial work focused on *E. coli*. The natural environment of enteric bacteria (the gastrointestinal tract of humans and animals) contains bile salts and fatty acids. Ma and colleagues⁸⁷ showed that *E. coli* that lack *acrAB* are

hypersusceptible to these agents, as are other species of enteric bacteria that lack homologues of AcrAB. In addition, the authors found that exposure of *E. coli* to 5 mM *n*-decanoate (a ten-carbon-atom fatty acid) results in an increase in the expression of *acrAB*. Together, these findings led them to propose that AcrAB exports these agents, thereby allowing enteric bacteria to survive in the presence of these naturally occurring, hydrophobic inhibitors. Although Ma *et al.*⁸⁷ did not report on the role of TolC in resistance to bile, Bina and Mekalanos⁷⁷ have shown that TolC is required for the resistance of *V. cholerae* to bile. In addition, the *C. jejuni* efflux pump CmeABC has been shown to confer resistance to bile^{47,48}. Lin and colleagues⁵⁰ have also shown that this efflux pump is essential for the growth and survival of *C. jejuni* in chicken intestinal extracts, and their report indicates that, through mediating resistance to bile salts in the intestinal tract, CmeABC allows *C. jejuni* to colonize chickens successfully. Similarly, *S. typhimurium* AcrB and/or TolC have also been shown to be essential for resistance to bile^{66,68,88,89}.

The RND-family efflux pump AcrAB of the phytopathogen *E. amylovora* has been shown to be similarly important. Burse *et al.*⁷¹ found that *E. amylovora* lacking *acrB* is more susceptible to host-defence compounds produced by apple plants, particularly to various isoflavonoids and phytoalexins.

It has been suggested that certain efflux pumps are often poorly expressed *in vitro* and that an inducer of expression is only present when the bacterium is growing in its natural environment or host. However, only a few of these natural inducers have been identified: bile salts and fatty acids for enteric bacteria (as discussed earlier); and Triton X-100 (as an example of a hydrophobic agent), which induces expression of the *mtrCDE* locus of *N. gonorrhoeae*, as shown by Shafer and colleagues⁹⁰.

In addition, subsequent studies⁷⁰ by Shafer’s research group indicate that this upregulation of expression of *mtrCDE* is important: not only was the MtrCDE system found to be important for the survival of gonococci in the genito-urinary tract of female mice but also an intact MtrCDE system was found to be required for gonococci to survive in mice that secrete gonadal hormones. These data indicate that this RND-family pump might export such hormones, and in support of this hypothesis, Elkins and Mullis⁹¹ have recently shown that mammalian steroids (such as oestradiol, progesterone and cortisol) are substrates

of another RND-family efflux pump, AcrAB-TolC of *E. coli*.

Gonococci with an intact MtrCDE efflux pump have also been shown, by Shafer *et al.*⁹², to resist endogenous antimicrobial peptides such as porcine protegrin-1 and human LL-37 (also known as cathelicidin antimicrobial peptide), indicating that these molecules are substrates of the MtrCDE efflux pump. Furthermore, Shafer and colleagues⁹² postulated that LL-37 was likely to be present at the mucosal surface of the genito-urinary tract during gonococcal infection and that the MtrCDE efflux pump would therefore allow gonococci to survive this host-defence mechanism. This led to the suggestion that the substrate profile of the MtrCDE efflux pump has evolved to allow *N. gonorrhoeae* to resist not only the antibacterial effects of fatty acids and bile salts present at the mucosal surface of the rectum but also those of the endogenous antibacterial peptides found in the genito-urinary mucosae⁹².

Taking into account the available data regarding the various efflux pumps in bacteria that cause diverse infections in humans, animals and plants, it is clear that these systems have a fundamental role in allowing bacteria to survive in their ecological niche.

