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MINIREVIEW

Efflux-Mediated Resistance to Fluoroquinolones in Gram-Negative Bacteria

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The introduction of the fluoroquinolones (FQs) in the 1980s provided clinicians with a class of broad-spectrum agents applicable to a range of gram-negative infections including urinary tract infections, gastrointestinal infections, respiratory tract infections, sexually transmitted diseases, bone and joint infections, and infections of the skin and soft tissue (reviewed in references 10 and 43). Targeted microorganisms include the family Enterobacteriaceae, Haemophilus spp., Neisseria spp., and Moraxella spp., which are highly susceptible to these agents, as well as important nosocomial pathogens such as Pseudomonas aeruginosa and Acinetobacter spp. FQs are less active but still clinically useful against Legionella spp.

Given this broad spectrum of activity, it is unfortunate that resistance to FQs has increased in a number of gram-negative organisms, most notably in P. aeruginosa but in virtually all organisms where FQs have been employed (1, 43). Resistance is due usually to mutations in the genes for the bacterial targets of the FQs (DNA gyrase [GyrA] and topoisomerase IV [ParC]) or to active efflux of the agents via antibiotic efflux pumps (59). This review focuses on efflux mechanisms of FQ resistance, their distribution and clinical significance in gram-negative pathogens, the possible natural function(s) of these, and, finally, the potential therapeutic value of efflux pump inhibitors.

ANTIBIOTIC EFFLUX

Efflux as a mechanism of antibiotic resistance was first reported in the early 1980s, for tetracycline, by two groups of researchers (11, 85). Since then, efflux-mediated resistance to several antimicrobial agents, including FQs, has been reported in a variety of bacterial species, and a number of efflux determinants have been cloned and sequenced (109) (Table 1). In most instances efflux was identified as the resistance mechanism because of an observed increase in FQ accumulation in FQ-resistant strains that was, when examined, compromised upon the addition of an energy inhibitor such as carbonyl cyanide m-chlorophenylhydrazone (CCCP). Unfortunately, a CCCP-promoted increase in FQ accumulation has been observed in the absence of an efflux mechanism (35, 100). As such, demonstration of CCCP-enhanced FQ accumulation in bacterial cells alone is insufficient to support the existence of an FQ efflux mechanism. The demonstration of FQ efflux, directly, or of reduced FQ accumulation in FQ-resistant relative to -susceptible strains is necessary before claims of an efflux mechanism can be made.

An interesting feature of strains expressing efflux-mediated quinolone resistance is their cross-resistance to a number of structurally unrelated antimicrobial agents (6, 15, 16, 18–20, 24, 25, 33, 36, 44, 66, 67, 77, 82, 99, 112, 117, 120, 123, 125, 149). This is due to the broad substrate specificity of the FQ efflux systems, which are capable of accommodating a variety of clinically relevant antimicrobial agents in addition to FQs. As such, FQ use and the attendant development of FQ resistance threatens to increase the incidence of multidrug resistance (MDR) in a number of important human pathogens. This is especially true for an organism like P. aeruginosa, where efflux-mediated resistance to FQs seems to predominate as a mechanism of resistance to these agents (50, 51, 62, 144).

