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MULTIDRUG EFFLUX SYSTEMS IN GRAM-NEGATIVE BACTERIA

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REVIEW

ABSTRACT

Multidrug efflux mechanisms in bacteria contribute significantly to intrinsic and acquired resistance to antimicrobial agents. Genome analysis have confirmed the broad distribution of these systems in Gram-negative as well as in Gram-positive bacteria. Among resistance mechanisms, the multidrug efflux system or pump deserves special attention, since a cell that has acquired it can simultaneously diminish or even suppress the susceptibility to a wide range of antimicrobials. The efflux system is mediated by transport proteins which confer resistance to toxic compounds. In Gram-negative bacteria, a tripartite efflux system is necessary to expel the drug to the outer medium: a protein localized in the cytoplasmic membrane; another in the periplasmatic space (membrane fusion protein – MFP); and a third in the outer membrane (outer membrane factor – OMF). The drug transport is active, and depends either on the energy provided by ATP hydrolysis or is directly driven by the proton motive force. The transport proteins are grouped in families, according to the homology of the amino acid sequences and to similarity of mechanisms. Among Gram-negative bacteria, *Escherichia coli* and *Pseudomonas aeruginosa* have most of the hitherto identified and studied multidrug efflux systems.

Key words: Gram-negative bacteria, multidrug efflux systems, antimicrobials, transport proteins, multiresistance

INTRODUCTION

Bacteria resistant to antimicrobial agents have been frequently isolated around the world, possibly as a consequence of their indiscriminate use (15,120). Antimicrobials are not only fundamental for the treatment of infections in humans and animals, but are also essential substances in agriculture and animal husbandry, where they are applied at subtherapeutic levels as growth promoters in bird, swine, beef, and fish food (112). The emergence of multiresistant bacteria has become a problem for the treatment of infectious diseases (67).

Throughout their evolution, microorganisms have developed versatile resistance mechanisms to toxic effects of antibiotics as well as other drugs (28,69). One such mechanism involves the production of antibiotic-hydrolyzing enzymes, as for example the β-lactamases (22), or of enzymes which transform antibiotics into inactive derivatives (17), such as the enzymes which phosphorylate, adenylate or acetylate aminoglycosides (109). Another mechanism is the alteration of cell targets by mutation or enzymatic modification, so that the drug affinity to the target is reduced (110). A third resistance mechanism is the inhibition of the drug's entry into the cell due to the low-permeability of the outer membrane of the Gram-negative bacteria and to the Gram-positive mycobacteria barrier, where the drug diffusion through the cell envelope is reduced (39,74,77).

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Bacterial cells, as well as eucaryotic cells, have cytoplasmic membrane-localized transport systems, which are involved in vital cell functions like nutrient uptake, excretion of toxic compounds, and maintenance of the cell homeostasis (93,101). Many of these systems confer resistance to toxic compounds such as antibiotics, antiseptics, chemotherapeutics, and others (76,84,102). Isolates of *Pseudomonas putida* are able to grow in organic solvents, like toluene, xylene, and styrene, owing mainly to the efflux pumps, which remove these solvents from the cell membrane (98).

Some prokaryote efflux systems – among which the best-studied example is the TetB protein of *E. coli*, which transports tetracycline and its chemical analogs (47) – are known as drug-specific transport systems. Efflux systems have been identified which are able to export a broad variety of structurally different compounds, and for this reason are known as multidrug efflux pumps or multidrug exporters (48). The multidrug efflux pumps have emerged as one of the main problems in medicine (48,74,125), since one single multidrug efflux system in a cell can diminish its susceptibility to a wide range of chemotherapeutic drugs (84). Multidrug efflux pumps (MDRs) have been defined as membrane translocases with a surprising capacity to expulse a variety of non-related drugs from the cell (48).

The objective of this minireview is to provide the reader with an overall vision of the main aspects underlying multidrug efflux systems in Gram-negative bacteria: origin and mechanisms of the multidrug transporters; families, organization, and energy sources of the transporters; examples of the main proton motive force-driven multidrug transporters in Gram-negative bacteria and their substrates; and their genetic organization, expression, and regulation.