Concluding remarks

It is increasingly evident that, for certain bacterial species, MDR efflux pumps confer clinically relevant resistance to antibiotics that are used to treat human disease. Owing to the broad range of substrates of MDR efflux pumps, bacteria that overexpress these pumps can be selected for by numerous agents. These include not only antibiotics but also agents that are increasingly used in the home, on farms and in food-processing plants. Examples include triclosan, which is a biocide present in many household items such as soaps and hand-washing lotions, and nonoxynol-9, which is widely available as a spermicide and can select mutants that overexpress *mtrCDE* in gonococcal populations⁹¹. Such widespread use of non-antimicrobial substrates of efflux pumps (such as triclosan and nonoxynol-9) could inadvertently lead to the selection of mutants overexpressing MDR efflux pumps.

With the increasing evidence that MDR efflux pumps have a role in bacterial pathogenicity, research is urgently required to elaborate this role and to investigate whether mutants that overexpress MDR efflux pumps have increased virulence. This knowledge is important for the development of MDR

efflux-pump inhibitors, which is an ongoing area of drug development; however, owing to the demise of antibacterial drug development by many of the major pharmaceutical companies, such research is mostly carried out by academic research laboratories or smaller biotechnology companies. Our current understanding of MDR efflux pumps indicates that such inhibitors would be invaluable tools to help clear bacterial infection because of their dual function: restoration of the activity of agents to which efflux pumps confer resistance and reduction in the ability of bacteria to colonize their host or even to cause infection.