RND-Type Efflux Systems

P. aeruginosa. Organisms with known FQ efflux systems of the MFP-RND-OEP type are highlighted in Table 1. In P. aeruginosa, four FQ-MDR efflux systems have been described to date, although numerous homologues are identifiable in the recently completed genome sequence (http://www.pseudomonas.com). The first to be reported, encoded by the mexAB-oprM operon (39, 69, 114, 115), is expressed constitutively in wild-type cells cultivated under usual laboratory conditions, where it contributes to intrinsic resistance to quin-
<table>
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<tr>
<th>Organism</th>
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<tr>
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<td>B. cepacia</td>
<td>CeoA, CeoB, OpeM</td>
<td></td>
<td>wt</td>
<td>mutant +</td>
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<td>C. jejuni</td>
<td>?</td>
<td></td>
<td>?</td>
<td>?</td>
<td>20</td>
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<td>?</td>
<td></td>
<td>?</td>
<td>?</td>
<td>94, 134a</td>
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<td>N. gonorrhoeae</td>
<td>MtrC</td>
<td></td>
<td>?</td>
<td>?</td>
<td>25, 79</td>
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<td>P. vulgaris</td>
<td>?</td>
<td></td>
<td>?</td>
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<td>49</td>
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<td>P. aeruginosa</td>
<td>MexA, MexB, OprM</td>
<td>mcrR</td>
<td>wt +</td>
<td>mutant ++; mcrC&lt;sup&gt;d&lt;/sup&gt; + +</td>
<td>18, 19, 33, 39, 67, 86, 114, 115, 117, 120, 145</td>
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<tr>
<td></td>
<td>MexC</td>
<td></td>
<td></td>
<td>?</td>
<td>113</td>
</tr>
<tr>
<td></td>
<td>MexE, MexF, OprN</td>
<td>mcrT</td>
<td>wt −</td>
<td>mutant ++</td>
<td>61</td>
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<tr>
<td></td>
<td>MexX (AmrA), MexY (AmrB), OprM</td>
<td>mcrZ (amrR)</td>
<td>wt +</td>
<td>?</td>
<td>3, 87, 139</td>
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<tr>
<td>S. enterica serovar Typhimurium</td>
<td>AcrA, AcrB</td>
<td>?</td>
<td>wt +; mutant ++</td>
<td>?</td>
<td>Antibiotics, dyes, lipophilic peptides</td>
</tr>
<tr>
<td>S. dysenteriae</td>
<td>?</td>
<td></td>
<td>?</td>
<td>?</td>
<td>2, 36</td>
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<td>S. maltophilia</td>
<td>SmcA, SmeB, SmeC</td>
<td>smcRS</td>
<td>?</td>
<td>?</td>
<td>6, 14, 9, Li et al., unpublished data</td>
</tr>
<tr>
<td>V. parahaemolyticus</td>
<td>NorM</td>
<td>?</td>
<td>?</td>
<td>Antibiotics, dyes, lipophilic cations</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> In organisms in which FQ efflux systems have been identified, components are identified as members of the single-component MATE or MFS group of efflux pumps or as members of the three-component RND-MFP-OEP group. In some instances the OEP component has yet to be confirmed.

<sup>b</sup> wt +, efflux system is expressed in wild-type cells (under laboratory growth conditions); mutant + +, expression is enhanced in resistant strains; wt −, efflux system is not expressed in wild-type; mutant −, efflux system is expressed in resistant strains. Where the nature of the mutation leading to enhanced efflux gene expression is known, the gene is indicated along with the relative level of gene expression (+ +, somewhat enhanced; ++, very enhanced).

<sup>c</sup> Where the identity of an FQ efflux system has yet to be made, references supporting the existence of FQ efflux mechanisms are supplied. Where FQ efflux systems have been identified, the more general references in support of efflux are indicated in boldface type opposite the organism name, and references pertaining to specific FQ efflux systems are indicated in lightface type opposite the relevant efflux system. Only the initial description of the latter is referenced here. Details of the regulation and substrate specificity are cited in the text.

<sup>d</sup> The nalC and nfsC genes have not yet been identified.

<sup>e</sup> ?, uncertain.
FIG. 1. Schematic demonstrating the organization and operation of antimicrobial efflux pumps of gram-negative bacteria. Although some MFS pumps work in conjunction with MFP and OEP counterparts, FQ efflux via a MFS-MFP-OEP tripartite pump has yet to be demonstrated. Abbreviations: PP, periplasmic space; CM, cytoplasmic membrane.

Lones and other antibiotics (60, 116, 131). The system is also hyperexpressed in so-called nalB mutants, which display elevated resistance to FQs and a variety of other antimicrobials (60, 82, 83, 116, 117). nalB strains carry mutations in a gene, mexR, which occurs immediately upstream of the efflux operon and encodes a repressor of mexAB-oprM expression (53, 116, 122, 132, 152). MexAB-OprM hyperexpression independent of mutations in mexR and the mexR mexAB-oprM intergenic region have also recently been described (132, 152). Dubbed nalC mutants (132), these presumably carry a mutation in a hitherto unidentified regulator of mexAB-oprM expression. The MexAB-OprM system is also growth phase regulated, its expression increasing in late log phase (30). Thus, this FQ-MDR efflux system is highly regulated in P. aeruginosa.