Origin and molecular mechanisms of multidrug transporters

Multidrug efflux systems in bacteria were subject of several outstanding reviews, among them are Levy (47), Lewis (48), George (29), Nikaido (75), Paulsen *et al.* (84), Van Veen and Konings (122), Putman *et al.* (96), Van Bambeke *et al.* (118), and Poole (89).

According to Neyfakh (71), there are two possible paradigms for the evolution of multidrug transporters. The first claims that because bacteria live in chemically complex environments they are constantly being challenged by other bacteria as well as by higher microorganisms. The evolution process created a molecular mechanism that would recognize non-specific toxic compounds and thus brought forth multidrug transporters. The other hypothesis postulates that each multidrug transporter is involved with physiologically substrate-specific efflux of a structurally related group of compounds. According to this latter hypothesis, the toxin-exporting capacity of multidrug transporters is merely opportunist, and only occurs in bacteria under drug exposure in experimental or clinic situations (71). A specific substrate transporter, for example, with a large and accessible substrate recognition site could potentially not only interact with the substrate itself, but also with multiple drug molecules, since they are recognized by different domains (71). Therefore, the specific substrate transporter can potentially exclude different drugs although its primary function is another (71). A third hypothesis, encompassing elements of the other two, cannot be rule out.

Recent progress in the structural analysis of a series of multidrug transport soluble proteins has shown that they possess large hydrophobic binding sites and bind to their substrates by a combination of hydrophobic interactions and electrostatic attraction preferentially to hydrogen bonds or other specific interactions which are characteristic of enzymes and receptors (70). Multidrug transporter topology suggests similar binding sites, with similar substrate recognition principles. This would not only explain the broad substrate specificity, but also their evolutionary relations and their apparent multiplicity in genomes of organisms of all kingdoms, although further studies will be necessary to prove this hypothesis (70).

The most well characterized multidrug efflux pump may be the Permease-glycoprotein (P-glycoprotein), encoded by the *mdr*1 gene (multidrug resistance), identified in humans and in rodents, which is responsible for resistance to a wide range of cytotoxic drugs via ATP dependent efflux (32). The efflux was identified through an analysís of the mechanism that makes human cancer cells acquire resistance to chemotherapeutic agents – this transport system diminishes the uptake and increases the efflux of anticancer drugs (32). Pioneers of this work were Dano (21) and Juliano and Ling (40), who worked with resistant Ehrlich ascites tumor cells and with hamster ovary cell mutant, respectively. Physiological alteration (diminished drug accumulation caused by efflux) was identified as well as biochemical changes (increase of P-glycoprotein on the cell surface) in multiresistant cells selected with colchicine – an antimitotic drug which inhibits microtubules formation by linking to the protein tubulin (2). The genetic study of multidrug efflux systems that protect cells against anticancer chemotherapy is an attractive target in the development of disease treatments, as well as for the treatment of congenital cell metabolism anomalies (2).

In prokaryotes, the first evidence of the presence of multidrug efflux pumps was shown by Tennent *et al.* (113), Rouch *et al.* (101), Littlejohn *et al.* (50), and Littlejohn *et al.* (51) in quaternary ammonium compounds – resistant *Staphylococcus* isolates. Those authors observed that the QacA protein was a membrane translocase which also actively transported ethidium bromide, among other drugs, out of the cell. Moreover, the transport of these substances is an antiport, drug/H^+, and the gene encoding the QacA protein is in a plasmid which also contains other antimicrobial resistance genes.