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- Borges-Walmsley, M. I., McKeegan, K. S. & Walmsley, A. R. Structure and function of efflux pumps that confer resistance to drugs. *Biochem. J.* **376**, 313–338 (2003).
- Paulsen, I. T. Multidrug efflux pumps and resistance: regulation and evolution. *Curr. Opin. Microbiol.* **6**, 446–451 (2003).
- Li, X. & Nikaido, H. Efflux mediated drug resistance in bacteria. *Drugs* **64**, 159–204 (2004).
- Poole, K. Efflux mediated multi-resistance in Gram-negative bacteria. *Clin. Microbiol. Infect.* **10**, 12–26 (2004).
- Poole, K. Efflux mediated antimicrobial resistance. *J. Antimicrob. Chemother.* **56**, 20–51 (2005).
- Hooper, D. C. Efflux pumps and nosocomial antibiotic resistance: a primer for hospital epidemiologists. *Clin. Infect. Dis.* **40**, 1811–1817 (2005).
- Piddock, L. J. V. Clinically relevant bacterial chromosomally encoded multi-drug resistance efflux pumps. *Clin. Microbiol. Rev.* **19**, 382–402 (2006).
- Ramos, J. L. *et al.* The TetR family of transcriptional repressors. *Microbiol. Mol. Biol. Rev.* **69**, 326–356 (2005).
- Koronakis, V., Eswaran, J. & Hughes, C. Structure and function of TolC: the bacterial exit duct for proteins and drugs. *Annu. Rev. Biochem.* **73**, 467–489 (2004).
- Aires, J. R. & Nikaido, H. Aminoglycosides are captured from both periplasm and cytoplasm by the AcrD multidrug efflux transporter of *Escherichia coli*. *J. Bacteriol.* **187**, 1923–1929 (2005).
- Eswaran, J., Koronakis, E., Higgins, M. K., Hughes, C. & Koronakis, V. Three's company: component structures bring a closer view of tripartite drug efflux pumps. *Curr. Opin. Struct. Biol.* **14**, 741–747 (2004).
- Li, X. Z., Livermore, D. M. & Nikaido, H. Role of efflux pump(s) in intrinsic resistance of *Pseudomonas aeruginosa*: resistance to tetracycline, chloramphenicol, and norfloxacin. *Antimicrob. Agents Chemother.* **38**, 1732–1741 (1994).
- Poole, K., Krebs, K., McNally, C. & Neshat, S. Multiple antibiotic resistance in *Pseudomonas aeruginosa*: evidence for involvement of an efflux operon. *J. Bacteriol.* **175**, 7363–7372 (1993).
- Livermore, D. L. Linezolid *in vitro*: mechanism and antibacterial spectrum. *J. Antimicrob. Chemother.* **51**, 9–16 (2003).
- Johnson, K. W., Lofland, D. & Moser, H. E. PDF inhibitors: an emerging class of antibacterial drugs. *Curr. Drug Targets Infect. Disord.* **5**, 39–52 (2005).
- Buyse, J. M. *et al.* Mutation of the AcrAB antibiotic efflux pump in *Escherichia coli* confers susceptibility to oxazolidinone antibiotics. Abstract C-42. 36th Interscience Conference on Antimicrobial Agents and Chemotherapy (New Orleans, Louisiana, USA, 15–18 Sep 1996).
- Chollet, R., Chevalier, J., Bryskier, A. & Pages, J. M. The AcrAB-TolC pump is involved in macrolide resistance but not in tetracycline efflux in *Enterobacter aerogenes* and *Escherichia coli*. *Antimicrob. Agents Chemother.* **48**, 3621–3624 (2004).
- Peric, M., Bozdogan, B., Jacobs, M. R. & Appelbaum, P. C. Effects of an efflux mechanism and ribosomal mutations on macrolide susceptibility of *Haemophilus influenzae* clinical isolates. *Antimicrob. Agents Chemother.* **47**, 1017–1022 (2003).
- Sanchez, L., Pan, W., Vinas, M. & Nikaido, H. The *acrAB* homolog of *Haemophilus influenzae* codes for a functional multidrug efflux pump. *J. Bacteriol.* **179**, 6855–6857 (1997).
- Dean, C. R., Visalli, M. A., Projan, S. J., Sum, P. E. & Bradford, P. A. Efflux-mediated resistance to tigecycline (GAR-936) in *Pseudomonas aeruginosa* PAO1. *Antimicrob. Agents Chemother.* **47**, 972–978 (2003).