The MexCD-OprJ (113) and MexEF-OprN (61) systems are apparently not expressed in wild-type cells under normal laboratory conditions (45, 61, 131) but are hyperexpressed in nfxB (42, 83, 113) and nfxC (33, 61, 83) mutants, respectively. NfxB mutants carry mutations in a gene, nfxB (105, 106), which is located upstream of the efflux genes and encodes a repressor of mexCD-oprJ expression (113). Two classes of nfxB mutants have been described, expressing moderate (type A) or high (type B) levels of the efflux system, with resistance levels correlating with efflux gene expression (81). The nature of mutations leading to MexEF-OprN hyperexpression in nfxC strains has yet to be elucidated. MexEF-OprN hyperexpression is, however, dependent upon the mexT gene, which is located upstream of mexEF-oprN and encodes a positive regulator of mexEF-oprN expression (65, 102). Unlike the aforementioned efflux operons, the recently described mexXY system (also called amrA [139]) lacks a linked OM gene (87), reminiscent of the acrAB FQ-MDR efflux operon of E. coli (see below). Still, MexXY appears to utilize the product of the oprM gene as its OM constituent (3, 87), consistent with an earlier observation that OprM is functional in efflux-mediated MDR in the absence of MexAB (151). Given that the OM efflux proteins are not functional in the absence of RND-MFP counterparts (i.e., these pumps operate as tripartite pumps only) (141), this was interpreted as an indication that OprM functioned as the OM efflux component for additional efflux systems (151). Again, this was reminiscent of E. coli, in which the TolC OM component of the AcrAB-TolC FQ-MDR efflux system (see below) also functioned as the OM constituent of several other three-component pumps involved in the export of uncouplers (97), colicin V (46) and hemolysin (138). MexXY-OprM-mediated resistance to FQs has only been demonstrated with the cloned genes in E. coli (87) and P. aeruginosa (3). Thus, it is not clear to what extent the chromosomal mexXY genes promote FQ resistance in P. aeruginosa. Inactivation of chromosomal mexXY enhances the organism’s susceptibility to several antibiotics, including aminoglycosides, but not to FQs (3), and FQ or MDR mutants hyperexpressing this system have yet to be described. A gene, mexZ (also called amrR [139]), has been identified upstream of mexXY and apparently encodes a repressor of mexXY (amrAB) expression (3, 139).

These three-component efflux systems include an inner membrane, RND-type presumed drug-proton antiporter (MexB, MexD, MexF, or MexY); a periplasmic link or membrane fusion protein (MexA, MexC, MexE, or MexX); and an OM, presumed channel-forming protein (OprM, OprJ, or OprN). The inner membrane and OM components function in the export of antibiotics across the corresponding membrane, while the MFP apparently couples antibiotic export across both membranes by bringing these export components (or the membranes [147]) into close apposition (76, 97). Although drug-proton antiporter has not been demonstrated for the RND pump components of P. aeruginosa, their amino acid sequence homology to a heavy metal-proton antiporter in Alcaligenes eutrophus (Ralstonia eutrophia) (95) and an MDR-type drug-proton antiporter in E. coli (147) and their inhibition by uncouplers such as CCCP (69) favor this mechanism of action. The MFP components, though periplasmic, are anchored to the cytoplasmic membrane, probably via the acyl moiety of these presumed lipoproteins. The MexA MFP has, in fact, been shown to be acylated, although this acylation appears to be unnecessary for its activity (142). Channel-forming activity has yet to be demonstrated for the OEP components, which appear to be distinct from the porin class of OM channel-forming proteins (54). Moreover, the OEP components contain so-called lipoprotein boxes, typical sites of acylation in lipoproteins, at their N termini, and indeed, acylation of OprM has been demonstrated (N. Bianco and K. Poole, unpublished data). The observation that tonB mutants are compromised with respect to MexAB-OprM-mediated resistance suggests that OprM, at least, is a “corked” channel whose opening is dependent on energy and the TonB protein (150).