Several models have been proposed to explain possible routes of carrier mediated transport (14). The *Hydrophobic*

vacuum cleaner hypothesis assumes that multidrug transporters are embedded in the phospholipid bilayer membrane, the drug molecule binds to the carrier protein from the inner or outer leaflet of the bilayer, and is transported from there outwards. Their physical properties would enable them to recognize lipophilic drugs (97). According to the *Flippase* theory, the drug would simply pass from the inner to the outer membrane leaflet and diffuse outwards (38). The *Aqueous pore* hypothesis claims that the drug translocation could involve the substrate transport from the cytoplasm to the outer medium via aqueous pores with flexible substrate recognition sites (4). It has been observed that the multidrug transporters carry their substrates from the phospholipid bilayer before reaching the cytoplasmic aqueous phase (38,97,116). The most convincing evidences of this are transport studies of fluorescent compounds such as TMA-DPH (1-[4-(trimethylamino)phenyl]-6-phenylhexa-1,3,5-triene) and LDS 751 (2-{-4[-4(dimethylamine)-phenyl]-1,3-butadienyl}-3-ethylbenzo-thiazolium-perchlorate) in *Lactococcus lactis* (12,13), *P. aeruginosa* (81), *Staphylococcus aureus* (61), and in mammalian cells (106,107,108).

Multidrug efflux systems in bacteria are of particular concern for the treatment of patients with infectious diseases, since the substrates of many multidrug transporters include antimicrobials used for therapy (96,119). Exposure to one substance that is a substrate of the efflux pump can favor its overexpression and the consequence may be cross-resistance to all other substrates which may include clinically relevant antimicrobials (124).

Although genes encoding efflux pumps may be present in plasmids, those found in chromosome are often related to the intrinsic resistance mechanisms and enable the bacteria to survive in hostile environments, as for instance in the presence of antimicrobials (124). Multidrug transporters can therefore be associated to intrinsic and to acquired antimicrobial resistance (96,124). Acquired multidrug resistance can occur via three mechanisms: (a) mutation and amplification of genes encoding multidrug transporters which alter their level of expression (73) or their activity level (42); (b) mutation in specific genes or in global regulatory genes, resulting in an increased expression of multidrug transporters, as for example the mutation in *mexR* of *P. aeruginosa* OCR1 (93); and (c) intercellular transfer of resistance genes, such as *qacE*, by plasmids or transposons (41).

Families, energy sources and organization of transporters

Bacterial drug efflux systems are grouped in transport protein families based on the homology of amino acid sequences and the similarities in secondary structure and in size (14,95). Two are large families and it is believed that they had a common origin. These are the super-families ATP Binding Cassete (ABC) (37) and Major Facilitador Super-family, MFS (33,55). Two other, smaller families are designated Small Multidrug Resistance (SMR) (87) and Resistance, Nodulation, Division (RND) (84,103). A fifth family, identified by Brown *et al.* (16), was denominated Multidrug and Toxic Compound Extrusion (MATE).

Members of the MFS family are transport proteins found in Gram-negative and Gram-positive bacteria and in higher eukaryotes, involved in the transport (uniport, symport, and antiport) of various substrates, such as sugars, intermediates of the Krebs cycle, phosphate esters, oligosaccharides, and antibiotics (55). They have 12 to 14 transmembrane segments (TMS) (86) and some examples are the proteins Bcr (7), EmrB (52), and EmrD (68) of *E. coli*; NorA (129) and QacA (101) of *S. aureus*; and Bmr of *Bacillus subtilis* (72).

The transport proteins of the SMR family are smaller, have approximately 107 amino acids and generally four TMS (87). Owing to their size, these proteins might function as homooligomeric complexes (83,85). The first gene encoding a protein of the SMR family was detected in plasmids present in clinic isolates of *Staphylococcus* (45,46,50). Examples of proteins of this family are Mmr of *Mycobacterium tuberculosis* (23), QacE of *Klebsiella aerogenes* (85), and EmrE of *E. coli* (65,95,128).

The RND family consists of transport proteins which interact with the membrane fusion protein and with an outer membrane protein (48,74,76). Members of this family have 12 TMS, with two large loops between TMS 1 and TMS 2, and TMS 7 and TMS 8, respectively (103), Examples of these proteins are AcrB (53) of *E. coli*, *Salmonella* (78), and *Enterobacter aerogenes* (94); Mex B (92), MexD (90), MexF (43), and MexY (60) of *P. aeruginosa*; AmrB of *Burkholderia pseudomallei* (63); and MtrD of *Neisseria gonorrhoeae* (36).