- Visalli, M. A., Murphy, E., Projan, S. J. & Bradford, P. A. AcrAB multidrug efflux pump is associated with reduced levels of susceptibility to tigecycline (GAR-936) in *Proteus mirabilis*. *Antimicrob. Agents Chemother.* **47**, 665–669 (2003).
- Poole, K. & Srikumar, R. Multidrug efflux in *Pseudomonas aeruginosa*: components, mechanisms and clinical significance. *Curr. Top. Med. Chem.* **1**, 59–71 (2001).
- Matsuda, N. *et al.* Substrate specificities of MexAB-OprM, MexCD-OprJ, and MexXY-OprM efflux pumps in *Pseudomonas aeruginosa*. *Antimicrob. Agents Chemother.* **44**, 3522–3527 (2000).
- Ziha-Zarif, I., Llanes, C., Köhler, T., Pechère, J.-C. & Plésiat, P. *In vivo* emergence of multidrug-resistant mutants of *Pseudomonas aeruginosa* overexpressing the active efflux system MexA–MexB–OprM. *Antimicrob. Agents Chemother.* **43**, 287–291 (1999).
- Oh, H., Stenhoff, J., Jalal, S. & Wretling, B. Role of efflux pumps and mutations in genes for topoisomerases II and IV in fluoroquinolone-resistant *Pseudomonas aeruginosa* strains. *Microb. Drug Resist.* **9**, 323–328 (2003).
- Hocquet, D., Bertand, X., Köhler, T., Talon, D. & Plésiat, P. Genetic and phenotypic variations of a resistant *Pseudomonas aeruginosa* epidemic clone. *Antimicrob. Agents Chemother.* **47**, 1887–1894 (2003).
- 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.* **178**, 306–308 (1996).
- Nikaido, H. Multiple antibiotic resistance and efflux. *Curr. Opin. Microbiol.* **1**, 515–523 (1998).
- Fernandes, P., Ferreira, B. S. & Cabral, J. M. Solvent tolerance in bacteria: role of efflux pumps and cross-resistance with antibiotics. *Int. J. Antimicrob. Agents* **22**, 211–216 (2003).
- White, D. G., Goldman, J. D., Demple, B. & Levy, S. B. Role of the *acrAB* locus in organic solvent tolerance mediated by expression of *marA*, *soxS*, or *robA* in *Escherichia coli*. *J. Bacteriol.* **179**, 6122–6126 (1997).
- Rosenberg, E. Y., Ma, D. & Nikaido, H. AcrD of *Escherichia coli* is an aminoglycoside efflux pump. *J. Bacteriol.* **182**, 1754–1756 (2000).
- Zgurskaya, H. I. & Nikaido, H. Multidrug resistance mechanisms: drug efflux across two membranes. *Mol. Microbiol.* **37**, 219–225 (2000).
- Nishino, K. & Yamaguchi, A. Analysis of the complete library of putative drug transporter genes in *Escherichia coli*. *J. Bacteriol.* **183**, 5803–5812 (2001).
- Mazzariol, A., Tokue, Y., Kanegawa, T. M., Cornaglia, G. & Nikaido, H. High level fluoroquinolone resistant clinical isolates of *Escherichia coli* over produce multidrug efflux protein AcrA. *Antimicrob. Agents Chemother.* **44**, 3441–3443 (2000); erratum in **45**, 647 (2001).
- Oethinger, M., Kern, W. V., Jellen-Ritter, A. S., McMurry, L. M. & Levy, S. B. Ineffectiveness of topoisomerase mutations in mediating clinically significant fluoroquinolone resistance in *Escherichia coli* in the absence of the AcrAB efflux pump. *Antimicrob. Agents Chemother.* **44**, 10–13 (2000).
- Webber, M. A. & Piddock, L. J. V. Absence of mutations in *marAB* or *soxRS* in *acrB*-overexpressing fluoroquinolone-resistant clinical and veterinary isolates of *Escherichia coli*. *Antimicrob. Agents Chemother.* **45**, 1550–1552 (2001).
- Everett, M. J., Jin, Y.-F., Ricci, V. & Piddock, L. J. V. Contribution of individual mechanisms to fluoroquinolone resistance in 36 *Escherichia coli* isolated from humans and animals. *Antimicrob. Agents Chemother.* **40**, 2380–2386 (1996).
- Eaves, D. J., Ricci, V. & Piddock, L. J. Expression of *acrB*, *acrF*, *acrD*, *marA*, and *soxS* in *Salmonella*

