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Efflux-Mediated Antibiotic Resistance in *Acinetobacter* spp.

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ABSTRACT

Among Acinetobacter spp., A. baumannii is the most frequently implicated in nosocomial infections, in particular in intensive care units. It was initially thought that multidrug resistance (MDR) in this species was mainly due to horizontal acquisition of resistance genes. However, it has become recently obvious that increased expression of chromosomal genes for efflux systems plays a major role in MDR. Among the five super families of pumps, resistance-nodulation-division (RND) systems are the most prevalent in multiply resistant A. baumannii. RND pumps typically exhibit a wide substrate range that can include antibiotics, dyes, biocides, detergents, and antiseptics. Overexpression of AdeABC, secondary to mutations in the adeRS genes encoding a two-component regulatory system, constitutes a major mechanism of multi-resistance in A. baumannii. AdeIJK, intrinsic to this species, is responsible for natural resistance but, since overexpression above a certain threshold is toxic for the host, its contribution to acquired resistance is minimal. Recently described AdeFGH, probably regulated by a LysR-type transcriptional regulator, also confers multidrug resistance when overexpressed. Non-RND efflux systems, such as CraA, AmvA, AbeM, and AbeS have been also characterized in A. baumannii as well as AdeXYZ and AdeDE in other Acinetobacter spp. Finally, acquired narrow spectrum efflux pumps, such as the MFS TetA, TetB, CmlA, FloR, and the SMR QacE in Acinetobacter spp. have been detected and mainly encoded by mobile genetic elements.
Acinetobacter baumannii, a gram-negative coccobacillus, is a worldwide nosocomial pathogen responsible for opportunistic infections such as pneumonia, infections of the urinary tract, bloodstream, and skin and soft tissue. A. baumannii constitutes a major public health problem due to its propensity to develop resistance to numerous drugs (17, 50) and isolates exhibiting multidrug, sometimes pandrug, resistance are emerging in clinical settings. Antibiotic resistance combined with the ability to persist in hospital environments are responsible for small epidemics of A. baumannii clones. This species exhibits broad intrinsic resistance, mainly conferred by a chromosomally-encoded cephalosporinase, basal level expression of efflux pumps, and a low membrane permeability (66). The most common mechanisms for resistance involve enzymatic degradation of the drugs, modification or protection of the target, and decreased permeability or active efflux of the antibiotics. At the genetic level, resistance is acquired either by horizontal transfer of genetic elements carrying resistance determinants or by mutation in endogenous genes leading to inactivation, modification, or overexpression of cellular functions. Of particular importance are mechanisms leading to multidrug resistance following a single genetic event: (i) horizontal acquisition of an element carrying several resistance genes or (ii) overexpression of a chromosomally-encoded efflux system. Mobile genetic elements, such as plasmids and transposons, are a common feature of multidrug resistance in A. baumannii (19) and resistance islands, i.e. acquired elements inserted in the chromosome and organized in a mosaic structure, have been described to carry as many as 45 resistance genes (18). Although mobility of the latter has not been demonstrated, there is indirect evidence for their dissemination among clinical isolates (60).

Efflux-mediated resistance has been reported in many bacterial genera (44, 45). Overexpression of an efflux system, responsible for reduction in the accumulation of the antibiotic, is an efficient mechanism for drug resistance (44). Genes encoding these systems
are carried either by genetic elements, e.g. the TetA and CmlA efflux pumps for resistance to tetracycline and chloramphenicol, respectively, or by the chromosome and thus can be responsible for acquired or intrinsic resistance when overexpressed. Five super families of efflux systems are associated with drug resistance: the ATP-binding cassette (ABC) transporters, the small multidrug resistance (SMR), multidrug and toxic compound extrusion (MATE), major facilitator superfamily (MFS), and the Resistance-Nodulation-cell Division (RND) family. As opposed to single component efflux systems that confer resistance to a small number of compounds, such as the tetracycline transporters, the RND systems, composed of an inner membrane protein (RND pump) linked by a major fusion protein (MFP) to an outer membrane factor (OMF), are able to extrude a wide range of substrates often unrelated in structure (44, 46, 52). They are the most clinically relevant pumps conferring multidrug resistance in gram-negative bacteria as they allow to cross both the inner and the outer membranes.

This review is devoted to efflux-mediated resistance in *Acinetobacter* spp., mainly focusing on RND systems in *A. baumannii*, their substrates, clinical importance, and regulation of expression. Other chromosomally-encoded efflux systems in *A. baumannii*, as well as intrinsic pumps in other *Acinetobacter* spp. and acquired efflux systems, will be mentioned.