NorM of *Vibrio parahaemolyticus* and YdhE of *E. coli* are members of the MATE family and possess 12 TMS (16,66).

The efflux systems are characteristically energy dependent and can be classified as primary or as secondary active transports (47,74): those of the primary active transport use the energy derived from ATP hydrolysis, and those of the secondary active transport directly use the energy generated by the electrochemical proton gradient, in other words, the proton motive force (2,96).

The primary active transport, driven by ATP hydrolysis, and the related transport proteins characterize the superfamily ABC, also referred to as Traffic ATPases (5,37). The first evidence on an ATP dependent transport system which conferred multidrug resistance in prokaryotes was obtained from studies of *L. lactis* (10). The gene *lmrA* in *L. lactis* encodes a transporter protein which carries ethidium bromide and daunomycin (121). It is interesting that resistance to ethidium bromide in *L. lactis* can be traced to at least two export systems, driven by different energy sources: LmrP (11), which depends on the proton motive force, and LmrA that is ATP dependent (121).

The secondary active transport, directed by an electrochemical transmembrane gradient of protons is common

in bacteria. The direct role that the proton motive force plays in the transport of antibiotics was demonstrated by McMurry *et al.* (59) and Yamaguchi *et al.* (127), concerning the tetracycline transporter TetA in *E. coli*. Bolhuis *et al.* (11), when studying the multidrug transporter LmrP of *Lactococcus*, demonstrated an antiport, with ethidium bromide being exchanged for a proton. A similar mechanism was suggested by Grinius and Goldberg (34) and Yerushalmi *et al.* (128) for the multidrug transporters Smr and EmrE, respectively. EmrE is an *E. coli* pump that exchanges toxic cations and H^+ (128) and Smr is a staphylococci pump that exchanges H^+ for multiple drugs, incluiding quaternary ammonium compounds and ethidium bromide (34). Other multidrug transporters, and also specific drug transporters can use proton motive force driven transport.

Evidence of the presence of efflux pumps in *E. coli* and *Enterobacter cloacae* isolates obtained from poultry carcasses was observed through the difference between the minimum inhibitory concentration (MIC) of several antimicrobials in the absence and presence of carbonyl cyanide m-chorophenylhidrazone, CCCP, an energy uncoupler (64). In some of these isolates, MIC values of the antimicrobials cefaclor, furazolidone, nitrofurantoin, tetracycline, chloramphenicol and sulphamethoxazole/trimethoprim presented a reduction after addition of the uncoupler. This is an evidence of an energy dependent resistance mechanism. In further studies we confirmed the active export of [H^3] chloramphenicol from those *E. coli* cells (unpublished data).

Gram-negative bacteria are generally more resistant to a greater number of antimicrobials than the Gram-positive (75). This may be due to the Gram-negative bacteria topology, with the outer membrane functioning as one more barrier that prevents the access of antimicrobial agents to their targets inside the cell (117).

Ordinarily, the efflux systems consist in monocomponent proteins with several TMS, approximately 12 (119). In Gram-negative bacteria, however, the transport system is complex because of the structure of its outer membrane. This is also the reason why they require a tripartite exportation system: one plasmic membrane protein, one membrane fusion protein, and an outer membrane factor (48,74,76). Some drug transporters in these bacteria are therefore also found in associations with an accessory protein which crosses the periplasmatic space and interacts with the outer membrane (48,74,76). This structure was first proposed to describe proteins that secrete hemolysin directly to the outer medium (123). Due to their capacity to transport the drugs directly to the medium, these pumps could produce significant resistance levels in Gram-negative bacteria, even when functioning at low levels (75). This adds further to the reduced uptake of drugs by Gram-negative bacteria in consequence of the outer membrane barrier.