FQ-MDR clinical isolates of the nalB (53, 152), nfxB (51, 53), and nfxC (34) type have been described, although clinical FQ-resistant strains typically display target site mutations (17, 93, 143). It is unlikely, however, that efflux mechanisms were assessed in many of these strains. Still, a recent report indicates that hyperexpression of MexCD-OprJ or MexEF-OprN is the predominant mechanism of FQ resistance in strains isolated from the lungs of cystic fibrosis patients (52). The presence of both efflux and gyrase or topoisomerase mutations in the same strain has, for example, been reported in a number of clinical strains, particularly those exhibiting high-level FQ resistance (18, 47, 53, 145, 148). Given that high-level FQ resistance is invariably associated with multiple mutations (53), it is likely that efflux is a significant contributing factor in many instances. Interestingly, selection of FQ-resistant strains in vitro at FQ concentrations two to four times the MIC in a recent study yielded efflux mutants in >90% of the cases (62), a trend noted previously for the FQs (50, 51, 144) and distinct from the pattern seen for nalidixic acid-resistant strains (where gyrase
The predominant FQ efflux system of *E. coli* is encoded by the *acrAB-tolC* genes (32, 74, 76). The AcrAB proteins are highly homologous to the Mex proteins of *P. aeruginosa*, while TolC has limited homology to the OEPs of *P. aeruginosa*. TolC has, however, been shown to form channels in planar lipid bilayer membranes (12), consistent with a role in the export of antibiotics across the OM of *E. coli*. As with the FQ efflux mechanisms of *P. aeruginosa*, this system is broadly specific and accommodates a number of clinically relevant antimicrobials in addition to FQs (74). This system is expressed in wild-type cells under normal laboratory growth conditions, where it provides for intrinsic resistance, and its hyperexpression in mutants results in elevated resistance to FQs and other agents (74, 97). A related MDR efflux system, encoded by the *ermEF* (76) (previously called *envCD* [58]) genes is not expressed in wild-type cells (76) but does accommodate the same antibiotics, including FQs, as does AcrAB (J. Hwang and H. Nikaido, personal communication). Acylation of EnvC-AcrE has been demonstrated (127) although this does not appear to be essential for the function of the MFPs (146). Homologues of these systems have been described in both *S. enterica* serovar Typhimurium (37, 65, 99) and *Haemophilus influenzae* (123), although an OM constituent has yet to be confirmed. While the *S. enterica* serovar Typhimurium counterpart has a demonstrated role in efflux and FQ resistance (99), the *H. influenzae* AcrAB homologue does not (123). Still, the latter organism possesses an OM of comparatively high permeability (124), and it is known that the OM barrier is an important determinant of resistance mediated by FQ-MDR efflux systems (i.e., they function synergistically) (41, 76, 98). Despite the presence of highly homologous FQ-MDR efflux systems in *P. aeruginosa* (MexAB-OprM) and *E. coli* (AcrAB-TolC), for example, the former provides resistance to a broader range of compounds, in particular small hydrophilic antibiotics (131). Moreover, expression of MexAB-OprM in *E. coli* affords resistance to a narrower range of antibiotics than it does in *P. aeruginosa* (130). This is apparently because the OM of *E. coli* is more permeable to these agents than is *P. aeruginosa*’s (96), and their rate of influx, then, is sufficient to overcome the effects of efflux (76). Similarly, disruption of the OM in *P. aeruginosa* compromises antibiotic resistance mediated by the MexAB-OprM pump (70a, 98). Thus, while the AcrAB system of *H. influenzae* may well pump FQs, the rather permeable OM of this organism (i.e., enhanced influx) would likely, as H. Nikaido has stated (123), negate this effect. In this vein, *Neisseria gonorrhoeae* also possesses a homologue of the AcrAB-MexAB FQ-MDR efflux systems, MtrCDE (40), that functions as an efflux pump but does not provide resistance to FQs. Again, the OM of this organism is apparently more permeable than that of *E. coli* (and *P. aeruginosa*) (123).

