Journal of Antimicrobial Chemotherapy (2003) **51**, 9–11
DOI: 10.1093/jac/dkg050

JAC

The importance of efflux pumps in bacterial antibiotic resistance

M. A. Webber and L. J. V. Piddock*

Antimicrobial Agents Research Group, Division of Immunity and Infection, University of Birmingham, Birmingham B15 2TT, UK

Keywords: efflux, multiple antibiotic resistance

Efflux pumps are transport proteins involved in the extrusion of toxic substrates (including virtually all classes of clinically relevant antibiotics) from within cells into the external environment. These proteins are found in both Gram-positive and -negative bacteria as well as in eukaryotic organisms.[1] Pumps may be specific for one substrate or may transport a range of structurally dissimilar compounds (including antibiotics of multiple classes); such pumps can be associated with multiple drug resistance (MDR). In the prokaryotic kingdom there are five major families of efflux transporter:[2] MF (major facilitator), MATE (multidrug and toxic efflux), RND (resistance-nodulation-division), SMR (small multidrug resistance) and ABC (ATP binding cassette). All these systems utilize the proton motive force as an energy source,[3] apart from the ABC family, which utilizes ATP hydrolysis to drive the export of substrates. Recent advances in DNA technology and the advent of the genomic era have led to the identification of numerous new members of the above families, and the ubiquitous nature of efflux pumps is remarkable. Transporters that efflux multiple substrates, including antibiotics, have not evolved in response to the stresses of the antibiotic era. All bacterial genomes studied contain several different efflux pumps; this indicates their ancestral origins. It has been estimated that ~5–10% of all bacterial genes are involved in transport and a large proportion of these encode efflux pumps.[2,4]

There is some debate as to the 'normal' physiological role of efflux transporters, as antibiotic susceptible as well as resistant bacteria carry and express these genes. In many cases, efflux pump genes are part of an operon, with a regulatory gene controlling expression. Increased expression is associated with resistance to the substrates, e.g. resistance to bile salts and some antibiotics in *Escherichia coli* is mediated by over-expression of *acrAB*.[5] Although genes encoding efflux pumps can be found on plasmids, the carriage of efflux pump genes on the chromosome gives the bacterium an intrinsic mechanism that allows survival in a hostile environment (e.g. the presence of antibiotics), and so mutant bacteria that over-express efflux pump genes can be selected without the acquisition of new genetic material. It is probable that these pumps arose so that noxious substances could be transported out of the bacterium, allowing survival. Indeed it is now widely accepted that the 'intrinsic resistance' of Gram-negative bacteria to certain antibiotics relative to Gram-positive bacteria is a result of the activity of efflux systems.[6] Efflux systems that contribute to antibiotic resistance have been described from a number of clinically important bacteria, including *Campylobacter jejuni* (CmeABC[7,8]), *E. coli* (AcrAB-TolC, AcrEF-TolC, EmrB, EmrD[9]), *Pseudomonas aeruginosa* (MexAB-OprM, MexCD-OprJ, MexEF-OprN and MexXY-OprM[9]), *Streptococcus pneumoniae* (PmrA[10]), *Salmonella typhimurium* (AcrB[11]) and *Staphylococcus aureus* (NorA[12]). All of these systems efflux fluoroquinolones and the RND pumps (CmeB, AcrB and the Mex pumps) also export multiple antibiotics.

Over-expression of efflux pumps can result from mutations within local repressor genes[13–15] or may result from activation of a regulon regulated by a global transcriptional regulator such as MarA or SoxS of *E. coli*.[16,17] The broad substrate range of efflux systems is of concern, as often over-expression of a pump will result in resistance to antibiotics of more than one class as well as some dyes, detergents and disinfectants (including some commonly used biocides). Cross-resistance is also a problem; exposure to any one agent that belongs to the substrate profile of a pump would favour over-expression of that pump and consequent cross-resistance to all other substrates of the pump. These may include clinically relevant antibiotics. An example of this is seen again with the *mexAB* system of *P. aeruginosa*; mutants that over-produce MexAB are less susceptible, if not fully resistant to a range of antibiotics (fluoroquinolones, β-lactams, chloramphenicol and trimethoprim) but also triclosan, a commonly used household biocide.[18] The potential misuse of biocides and possible selection of bacteria cross-resistant