- enterica* serovar Typhimurium: role in multiple antibiotic resistance. *Antimicrob. Agents Chemother.* **48**, 1145–1150 (2004).
39. Baucheron, S. *et al.* AcrAB–TolC directs efflux-mediated multidrug resistance in *Salmonella enterica* serovar Typhimurium DT104. *Antimicrob. Agents Chemother.* **48**, 3729–3735 (2004).
40. Nishino, K., Latifi, T. & Groisman, E. A. Virulence and drug resistance roles of multidrug efflux systems of *Salmonella enterica* serovar Typhimurium. *Mol. Microbiol.* **59**, 126–141 (2006).
41. Giraud, E., Cloeckaert, A., Kerboeuf, D. & Chaslus-Dancla, E. Evidence for active efflux as the primary mechanism of resistance to ciprofloxacin in *Salmonella enterica* serovar Typhimurium. *Antimicrob. Agents Chemother.* **44**, 1223–1228 (2000).
42. Piddock, L. V. J., White, D. G., Gensberg, K., Pumbwe, L. & Griggs, D. J. Evidence for an efflux pump mediating multiple antibiotic resistance in *Salmonella enterica* serovar Typhimurium. *Antimicrob. Agents Chemother.* **44**, 3118–3121 (2000).
43. Piddock, L. V. J., Griggs, D. J., Hall, M. C. & Jin, Y. F. Ciprofloxacin resistance in clinical isolates of *Salmonella typhimurium* obtained from two patients. *Antimicrob. Agents Chemother.* **37**, 662–666 (1993).
44. Wain, J. *et al.* Quinolone-resistant *Salmonella typhi* in Viet Nam: basis of resistance and clinical response to treatment. *Clin. Infect. Dis.* **25**, 1404–1410 (1997).
45. Molbak, K. *et al.* An outbreak of multidrug-resistant, quinolone-resistant, *Salmonella enterica* serotype Typhimurium DT104. *N. Engl. J. Med.* **341**, 1420–1425 (1999).
46. Pumbwe, L. & Piddock, L. J. V. Identification and molecular characterisation of CmeB, a *Campylobacter jejuni* multidrug efflux pump. *FEMS Microbiol. Lett.* **206**, 185–189 (2002).
47. Lin, J., Overbye, L. M. & Zhang, Q. CmeABC functions as a multidrug efflux system in *Campylobacter jejuni*. *Antimicrob. Agents Chemother.* **46**, 2124–2131 (2002).
48. Pumbwe, L., Randall, L. P., Woodward, M. J. & Piddock, L. J. V. Expression of the efflux pump genes *cmeB*, *cmeF* and the porin gene *porA* in multiply antibiotic-resistant *Campylobacter* spp. *J. Antimicrob. Chemother.* **54**, 341–347 (2004).
49. Pumbwe, L., Randall, L. P., Woodward, M. J. & Piddock, L. J. V. Evidence for multiple antibiotic resistance in *Campylobacter jejuni* not mediated by CmeB or CmeF. *Antimicrob. Agents Chemother.* **49**, 1289–1293 (2005).
50. Lin, J., Sahin, O., Michel, L. O. & Zhang, Q. Critical role of multi-drug efflux pump CmeABC in bile resistance and *in vivo* colonisation of *Campylobacter jejuni*. *Infect. Immun.* **71**, 4250–4259 (2003).
51. Veal, W. L., Nicholas, R. A. & Shafer, W. M. 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.* **184**, 5619–5624 (2002).
52. Neyfakh, A. A. The multidrug efflux transporter of *Bacillus subtilis* is a structural and functional homolog of the *Staphylococcus* NorA protein. *Antimicrob. Agents Chemother.* **36**, 484–485 (1992).
53. Yoshida, H., Bogaki, M., Nakamura, S., Ubukata, K. & Konno, M. Nucleotide sequence and characterization of the *Staphylococcus aureus* *norA* gene, which confers resistance to quinolones. *J. Bacteriol.* **172**, 6942–6949 (1990).
54. Neyfakh, A. A., Borsch, C. M. & Kaatz, G. W. Fluoroquinolone resistance protein NorA of *Staphylococcus aureus* is a multidrug efflux transporter. *Antimicrob. Agents Chemother.* **37**, 128–129 (1993).
55. Kaatz, G. W., Seo, S. M. & Ruble, C. A. Efflux-mediated fluoroquinolone resistance in *Staphylococcus aureus*. *Antimicrob. Agents Chemother.* **37**, 1086–1094 (1993).
56. Ng, E. Y., Truckis, M. & Hooper, D. C. Quinolone resistance mediated by *norA*: physiologic characterization and relationship to *flqB*, a quinolone resistance locus on the *Staphylococcus aureus* chromosome. *Antimicrob. Agents Chemother.* **38**, 1345–1355 (1994).