Fig. 1 depicts models of multidrug efflux systems in Gram-negative and in Gram-positive bacteria.

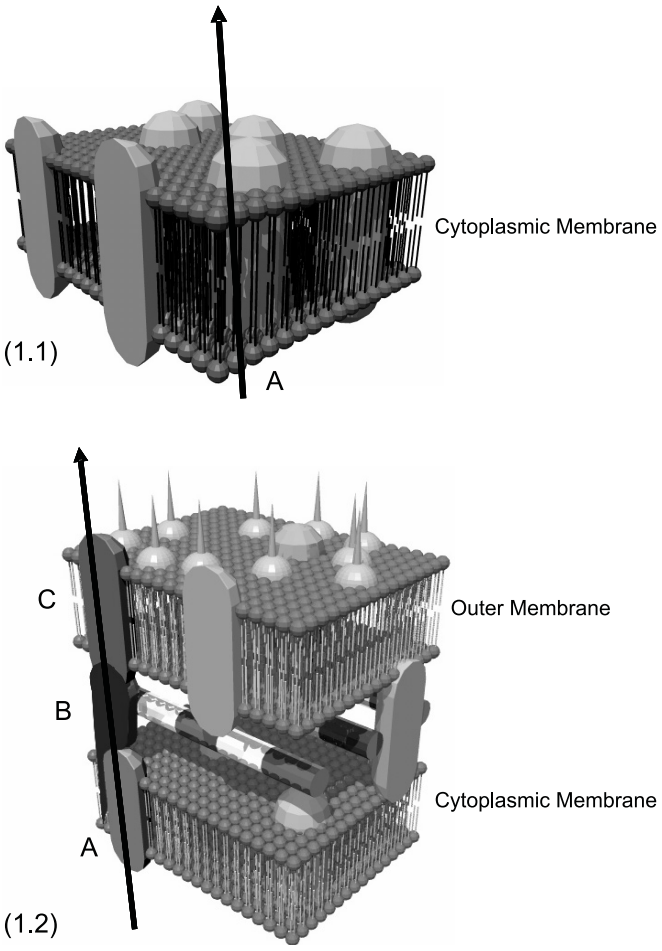

Figure 1. Diagram of the cytoplasmic membrane and the outer membrane of bacteria depicting multidrug efflux systems in Gram-positive bacteria (1.1) and Gram-negative bacteria (1.2). Cytoplasmic membrane protein (A); periplasmatic space protein (B) and outer membrane protein (C). In (1.2) the proteins A, B, and C form the tripartite system to transport the drug through the cytoplasmic membrane and the outer membrane of Gram-negative bacteria. In Gram-positive bacteria (1.1), only one protein is necessary (A) for the drug transport. The arrows indicate the drug efflux.

Concerning clinical aspects of resistance to antimicrobial drugs, RND seems to be the most important family of transporters (89), though others should also be mentioned, such as MATE, represented by the NorA protein of *V. parahaemolyticus*, and its homologue, YdhE, of *E. coli* (66); MFS, such as the *Bacteroides fragilis* protein homologous to NorA (62) and the MdfA of *E. coli* (9), among others.

Members of the RND family were formerly believed to be restricted to Gram-negative bacteria, however they were later

identified in the three domains: *Eukarya*, *Archaea,* and *Bacteria* (115). This family presents low substrate specificity (75), thus being able to transport a wide variety of compounds. The efflux system of the RND/MFP/OMF type was described in several bacteria, including *E. coli*, *Salmonella typhimurium*, *Haemophilus influenzae*, *Neisseria* spp., *P. aeruginosa*, *P. putida*, *Burkholderia* spp., and *Stenotrophomonas maltophilia* (75,89,105). Typically encoded in the chromosome, the system RND/MFP/OMF was found to be plasmid encoded in bacteria of activated sludge (24, 89). The sequences presented 66% of identity with MexC, 79% with MexD, and 67% with OprJ (24, 89), all of them from *P. aeruginosa*.