Expression of *acrAB* is governed primarily by AcrR, the product of a repressor gene located immediately adjacent to the efflux genes (73), and MarA, a positive regulator encoded by the *marA* gene of the *marAB* operon (4). AcrR is a member of a family of repressor proteins that includes AcrS, which apparently regulates *acrEF* expression (76), and MtrR, which regulates *mtrCDE* expression (40). Mutations in *acrR* (73) or *mtrR* (40) produce modest increases in efflux gene expression and, thus, modest increases in resistance to substrate antibiotics. The *marAB* operon, the so-called *mar* locus, has long been known to be the site of mutations in *E. coli* that produce a multiple antibiotic resistance (Mar) phenotype (4). The *marR* gene encodes a repressor of *marAB* expression (and is the site of mutation in most *mar* strains [4]), while the *marA* gene product activates a variety of genes associated with resistance to antibiotics and oxygen stress (4). Still, the predominant locus responsible for the MDR of *mar* mutants is *acrAB*. This was aptly demonstrated by observations that expression of *acrAB* is increased in *marR* mutants (which show elevated *marA* expression) (75) and the multiple antibiotic resistance of *marR* mutants is compromised in strains with *acrAB* deletions (107). Moreover, the cloned *marA* gene enhances expression of both *acrA* and *tolC* (9). Two additional genes, robA and souS, also increase *acrA* and *tolC* expression (9), highlighting the complexity of *acrAB-tolC* regulation in *E. coli*. Recently, a null mutation in the *mppA* gene encoding a periplasmic murein peptide-binding protein was shown to increase MarA production and, concomitantly, the antibiotic resistance of *E. coli* (68). Still, the mechanism by which MppA influences *marA* expression remains to be elucidated.

Intriguingly, MarA homologues have been identified in several bacteria, including *serovar Typhimurium* (21, 133), *Shigella* spp., *Klebsiella* spp., *C. freundii*, *Hafnia alvei*, *Enterobacter* spp. (21) and *P. vulgaris* (48), with *marA* expression associated with a mar phenotype in *Enterobacter agglomerans* and *S. typhimurium* (21). A Mar phenotype has also been reported in *P. aeruginosa* (148), although a mar locus has yet to be identified in this organism. It is possible, therefore, that these organisms possess FQ-MDR transporters of the AcrAB type. The observation that FQ resistance is increased in *K. pneumoniae* upon exposure to salicylate (27), a known inducer of the *mar* operon (23), also suggests that this organism possesses an AcrAB-type efflux system.

The clinical relevance of AcrAB-mediated resistance to FQs or any antibiotic is unclear, although the presence of *mar* mutations in clinical FQ-resistant strains (78, 104) and the observation that *mar* mutants are less sensitive to the bactericidal effects of FQs (38) and mutate more readily to high-level FQ resistance (4) suggest it may be important. Moreover, clinical strains of *E. coli* resistant to FQs and exhibiting decreased accumulation of ciprofloxacin have been described, many of which show very marked increases in ciprofloxacin accumulation upon treatment with CCCP (31). These strains also carry mutations in *gyrA* and *parC*, indicating, again, that FQ resistance often results from a combination of efflux and target site mutations (see also reference 56).

*Burkholderia spp.* First identified as a determinant of chloramphenicol resistance in a clinical strain (15), the *ceoAB-opcm* operon (GenBank accession number U97042) of *B. cepacia* encodes an MDR efflux pump which accommodates and, thus, provides resistance to FQs (16; J. L. Burns, C. Pritzlaff, J. Barry, M. Charon, and M. Cieri, Abstr. 98th Gen. Meet. Am. Soc. Microbiol., abstr. V-108, 1998). Associated with the MDR of a clinical strain, it is unclear if this homologue of the RND-MFP-OEP efflux systems of *P. aeruginosa* is only expressed in mutant strains (like the *nfxB* and *nfxC* mutants of *P. aeruginosa*) or is expressed constitutively and, thus, contributes to the well-known intrinsic resistance of this organism to many antibiotics. A regulatory gene has not been reported for this efflux system, although salicylate induction of FO-MDR in *B. cepacia* (14) is suggestive of a mar locus in this organism (see below). An RND-MFP-OEP homologue, *AmrAB-OprA*, has recently been described in *Burkholderia pseudomallei* (91). Also an MDR transporter, this system exports and provides resistance to aminoglycosides and macrolides but not FQs.