**CHROMOSOMALLY-ENCODED EFFLUX SYSTEMS IN *A. BAUMANNII***

Several *A. baumannii* genome sequences are available and indicate a high content of efflux genes. Depending on the strain and annotation, *A. baumannii* chromosome encodes 7 RND, more than 30 MFS, and several MATE, SMR, and ABC efflux systems (1, 32, 61, 65). To date, three RND, two MFS, and a member of the MATE and SMR families have been demonstrated to be involved in antibiotic efflux.
RND efflux systems

AdeABC. AdeABC (for *Acinetobacter* drug efflux) is the first characterized RND system in *A. baumannii* (38). The adeABC operon encodes the AdeA MFP, the multidrug transporter AdeB, and the AdeC OMF (Fig. 1). This operon is not expressed in natural isolates *A. baumannii* and the MDR phenotype is due to overexpression of the pump (38). Expression of adeABC is tightly regulated by the two-component regulatory system AdeR-AdeS (40) encoded by the adeRS operon located upstream from adeABC and transcribed in the opposite direction (Fig. 1).

Resistance phenotypes associated with AdeABC. Inactivation experiments in an overexpressing clinical isolate indicated that AdeABC confers resistance by extruding aminoglycosides, β-lactams, fluoroquinolones, tetracyclines, tigecycline, macrolides, chloramphenicol, and trimethoprim (Table 1) (14, 38). Decreased susceptibility to netilmicin has been correlated with overexpression of AdeABC in a large collection of clinical strains (43). Cefepime, cefpirome, and cefotaxime are the most affected β-lactams with little impact on other members of this drug class (7, 23). The role of AdeABC in carbapenem resistance is controversial. Susceptibility testing, with and without efflux pump inhibitors (EPI) such as carbonyl cyanide *m*-chlorophenylhydrazone (CCCP), reserpine, 1-(1-naphthylmethyl)-piperazine (NMP), and phenyl-arginine-β-naphtylamide (PAβN), showed no differences in carbapenem activity (6, 53, 54), whereas a two- to eight-fold reduction in resistance was found in other works when an EPI was added, suggesting involvement of efflux (27, 33, 34, 48). Overexpression of AdeABC contributes to significantly higher level carbapenem resistance, notably to imipenem and meropenem, when associated with various class D carbapenemases (23). However, contribution of AdeABC to resistance to meropenem but not to imipenem was observed following inactivation of the pump in OXA-23-producing strains.
Evidence for AdeABC-mediated efflux of carbapenems is further suggested by the correlation between overexpression of the adeB gene and resistance level in clinical isolates (28, 34). A decrease in imipenem MIC in the presence of CCCP was detected against in vitro mutants and clinical strains (27). However, no overexpression of adeB was detected in these strains indicating that, either another system extrudes carbapenems, or that a mutation in AdeABC expands the substrate profile but not the level of expression of the system. Thus, AdeABC overexpression contributes to carbapenem resistance but other efflux mechanisms are also probably involved. Efflux does not itself confer high-level resistance but increases weakly the MICs allowing bacteria to reach high-level resistance when associated with other mechanisms.

Upon exposure to NaCl, A. baumannii expresses low-level resistance to carbapenems, aminoglycosides, quinolones, and colistin associated with overexpression of adeA, together with that of other pump genes (25). Osmotic stress could represent a natural inducer for expression of efflux systems.

**Epidemiology of resistance due to AdeABC.** The adeABC operon is present in ca. 80% (from 53% to 97%) of A. baumannii (7, 9, 29, 31, 36, 43, 63). It has not been found in environmental strains (30) and appears to be mainly associated with clinical isolates. A sequence-based typing of adeB has been proposed as an epidemiological tool for clinical strains (30). Correlation between overexpression of adeB and decreased susceptibility to tigecycline was found in 5/40 (12.5%) MDR isolates (7). AdeABC-overexpressing strains have been selected in vivo under treatment by tigecycline (49) and also probably by ciprofloxacin (24); one-step in vitro mutants have been obtained with subinhibitory concentrations of tigecycline (49), moxifloxacin (47), and gentamicin (12, 40). These data highlight the propensity of A. baumannii to achieve multidrug resistance by overexpression of the pump when exposed to its substrates. Screening for expression of antibiotic resistance
genes using a microarray found overexpression of adeABC, associated with acquired resistance genes, in all the three MDR clinical isolates tested (12).

AdeC has been shown not to be essential for the MDR phenotype conferred by the pump, as an adeC-inactivated mutant displays resistance to the substrates of AdeABC similar to that of the parental strain (40). The gene for the outer membrane protein was not found in ca. 41% (48/116) of clinical isolates carrying adeRS-adeAB (43) suggesting that AdeAB could recruit another outer membrane protein to form a functional tripartite complex, as observed for the MexXY pump with OprM in *P. aeruginosa* (3, 41).