Proton motive force-driven multidrug transporters of Gram-negative bacteria

In prokaryotes proton motive force driven transports have been the most frequently found and studied.

Five multidrug efflux systems have been described for *P. aeruginosa*: MexAB (92), MexCD (90), MexEF (43), MexXY (60), and AmrAB (126), all belonging to the RND family (96,125). The first component of each system is the MFP, and the second is the cytoplasmic membrane protein that belongs to the transporter family.

Poole *et al*. (92) studied spontaneous mutants of *P. aeruginosa* and observed the production of one outer membrane protein, OprK, which was associated to the increased resistance of that bacterium to a large number of antimicrobial agents bearing very distinct chemical structures, such as ciprofloxacin, nalidixic acid, tetracycline, chloramphenicol, and streptonigrin. The gene encoding OprK, previously identified as *orfC*, is a component of the *orfABC* operon, related to the secretion of pyoverdine, a siderophore produced by *P. aeruginosa* (91). Mutants defective in one of these genes presented increased sensitivity to those antimicrobials (92). The authors designated *mexAB* for the o*rfAB*, since the proteins ORFA and ORFB present homology with the export proteins usually located in the periplasmic space and the cytoplasmic membrane, respectively, while the *orfC* gene encodes a product homologous to the efflux related outer membrane proteins. It was later demonstrated that the outer membrane component of this system is OprM rather than OprK (31). Besides the aforementioned antimicrobials, this system, MexAB-OprM, transports other antibiotics of the classes quinolones, macrolides, and β-lactams, and also transports lincomycin and novobiocin (57). The efflux system MexAB-OprM is highly conserved in environmental and clinic isolates of *P. aeruginosa*, which indicates that the system plays an important role in the intrinsic resistance of this bacterium, independently of its source (8).

Poole *et al.* (90) identified a second efflux operon in *P. aeruginosa*, encoding MexCD-OprJ. That operon may be relevant in providing resistance to newer fluroquinolone antibiotics in clinical strains (90). The system MexCD-OprJ expels from the cell drugs as diverse as quinolones, macrolides, tetracyclines, lincomycin, chloramphenicol, novobiocin and some β-lactams (57)

Kohler *et al.* (43) described the efflux system named MexEF-OprN in *P. aeruginosa*. Isolates which overexpress the proteins of this system increase their resistance to quinolones, chloramphenicol, trimethoprim, and to imipenem, whereas tetracycline is also transported reasonably well by this system (43). Resistance to fluoroquinolones was verified in *P. aeruginosa* isolates which express MexEF-OprN, but there was sensitivity to novobiocin, ceftazidime, cefotaxime, cefoperazone, cefpirone, cefozopran, carbenicillin, aztreonam, and meropenem (49,56). Kohler *et al.* (43) suggested that CCCP, an uncoupler of the proton motive force, could also be a substrate to MexEF-OprN, since the CCCP effect in that transporter was smaller than expected.

The presence of genes similar to *mexE* and *mexF* of *P. aeruginosa* was detected in *E. coli* and in *E. cloacae* by the Southern analysis with probes derived from the referred genes (64). Hybridization occurred with the DNA which corresponds to the chromosome, and the tested bacteria presented multiresistance profiles (64).

The MexXY system confers resistance to acriflavine, ethidium bromide, erythromycin and to the fluoroquinolones; besides, it conferred a certain degree of resistance to tetracycline, chloramphenicol, kanamycin, and to some β-lactams (60). MexXY expression was not observed in wild isolates of *P. aeruginosa*, but it can be induced by subinhibitory concentrations of its substrates, like tetracycline and gentamycin (57). Among the Mex systems, MexXY is the one that confers the strongest resistance to fluoroquinolones (60).

Plasmid curing demonstrated a possibly plasmid-encoded chloramphenicol efflux system, in one of the *E. coli* isolates, bearing homology to the MexXY system of *P. aeruginosa* (64). Loss of plasmid resulted in diminished resistance to chloramphenicol but not tetracycline (64).