*S. malophilia.* FQ selection of MDR strains of *S. malophilia* (then called *Xanthomonas malophilia*), reminiscent of FQ selection of efflux mutants in *P. aeruginosa* (see above), has been seen, although an efflux mechanism was not originally impli-
cated (66). More recently, FQ-MDR strains have been described which appear to possess efflux mechanisms responsible for the FQ-MDR (6, 149). Of significance, several clinical MDR strains of S. multophilia expressing homologues of the MexAB-OprM efflux system of P. aeruginosa have been reported (149). In fact, a MexAB-OprM-like efflux system, encoded by the smcABC operon (GenBank accession number AF173226), has been discovered recently in this organism and shown to accommodate FQs as well as other antibiotics (X.-Z. Li, L. Zhang, and K. Poole, unpublished data).

Genome projects. Homologues of the RND-MFP-type MDR systems with and without linked OEP genes are identifiable in the genomes of many bacteria, including Rickettsia prowazekii (7) and Helicobacter pylori (5, 136), where a hflABC operon has been identified by Bina and Hancock (accession number AF059041); as well as the cyanobacterium Synechocystis sp. (55) and Rhodobacter capsulatus (64). BLAST searching of the unfinished genome sequences available on-line (http://www.ncbi.nlm.nih.gov/BLAST/unfinishedgenome.html) also revealed numerous homologues in S. enterica serovar Typhi (four), Bordetella pertussis (four), Yersinia pestis (five), C. jejuni (one), and Vibrio cholerae (two). Whether any of these function in antibiotic efflux remains to be determined. Still, the possibility exists, at least, that FQ-MDR efflux systems are widespread in gram-negative bacteria, where they may play a significant role in resistance to this important class of antibiotic.

MFS- and MATE-Type Efflux Systems

The most common example of an MFS antibiotic efflux system in gram-negative bacteria is that encoded by the various tet genes associated with tetracycline efflux and resistance (119). Members of this family that efflux FQs are, in contrast, rare in gram-negative bacteria, including only the MdfA transporter of E. coli (28), although it appears to be a more effective pump for nonantibiotics. Originally described as MFS transporters, the NorM pump of V. parahaemolyticus (92) and its E. coli homologue YdhE (92) now appear to be members of the newly reported MATE family of transporters (13). These pumps facilitate resistance to multiple agents, including antibiotics and nonantibiotics, and appear to be better exporters of FQs than is MdfA. In the case of all three of the aforementioned transporters, however, efflux and antibiotic resistance were assessed using genes cloned on plasmids and expressed in E. coli. Thus, the relevance of the chromosomal counterparts to FQ resistance is uncertain. As with MFS-type exporters involved in FQ resistance in gram-positive bacteria FQ resistance attributable to NorM and YdhE is limited to the more hydrophilic FQs such as ciprofloxacin and norfloxacin (92).