*Corresponding author. Tel: +44-121-414-6966; Fax: +44-121-414-3599; E-mail: l.j.v.piddock@bham.ac.uk

to antibiotics has recently been debated in this journal and elsewhere.[19–22] Over-expression of a multidrug resistance efflux pump alone often does not confer high-level, clinically significant resistance to antibiotics. However, such bacteria are better equipped to survive antibiotic pressure and develop further mutations in genes encoding the target sites of antibiotics.[23] It has been shown that fluoroquinolone-resistant strains of *E. coli* are selected 1000-fold more readily from *mar* mutants than wild-type bacteria,[24] and highly fluoroquinolone-resistant *E. coli* contain mutations in genes encoding the target topoisomerase enzymes and have reduced accumulation and increased efflux (porin down-regulation and efflux pump over-expression).[14,15] Additive increases in MICs of antibiotics have also been seen after concurrent over-expression of more than one pump of different classes, also resulting in highly resistant *E. coli*.[25]

It has been demonstrated that expression of the Mex systems of *P. aeruginosa* and the *acrAB* efflux system of *E. coli* is greatest when the bacteria are stressed, e.g. growth in a nutrient-poor medium, growth to stationary phase or osmotic shock; these inhospitable conditions may be relevant to the situation within an infection.[26,27] Unregulated over-expression of efflux pumps is potentially disadvantageous to the bacterium as not only will toxic substrates be exported but also nutrients and metabolic intermediates may be lost. Work with *P. aeruginosa* has suggested that mutants over-expressing Mex pumps are less able to withstand environmental stress and are less virulent than their wild-type counterparts.[28] As a result the expression of pumps is tightly controlled. However, mutants and clinical isolates that over-express efflux pumps are stable and commonly isolated; it may be that such mutants accumulate compensatory mutations allowing them to grow as well as wild-type bacteria.

Recently, the use of efflux pump inhibitors has been investigated in order to improve and potentiate the activity of exported antibiotics. Such a strategy has been used to develop inhibitors that reduce the impact of efflux pumps on fluoroquinolone activity. As many efflux pumps possess significant structural homology, it is hoped that one inhibitor compound will be active against a range of pumps from different bacterial species. Most research has focused upon *P. aeruginosa* Mex efflux pumps and inhibitors of these. One such inhibitor lowered the MIC values of fluoroquinolones for both sensitive and resistant strains.[2] In addition the frequency of selection of fluoroquinolone-resistant strains was also lower in the presence of the inhibitor, suggesting that efflux may be important in the selection of fluoroquinolone resistance. Similar observations have been made for *S. pneumoniae* and *S. aureus*.[29,30] A requirement for an intact efflux system to allow the development of topoisomerase mutations and consequent fluoroquinolone resistance in *E. coli* has also been described.[31] The link between active efflux and mutations in genes encoding the target site proteins suggests that the use of such inhibitors, in association with substrate antibiotics, may be useful by increasing both the activity and the range of species for which a drug may be effective. The design of new drugs and modification of existing molecules should also now be carried out with efflux pumps in mind. Structural alterations that reduce the ability of an antibiotic to be effluxed without compromising its activity may lead to the development of more potent compounds, certainly the 'effluxability' of drugs must now be considered, as agents are developed with regard to their overall efficacy and the likelihood of development of resistance.

To conclude, there is increasing evidence that the role of efflux pumps in antibiotic resistance in bacteria is significant. Although high-level resistance may not occur as a result of MDR efflux pumps alone, the association of over-expression of these genes amongst highly resistant clinical isolates cannot be ignored. The intrinsic antibiotic resistance of certain species may also be largely due to efflux pumps. Selection of efflux mutants by biocides encountered in the environment is a potential concern; more work is needed to quantify the risk, if any, from such a process. Synergic increases in resistance seen with over-expression of efflux system(s) as well as target site mutations can lead to highly resistant bacteria that are hard to treat. The effect of efflux pumps needs to be considered in the design of future antibiotics and the role of inhibitors assessed in order to maximize the efficacy of current and future antibiotics.