Several efflux systems have been identified in *E. coli*, among which are those of the RND family, such as AcrAB (53,54), AcrD (99), and YhiUV (79); those of the MFS family, EmrAB (52), MdfA (25), and EmrKY (80); those of the SMR family, EmrE (MvrC) (65,95,128); and of the MATE family, YdhE (16,66). In some of these, MFP and OMF proteins have already been identified.

The AcrAB multidrug efflux system is the main efflux pump in *E. coli* and it is responsible for the acquisition of multiple antimicrobial resistance of the *mar* mutants, including resistance to tetracycline, chloramphenicol, ampicillin, nalidixic, and rifamicin (82). Initially, it was thought to be related to acriflavine transport (53). The AcrAB system expresses itself constitutively in *E. coli* (58), and the TolC protein, an OMP, participates in the drug transport to the outer medium together with AcrAB (27,44). Nikaido *et al.* (78) identified the AcrAB system in *S.*

typhimurium, which also transported β-lactams with lipophilic side chains.

Pradel and Pagés (94) identified AcrAB-TolC genes in *E. aerogenes*. Intergenic sequence analysis of *acr*A-*acr*B showed similarity between *E. coli* and *E. aerogenes*; in *E. aerogenes,* these two genes probably also form an operon (94).

Evidence of a system similar to AcrAB of *E. coli*, *Salmonella,* and *E. aerogenes* (76,78,94) was detected by the polymerase chain reaction, PCR in 40% of *E. cloacae* isolates obtained from poultry carcasses (64). The primers used were designed according to the sequences of *acrA* and *acrB* from the GenBank (number M94248). Isolates with the expected length of the amplified DNA with the appropriate *acrA* primers also presented expected size amplicons with the *acrB* derived primers (64). The isolates were multiresistant.

The transporter protein AcrD of *E. coli* takes part in the aminoglycosides efflux (99). Deletions in the *acrD* gene resulted in a two to eight-fold reduction of MICs of amikacin, gentamycin, neomycin, kanamycin, and tobramycin, and cells with these deletions accumulated high levels of [H^3]diidroestreptomycin and [H^3]gentamycin,when compared to the parental strain (99).

MdfA is a putative membrane protein with 419 amino acids, product of the *mdfA* gene, found in *E. coli* and is a member of the MSF family (25). The cells which express this protein are more resistant to several compounds such as ethidium bromide, rhodamine, benzalkonium, tetraphenylphosphonium, daunomycin, rifamycin, tetracycline, and puromycin (25). Besides, it also confers resistance to antimicrobials with non-related chemical structures which play a key role in clinical therapy, like chloramphenicol, erythromycin, and certain aminoglycosides and fluoroquinolones (25).

There is a certain degree of similarity among the amino acid sequences of proteins that form multidrug efflux systems from different bacteria (57,111). Mine *et al.* (57) determined the identity and similarity percentages of MexXY to five other multidrug efflux systems. The MexX protein has a 30% to 40% identity and 40% to 60% similarity with the membrane fusion proteins MexA, MexC, AcrA, AcrE, and YhiU; the MexY protein has a 40% to 50% identity and 60% to 70% similarity with the cytoplasmic membrane proteins MexB, MexD, MexF, AcrB, AcrD, and YhiV. Proteins AcrA of *E. aerogenes* and of *E. coli* present 85% identity, and the corresponding AcrB proteins are 87% identical (94).

In relation to substrate specificity, the MexAB system can transport a greater number of antimicrobials, while MexEF is more selective (43). The MexAB-OprM system contributes to intrinsic and acquired resistance in *P. aeruginosa*, whereas MexCD-OprJ and MexEF-OprN contribute only to the acquired resistance of this species (92).

The AmrAB pump is involved in aminoglycoside resistance in clinical isolates of *P. aeruginosa* (126). The *amrAB* genes seemed to be the same as *mexXY* (126).