FQ EFFLUX SYSTEMS EXHIBIT BROAD SUBSTRATE SPECIFICITY

The most striking feature of the RND-MFP-OEP MDR efflux systems of gram-negative bacteria is their incredibly broad substrate specificity, encompassing a variety of structurally unrelated antimicrobial agents, including clinically relevant antibiotics, dyes (28, 40, 74, 88, 92, 101, 123, 134), detergents (40, 74, 130, 135), disinfectants (84, 89), antimicrobial peptides (129), organic solvents (8, 57, 70, 71, 140), inhibitors of fatty acid synthesis (126), and homoserine lactones involved in bacterial cell-to-cell signalling (29, 111). The binding of multiple structurally varied substrates is uncommon in biology, and how this is achieved in gram-negative FQ-MDR transporters is as yet unknown. This broad substrate specificity contrasts with other examples of antibiotic efflux systems, which tend to be agent or class specific (e.g., the tetracycline [tet] [119] efflux systems). Interestingly, too, the FQ-MDR efflux systems are invariably chromosomally encoded and conserved in both sensitive and resistant strains, with resistance usually resulting from mutational upregulation of the efflux genes. The recently described plasmid-borne FQ resistance determinant (80) appears not to involve efflux, although the mechanism of resistance has yet to be elucidated (G. A. Jacoby, personal communication). Again, contrasts with the tetracycline efflux systems, which are generally plasmid-borne or transposon-encoded (119). This suggests that FQ-MDR efflux systems are an intrinsic part of the gram-negative bacterium and function independently of antibiotic efflux and resistance, while the others function uniquely in antibiotic efflux and resistance and their acquisition from outside sources provides for antibiotic resistance.

NATURAL FUNCTION OF FQ-MDR EFFLUX SYSTEMS

The natural role of FQ-MDR efflux systems is the subject of some debate, with support for export of and, thus, protection from exogenous antimicrobial agents available in some instances. The inducibility of the E. coli AcrAB system by toxic fatty acids (75) and the demonstrated role of AcrAB in the export of and resistance to bile salts (135) are consistent with a role for AcrAB in protecting the cell from the action of these agents in the gut (75). A protective function is also likely attributable to the MtrCDE system, which provides for resistance to fecal lipids in rectal isolates of N. gonorrhoeae (128) and, probably, bile salts known to bathe mucous membranes (40). Still, in none of these cases are antibiotics the intended substrate. The fact, too, that most pump genes have linked regulatory genes indicates that the efflux systems are highly regulated and, thus, likely respond to something environmental or cell associated. Although some cell-derived compounds have been identified as substrates for these efflux systems, including homoserine lactone autoinducers in P. aeruginosa (MexAB-OprM) (29, 111) and indole (a precursor of tryptophan) in E. coli (AcrEF) (K. Sato, K. Shibayama, T. Horii, Y. Arakawa, and M. Ohta, Abstr. 38th Intersci. Conf. Antimicrob. Agents Chemother., abstr. C-126, 1998), it is far from clear that these are the intended substrates. MexEF-OprN expression in P. aeruginosa, for example, is associated with a decrease in pyocyanin production (61), a phenotype also seen in nalB strains and attributed to homoserine lactone efflux by MexAB-OprM (29). Thus, homoserine lactones may be just another in a line of shared substrates for these highly accommodating MDR efflux systems in P. aeruginosa (K. Sato et al. 38th ICAAC).

THERAPEUTIC VALUE OF EFFLUX PUMP INHIBITORS

Given the contribution of FQ-MDR efflux systems to low- and high-level FQ resistance in clinical strains of a variety of important human pathogens, it seems logical that they be targets for therapeutic intervention. Indeed, a variety of genetic and inhibitor studies have confirmed the usefulness of pump inactivation in increasing bacterial susceptibility to FQs (and other antibiotics) and preventing emergence of FQ resistance. Mutants of P. aeruginosa with deletions of the genes coding for the three best-characterized FQ-MDR efflux systems of this organism were markedly FQ hypersusceptible, and FQ-resistant derivatives of this deletion strain could not be selected in vitro at clinically relevant concentrations of FQ (72). Moreover, elimination of the FQ-MDR efflux systems in this or-
CONCLUSIONS

Efflux mechanisms of FQ resistance are widely distributed among important gram-negative human pathogens, where they appear to be components of the normal genetic complement of these bacteria. The identification, too, of several homologues of the RND-MFP-OEP-type efflux systems in the genome sequences of a number of these organisms, in which efflux-mediated FQ resistance has yet to be studied, suggests that the potential for efflux to contribute to FQ resistance is particularly great in gram-negative bacteria. Although there is much debate and uncertainty regarding the natural function of these plentiful FQ-MDR efflux systems, it is clear that they are important contributors to intrinsic and acquired FQ resistance. Inhibition of efflux systems would seem, therefore, a prudent approach to combating and/or preventing FQ resistance.

REFERENCES