For those interested, there are a number of excellent review articles focusing on efflux pumps.[2,3,9,11,32,33]

References

1. Bambeke, V. F., Balzi, E. & Tulkens, P. M. (2000). Antibiotic efflux pumps. *Biochemical Pharmacology* **60**, 457–70.

2. Lomovskaya, O., Warren, M. S., Lee, A., Galazzo, J., Fronko, R., Lee, M. *et al.* (2001). Identification and characterization of inhibitors of multidrug resistance efflux pumps in *Pseudomonas aeruginosa*: novel agents for combination therapy. *Antimicrobial Agents and Chemotherapy* **45**, 105–16.

3. Paulsen, I. T., Brown, M. H. & Skurray, R. A. (1996). Proton-dependent multidrug efflux systems. *Microbiological Reviews* **60**, 575–608.

4. Saier, M. H. & Paulsen, I. T. (2001). Phylogeny of multidrug transporters. *Seminars in Cellular and Developmental Biology* **12**, 205–13.

5. Thanassi, D. G., Cheng, L. W. & Nikaido, H. (1997). Active efflux of bile salts by *Escherichia coli*. *Journal of Bacteriology* **179**, 2512–8.

6. Li, X. Z., Livermore, D. M. & Nikaido, H. (1994). Role of efflux pump(s) in intrinsic resistance of *Pseudomonas aeruginosa*—resistance to tetracycline, chloramphenicol, and norfloxacin. *Antimicrobial Agents and Chemotherapy* **38**, 1732–41.

7. Lin, J., Michel, L. O. & Zhang, Q. (2002). Cme ABC functions as a multidrug efflux system in *Campylobacter jejuni*. *Antimicrobial Agents and Chemotherapy* **46**, 2124–31.

8. Pumbwe, L. & Piddock, L. J. V. (2002). Identification and characterisation of CmeB, a *Campylobacter jejuni* multidrug efflux pump. *FEMS Microbiology Letters* **206**, 185–9.

9. Poole, K. (2000). Efflux-mediated resistance to fluoroquinolones in Gram-negative bacteria. *Antimicrobial Agents and Chemotherapy* **44**, 2233–41.

10. Gill, M. J., Brenwald, N. P. & Wise, R. (1999). Identification of an efflux pump gene *pmrA*, associated with fluoroquinolone resistance in *Streptococcus pneumoniae*. *Antimicrobial Agents and Chemotherapy* **43**, 187–9.

11. Nikaido, H. (2000). Preventing drug access to targets: cell surface permeability barriers and active efflux in bacteria. *Seminars in Cellular and Developmental Biology* **12**, 215–33.

12. Kaatz, G. W. & Seo, S. M. (1995). Inducible NorA-mediated multidrug resistance in *Staphylococcus aureus*. *Antimicrobial Agents and Chemotherapy* **39**, 2650–5.

13. Adewoye, L., Sutherland, A., Srikumar, R. & Poole, K. (2002). The MexR repressor of the *mexAB-oprM* multidrug efflux operon in *Pseudomonas aeruginosa*: characterization of mutations compromising activity. *Journal of Bacteriology* **184**, 4308–12.

14. Wang, H., Dzink-Fox, J. L., Chen, M. J. & Levy, S. B. (2001). Genetic characterization of highly fluoroquinolone-resistant clinical *Escherichia coli* strains from China: role of *acrR* mutations. *Antimicrobial Agents and Chemotherapy* **45**, 1515–21.

15. Webber, M. A. & Piddock, L. J. V. (2001). Absence of mutations in *marRAB* or *soxRS* in *acrB*-overexpressing fluoroquinolone-resistant clinical and veterinary isolates of *Escherichia coli*. *Antimicrobial Agents and Chemotherapy* **45**, 1550–2.

16. Alekshun, M. N. & Levy, S. B. (1997). Regulation of chromosomally mediated multiple antibiotic resistance: the *mar* regulon. *Antimicrobial Agents and Chemotherapy* **41**, 2067–75.

17. Pomposiello, P. J. & Demple, B. (2000). Identification of SoxS-regulated genes in *Salmonella enterica* serovar *typhimurium*. *Journal of Bacteriology* **182**, 23–9.

18. Chuanchuen, R., Beinlich, K., Hoang, T. T., Becher, A., Karkhoff-Schweizer, R. R. & Schweizer, H. P. (2001). 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. *Antimicrobial Agents and Chemotherapy* **45**, 428–32.