Genetic organization, expression, and regulation

Overexpression of the efflux pumps can be the result of mutations in a repressor gene (1,124) or the activation of a regulon controlled by a transcriptional regulator like MarA and SoxS of *E. coli* (3, 88).

The first multidrug efflux pump related operon described was *mexAB-oprM*, (92). It contains genes for all the three necessary components for efflux in Gram-negative bacteria (35). However, the three components of the system are not always present in a single operon. Operon *acrAB* of *E. coli*, for instance, does not comprise the gene encoding the outer membrane channel protein (114), but it is acknowledged that there is a channel where the drug is expelled into the extracellular space, the porine TolC (27,44).

The multiresistance phenotype is not always due to functions encoded in one single locus nor is it necessarily due to a specific multidrug exportation system (84). The *marRAB* operon of *E. coli,* for example*,* confers resistance to tetracycline, chloramphenicol, fluoroquinolones, nalidixic acid, rifamycin, penicillin, and others (19), although it does not encode a multidrug efflux system by itself. The expression of multiple resistance chromosomal genes in *E. coli* is regulated by MarA, a transcriptional activator encoded in the *marRAB* operon (3). The constitutive expression of the *marRAB* operon in *E. coli* produces a multiple drug resistance phenotype by means of multiple genetic loci expression, in response to the regulatory proteins of the operon (18). Besides controlling an adaptive response to antibiotics, the chromosomal locus *mar* of *E. coli* also controls the response to other toxic substances in the environment (3). The function of MarB is still unknown. MarR is a repressor, e.g. a negative regulatory protein (104). A specific DNA probe of the *mar* operon hybridizes with the DNAs of *Salmonella* spp., *Shigella* spp., *Klebsiella* spp., *Citrobacter* spp., *Hafnia* spp., and *Enterobacter* spp. under high stringency conditions (20). The *marRAB* operon encoded proteins affect distant chromosomal genes which encode as diverse proteins as the outer membrane porins (100), the drug exclusion system proteins, including efflux system AcrAB (82), as well as proteins involved in superoxide resistance (100). Interestingly, several of these genes are also affected by the expression of *soxRS* genes in response to oxidative stress (6,100).

The *ramA* gene of *Klebsiella pneumoniae*, also present in *E. cloacae* with over 97% of identity (30,29), confer the multiresistance phenotype to non-related antimicrobials, including chloramphenicol, tetracycline, nalidixic acid, ampicillin, norfloxacin, trimethoprim, and puromycin (30). The putative transcriptional activator, which is the product of this gene, is poorly related to the proteins MarA and SoxS of *E. coli* (30,29).

In *P. aeruginosa*, the regulation of the *mexAB-oprM* the operon maybe the best available example of the regulatory complexity of multidrug efflux (35). MexR, a member of the MarR protein family, acts as a transcription repressor of *mexAB-oprM*

(93). MexR auto-regulates its own gene expression by repression of the *mexR* promoter, besides controlling the transcription of the *mexA* promoter (26). A second promoter has been suggested, which would be responsible for the relatively high constitutive expression of *mexAB-oprM*, which contributes substantially to the high intrinsic antibiotics resistance of *P. aeruginosa* (35).

Multidrug transporters are a relatively recent subject of scientific reaserch. New findings and newly available data on genomics and proteomics of resistant bacteria are expected to bring valuable insights to define the role of this mechanism and its ecological and clinical relevance in antibiotic resistance in bacteria.

ABBREVIATIONS

Acr (acridine resistance); Bcr (bicyclomycin resistance); Bmr (multidrug resistance in *Bacillus*); Emr (multidrug resistance in *E. coli*); Lmr (multidrug resistance in *Lactococcus*); Mex (multiple efflux); Mmr (multidrug resistance in *Mycobacterium*); Mtr (multiple transferable resistance); Nor (norfloxacin resistance); Qac (quaternary ammonium compounds); Ram (multiple antibiotics resistance); Smr (multidrug resistance in *Staphylococcus*); Tet (tetracycline); OMR (outer membrane factor); MFP (membrane fusion protein).

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