57. Jones, M. E., Boenink, N. M., Verhoef, J., Kohrer, K. & Schmitz, F.-J. Multiple mutations conferring ciprofloxacin resistance in *Staphylococcus aureus* demonstrate the long term stability in an antibiotic-free environment. *J. Antimicrob. Chemother.* **45**, 353–356 (2000).
58. Noguchi, N., Okada, H., Narui, K. & Sasatsu, M. Comparison of the nucleotide sequence and expression of *norA* genes and microbial susceptibility in 21 strains of *Staphylococcus aureus*. *Microb. Drug Resist.* **10**, 197–203 (2004).
59. Schmitz, F.-J. *et al.* Relationship between mutations in the coding and promoter regions of the *norA* genes in 42 unrelated clinical isolates of *Staphylococcus aureus* and the MICs of norfloxacin for these strains. *J. Antimicrob. Chemother.* **42**, 561–563 (1998).
60. Oizumi, N. *et al.* Relationship between mutations in the DNA gyrase and topoisomerase IV genes and nadifloxacin resistance in clinically isolated quinolone-resistant *Staphylococcus aureus*. *J. Infect. Chemother.* **7**, 191–194 (2001).
61. Gill, M. J., Brenwald, N. P. & Wise, R. Identification of an efflux pump gene, *pmrA*, associated with fluoroquinolone resistance in *Streptococcus pneumoniae*. *Antimicrob. Agents Chemother.* **43**, 187–189 (1999).
62. Piddock, L. J. V., Johnson, M. M., Simjee, S. & Pumbwe, L. Expression of efflux pump gene *pmrA* in fluoroquinolone-resistant and -susceptible clinical isolates of *Streptococcus pneumoniae*. *Antimicrob. Agents Chemother.* **46**, 808–812 (2002).
63. Marrer, E. *et al.* Involvement of the putative ATP-dependent efflux proteins PatA and PatB in fluoroquinolone resistance of a multidrug-resistant mutant of *Streptococcus pneumoniae*. *Antimicrob. Agents Chemother.* **50**, 685–693 (2006).
64. Ambrose, K. D., Nisbet, R. & Stephens, D. S. Macrolide efflux in *Streptococcus pneumoniae* is mediated by a dual efflux pump (*mel* and *mef*) and is erythromycin inducible. *Antimicrob. Agents Chemother.* **49**, 4203–4209 (2005).
65. Stone, B. J. & Miller, V. L. *Salmonella enteritidis* has a homologue of *tolC* that is required for virulence in BALB/c mice. *Mol. Microbiol.* **17**, 701–712 (1995).
66. Lacroix, F. J. C. *et al.* *Salmonella typhimurium* *acrB*-like gene: identification and role in resistance to biliary salts and detergents and in murine infection. *FEMS Microbiol. Lett.* **135**, 161–167 (1996).
67. Baucheron, S., Mouline, C., Praud, K., Chaslus-Dancla, E. & Cloeckaert, A. TolC but not AcrB is essential for multidrug-resistant *Salmonella enterica* serotype Typhimurium colonization of chicks. *J. Antimicrob. Chemother.* **55**, 707–712 (2005).
68. Buckley, A. M. *et al.* The AcrAB–TolC efflux system of *Salmonella enterica* serovar Typhimurium plays a role in pathogenesis. *Cell. Microbiol.* **8**, 847–856 (2006).
69. Hirakata, Y. *et al.* Multidrug efflux systems play an important role in the invasiveness of *Pseudomonas aeruginosa*. *J. Exp. Med.* **196**, 109–118 (2002).
70. Jerse, A. E. *et al.* A gonococcal efflux pump system enhances bacterial survival in a female mouse model of genital tract infection. *Infect. Immun.* **71**, 5576–5582 (2003).
71. Burse, A., Weingart, H. & Ullrich, M. S. The phytoalexin-inducible multidrug efflux pump AcrAB contributes to virulence in the fire blight pathogen, *Erwinia amylovora*. *Mol. Plant Microbe Interact.* **17**, 43–54 (2004).
72. Burse, A., Weingart, H. & Ullrich, M. S. NorM, an *Erwinia amylovora* multidrug efflux pump involved in *in vitro* competition with other epiphytic bacteria. *Appl. Environ. Microbiol.* **70**, 693–703 (2004).
73. Koronakis, V. & Hughes, C. Synthesis, maturation and export of the *E. coli* hemolysin. *Med. Microbiol. Immunol. (Berl.)* **185**, 65–71 (1996).
74. Binet, R., Letoffe, S., Ghigo, J. M., Delpeleire, P. & Wandersman, C. Protein secretion by Gram-negative bacterial ABC exporters — a review. *Gene* **192**, 7–11 (1997).
75. Bhakdi, S. *et al.* The hemolysin of *Escherichia coli*. *Eur. J. Epidemiol.* **4**, 135–143 (1988).