**Regulation of adeABC expression by AdeRS.** The proteins encoded by adeR/adeS are highly similar to those of various two-component regulatory systems (38). The signal recognized by AdeS, the mechanism of regulation of adeABC by adeRS, and the DNA-binding site of AdeR remain to be investigated. However, mutations in AdeRS have been shown to be responsible for constitutive expression of AdeABC (12, 40) whereas inactivation of adeR or adeS confers susceptibility (40), suggesting that AdeR functions as a transcriptional activator. A proline to leucine substitution at position 116 of AdeR is responsible for adeABC overexpression likely due to modification in the structural conformation of the regulator (40), as observed in other two-component systems. Another adeABC-overexpressing mutant exhibits a threonine to methionine substitution at position 153 of AdeS (40). This residue is located in the histidine box, four amino acids downstream from the conserved histidine residue. Substitutions at the corresponding position in other sensor kinases abolish phosphatase activity and the mutation in AdeS is likely to result in lack of dephosphorylation of AdeR, leading to a constitutively activated system. A mutation at position 30 of AdeS, replacing a glycine by an aspartate, has been reported to lead to adeABC overexpression in a single-step mutant (12). This residue is located in the periplasmic loop of the sensor, potentially involved in the recognition of the signal that activates the system; the
AdeS. An adeABC-overexpressing clinical isolate obtained under tigecycline therapy exhibited an alanine to valine substitution at position 94 of AdeS when compared to the parental strain (26). This residue is located in the linker domain typically involved in signal transduction between the recognition domain and the histidine box and mutations in this domain in other sensors have been associated with constitutive phenotypes (16). A mutant highly resistant to tigecycline with higher overexpression of adeABC was obtained from this strain (26) and carried two additional substitutions in AdeS (a glycine to aspartate at position 103) and AdeR (an alanine to valine at position 91). However, the mutant was obtained after serial exposure to tigecycline and the role of every mutation has not been ascertained. Six amino acid differences have been found between AdeRS of two tigecycline-susceptible strains indicating that AdeR and AdeS display sequence variability and that only some mutations lead to overexpression of adeABC (48). Transposition of an ISAbal copy into adeS can also lead to overexpression of the system as observed in a clinical isolate (59). Transcriptional activation could be due to disruption of adeS or to the bringing by ISAbal of a strong promoter for adeABC expression. Finally, adeABC-overexpressing mutants that did not carry any mutations in adeRS when compared with their isogenic parents have been reported (26, 48) suggesting that other regulatory mechanisms can be involved. Mutations in the adeABC promoter region could affect the binding of AdeR.

AdeIJK. AdeIJK, encoded by the adeIJK operon (Fig. 1), is the second RND efflux system described in A. baumannii (14). This pump is specific for the species (36, 55) where it contributes to intrinsic resistance to β-lactams, such as ticarcillin, cephalosporins and aztreonam, fluoroquinolones, tetracyclines, tigecycline, lincosamides, rifampin, chloramphenicol, co-trimoxazole, novobiocin, and fusidic acid (Table 1) (14). Aminoglycosides are not substrates for the pump (12). AdeIJK was found to act in a
synergistic fashion with AdeABC to extrude compounds such as tigecycline: inactivation of AdeIJK or of overexpressed AdeABC confers 3- and 8-fold decrease in tigecycline MIC, respectively, whereas inactivation of both pumps leads to a 85-fold decrease (14).

It was initially thought that overexpression of AdeIJK was toxic for the host cell (14). However, spontaneous low-level resistant mutants overexpressing AdeIJK have been obtained on drug gradients of tetracycline or cefotaxime (12). Levels of adeIJK overexpression, detected by a transcriptomic microarray and quantitative RT-PCR, were always lower than those observed for adeABC overexpression, suggesting that AdeIJK can be overexpressed only under a certain threshold and is then toxic (12).

No regulatory genes are adjacent to the adeIJK operon and no mutations have been detected in the putative promoter region of adeIJK-overexpressing mutants (12). Thus, regulation of the pump and the genetic events leading to overexpression remain unknown.

**AdeFGH.** A third RND efflux pump, AdeFGH, encoded by the adeFGH operon (Fig. 1), confers multidrug resistance when overexpressed (12, 13). An ORF for a putative LysR-type transcriptional regulator (LTTR), named adeL, is located upstream from the adeFGH operon and transcribed in the opposite direction (Fig. 1) (13). A helix-turn-helix (HTH) DNA-binding motif, typical of the LTTR family, is present between residues 11 and 32. The sequence of the adeL-adeF intergenic region suggests the presence of overlapping promoters for adeL and adeFGH expression (13) including a TTA-N7-TAA motif typical of a LTTR box which is implicated in DNA-binding by LTTRs (37).