19. Fraise, A. P. (2002). Biocide abuse and antimicrobial resistance—a cause for concern? *Journal of Antimicrobial Chemotherapy* **49**, 11–2.

20. Gilbert, P., McBain, A. J. & Bloomfield, S. F. (2002). Biocide abuse and antimicrobial resistance: being clear about the issues. *Journal of Antimicrobial Chemotherapy* **50**, 137–9.

21. Fraise, A. P. (2002). Reply. *Journal of Antimicrobial Chemotherapy* **50**, 139–40.

22. Levy, S. B. (2001). Antibacterial household products: cause for concern. *Emerging Infectious Diseases* **7**, 512–5.

23. Kern, W. V., Oethinger, M., Jellen-Ritter, A. S. & Levy, S. B. (2000). Non-target gene mutations in the development of fluoroquinolone resistance in *Escherichia coli*. *Antimicrobial Agents and Chemotherapy* **44**, 814–20.

24. Cohen, S. P., McMurry, L. M., Hooper, D. C., Wolfson, J. S. & Levy, S. B. (1989). Cross-resistance to fluoroquinolones in multiple-antibiotic-resistant (Mar) *Escherichia coli* selected by tetracycline or chloramphenicol: decreased drug accumulation associated with membrane changes in addition to OmpF reduction. *Antimicrobial Agents and Chemotherapy* **33**, 1318–25.

25. Lee, A., Mao, W., Warren, M. S., Mistry, A. S., Hoshino, K., Okumura, R. *et al*. (2000). Interplay between efflux pumps may provide either additive or multiplicative effects on drug resistance. *Journal of Bacteriology* **182**, 3142–50.

26. Ma, D., Alberti, M., Lynch, C., Nikaido, H. & Hearst, J. E. (1996). The local repressor AcrR plays a modulating role in the regulation of *acrAB* genes of *Escherichia coli* by global stress signals. *Molecular Microbiology* **19**, 101–12.

27. Rand, J. D., Danby, S. G., Greenway, D. L. A. & England, R. R. (2002). Increased expression of the multidrug efflux genes *acrAB* occurs during slow growth of *Escherichia coli*. *FEMS Microbiology Letters* **207**, 91–5.

28. Sanchez, P., Ruiz-Diez, B., Campanario, E., Navas, A., Martinez, J. I. & Baquero, F. (2001). Hyperexpression of pumps in *nalB* and *nfxB* mutants of *Pseudomonas aeruginosa* decreases virulence in the *Caenorhabditis elegans* nematode model. In *Program and Abstracts of the Forty-first Interscience Conference on Antimicrobial Agents and Chemotherapy, Chicago, IL, USA, 2001*. Abstract C1-650. American Society for Microbiology, Washington, DC, USA.

29. Markham, P. N. (1999). Inhibition of the emergence of ciprofloxacin resistance in *Streptococcus pneumoniae* by the multidrug efflux inhibitor reserpine. *Antimicrobial Agents and Chemotherapy* **43**, 988–9.

30. Markham, P. N., Westhaus, E., Klyachko, K., Johnson, M. E. & Neyfakh, A. A. (1999). Multiple novel inhibitors of the NorA multidrug transporter of *Staphylococcus aureus*. *Antimicrobial Agents and Chemotherapy* **43**, 2404–8.

31. Oethinger, M., Kern, W. V., Jellen-Ritter, A. S., McMurry, L. M. & Levy, S. B. (2000). Ineffectiveness of topoisomerase mutations in mediating clinically significant fluoroquinolone resistance in *Escherichia coli* in the absence of the AcrAB efflux pump. *Antimicrobial Agents and Chemotherapy* **44**, 10–3.

32. Paulsen, I. T., Chen, J., Nelson, K. E. & Saier, M. H. (2001). Comparative genomics of microbial drug efflux systems. *Journal of Molecular Microbiology and Biotechnology* **3**, 145–50.

33. Poole, K. (2000). Efflux-mediated resistance to fluoroquinolones in gram-positive bacteria and the mycobacteria. *Antimicrobial Agents and Chemotherapy* **44**, 2595–9.