76. Gilson, L., Mahanty, H. K. & Koltter, R. Genetic analysis of an MDR-like export system: the secretion of colicin V. *EMBO J.* **9**, 3875–3894 (1990).
77. Bina, J. E. & Mekalanos, J. J. *Vibrio cholerae* *tolC* is required for bile resistance and colonization. *Infect. Immun.* **69**, 4681–4685 (2001).
78. Boardman, B. K. & Satchell, K. J. *Vibrio cholerae* strains with mutations in an atypical type I secretion system accumulate RTX toxin intracellularly. *J. Bacteriol.* **186**, 8137–8143 (2004).
79. Join-Lambert, O. F. *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.* **45**, 571–576 (2001).
80. Lee, V. T. & Schneewind, O. Protein secretion and the pathogenesis of bacterial infections. *Genes Dev.* **15**, 1725–1752 (2001).
81. Groisman, E. A., Eduardo, A. & Mouslim, C. Molecular mechanisms of *Salmonella* pathogenesis. *Curr. Opin. Infect. Dis.* **13**, 519–522 (2000).
82. Evans, K. *et al.* Influence of the MexAB–OprM multidrug efflux system on quorum sensing in *Pseudomonas aeruginosa*. *J. Bacteriol.* **180**, 5443–5447 (1998).
83. Keller, L. & Surette, M. G. Communication in bacteria: an ecological and evolutionary perspective. *Nature Rev. Microbiol.* **4**, 249–258 (2006).
84. Sanchez, P. *et al.* Fitness of *in vitro* selected *Pseudomonas aeruginosa* *nalB* and *nfxB* multidrug resistant mutants. *J. Antimicrob. Chemother.* **50**, 657–664 (2002).
85. Yang, S., Lopez, C. R. & Zechiedrich, E. L. Quorum sensing and multi-drug transporters in *Escherichia coli*. *Proc. Natl Acad. Sci. USA* **103**, 2386–2391 (2006).
86. Linares, J. F. *et al.* Overexpression of the multidrug efflux pumps MexCD–OprJ and MexEF–OprN is associated with a reduction of type III secretion in *Pseudomonas aeruginosa*. *J. Bacteriol.* **187**, 1384–1391 (2005).
87. Ma, D. *et al.* Genes *acrA* and *acrB* encode a stress-induced efflux system of *Escherichia coli*. *Mol. Microbiol.* **16**, 45–55 (1995).
88. Nikaido, H., Basina, V., Nguyen, V. & Rosenberg, E. Y. Multidrug efflux pump AcrAB of *Salmonella typhimurium* excretes only those β -lactam antibiotics containing lipophilic side chains. *J. Bacteriol.* **180**, 4686–4692 (1998).
89. Prouty, A. M., Brodsky, I. E., Falkow, S. & Gunn, J. S. Bile-salt-mediated induction of antimicrobial and bile resistance in *Salmonella typhimurium*. *Microbiology* **150**, 775–783 (2004).
90. Rouquette, C., Harmon, J. B. & Shafer, W. M. Induction of the *mtrCDE*-encoded efflux pump system of *Neisseria gonorrhoeae* requires MtrA, an AraC-like protein. *Mol. Microbiol.* **33**, 651–658 (1999).
91. Elkins, C. A. & Mullis, L. B. Mammalian steroid hormones are substrates for the major RND- and MFS-type tripartite multidrug efflux pumps of *Escherichia coli*. *J. Bacteriol.* **188**, 1191–1195 (2006).
92. Shafer, W. M., Qu, X.-D., Waring, A. J. & Lehrer, R. I. Modulation of *Neisseria gonorrhoeae* susceptibility to vertebrate antibacterial peptides due to a member of the resistance/modulation/division efflux pump family. *Proc. Natl Acad. Sci. USA* **95**, 1829–1833 (1998).
93. Sulavik, M. C. *et al.* Antibiotic susceptibility profiles of *Escherichia coli* strains lacking multidrug efflux pump genes. *Antimicrob. Agents Chemother.* **45**, 1126–1136 (2001).
94. Kaatz, G. W. & Seo, S. M. Mechanisms of fluoroquinolone resistance in genetically related strains of *Staphylococcus aureus*. *Antimicrob. Agents Chemother.* **41**, 2733–2737 (1997).
95. Hsieh, P.-C., Siegel, S. A., Rogers, B., Davis, D. & Lewis, K. Bacteria lacking a multidrug pump: a sensitive tool for drug discovery. *Proc. Natl Acad. Sci. USA* **95**, 6602–6606 (1998).

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Competing interests statement

The author declares no competing financial interests.

DATABASES

The following terms in this article are linked online to:

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