Inactivation of the pump in an adeFGH-overexpressing mutant indicated that it confers high level resistance to fluoroquinolones, chloramphenicol, trimethoprim, clindamycin and decreased susceptibility to tetracyclines, tigecycline, and sulfamethoxazole, without affecting β-lactams and aminoglycosides (Table 1) (13). The structural genes for the AdeFGH pump and its putative AdeL regulator have been found in the seven *A. baumannii* sequenced and in
40 out of 44 clinical isolates of *A. baumannii*, the four remaining strains lacking the *adeG*- *adeH* genes (13). AdeFGH does not contribute to intrinsic resistance since it is not constitutively expressed in wild-type strains.

Three spontaneous MDR mutants overexpressing *adeFGH* have been obtained on drug gradients of norfloxacin or chloramphenicol (13). Two of them exhibit alterations of the C-terminal domain of AdeL: a threonine to lysine substitution at position 319 or a deletion of the last 11 residues. Mutations in this region have been shown in other LTTRs to impair oligomerization and interaction of the regulator with the RNA polymerase (15). A third mutant carries a valine to glycine substitution at position 139, in a domain putatively involved in the recognition of the signal, that could be responsible for a signal-independent activation of AdeL.

**RND efflux pumps and tigecycline.** Tigecycline is a glycylcycline active against a broad range of gram-positive and gram-negative bacteria, including *Acinetobacter* spp. and anaerobes (51). In *A. baumannii*, tigecycline is a substrate for the three characterized RND pumps described above (13, 14). AdeABC is, however, the only system to date to have been involved in resistance of clinical isolates (26, 48, 58, 59). Ruzin et al. showed that, among a collection of 106 *A. baumannii*, overexpression of AdeABC is a prevalent mechanism for decreased susceptibility to tigecycline and reported a linear relationship between log-transformed *adeA* expression and tigecycline MIC in the range of 0.5 to 16 µg/ml (58). Increase in the MIC of tigecycline without expression of *adeA* has also been reported, indicating the involvement of another mechanism (58).

**RND systems and non-antibiotic compounds.** As already mentioned, RND efflux systems typically exhibit wide substrate ranges. AdeABC and AdeIJK extrude, in addition to antibiotics, several biocides, detergents, antiseptics, and dyes (14, 38, 55). AdeFGH is able to export SDS, acridine orange, safranine O, and ethidium bromide (13).
In a study on the susceptibility of *A. baumannii* to triclosan, none of the 20 clinical isolates with reduced susceptibility exhibited overexpression of *adeB* or *adeJ* (9). Resistance to triclosan was associated with either overexpression of *fabI*, encoding an enoyl-acyl carrier protein reductase, target of the biocide, or to point mutations in this gene. However, a decrease in resistance was observed in the presence of PAβN indicating the potential involvement of an efflux system.

**Non-RND efflux systems**

**CraA.** CraA (for chloramphenicol resistance *Acinetobacter*) is homologous to the MdfA efflux pump of *E. coli* that extrudes only chloramphenicol (57). Inactivation of CraA in *A. baumannii* results in a 128-fold decrease in chloramphenicol resistance. The system has been found in all 82 *A. baumannii* tested and is believed to contribute to intrinsic resistance to chloramphenicol but it is not known if it is constitutively expressed or confers resistance only after overexpression. The structural gene for the pump, together with other efflux genes, has been found overexpressed in response to exposure to NaCl (25).

**AmvA.** A second MFS pump, AmvA, has been characterized recently (55). This 14-transmembrane domain system extrudes mainly dyes, disinfectants, and detergents. Erythromycin is the only antibiotic significantly affected with a 4-fold decrease in MIC when the structural gene is inactivated. The *amvA* gene is present in all *A. baumannii* studied and overexpressed in isolates exhibiting higher drug resistance.

**AbeM.** AbeM, a member of the MATE family, extrudes aminoglycosides, fluoroquinolones, chloramphenicol, trimethoprim, ethidium bromide, and dyes (64). Its relevance to multidrug resistance of *A. baumannii* remains hypothetical since the system has been studied only in *E. coli*. The structural gene for AbeM was found in all clinical isolates
tested without any correlation with antibiotic resistance, even in isolates overexpressing the abeM gene (7, 9), suggesting a weak impact of this system.

AbeS. AbeS, a chromosomally-encoded SMR efflux pump displaying homology with the EmrE system of E. coli, has been recently characterized in a MDR clinical isolate of A. baumannii (62). Inactivation of and trans-complementation with the abeS gene indicated that it confers low-level resistance to chloramphenicol, fluoroquinolones, erythromycin, novobiocin (3- to 8-fold decrease when the system is inactivated) and resistance to dyes and detergents. Among four sequenced A. baumannii, abeS was found highly conserved in three and absent in the remaining strain.

CHROMOSOMALLY-ENCODED EFFLUX SYSTEMS IN OTHER ACINETOBACTER SPP.

The Acinetobacter genus comprises a minimum of 21 defined species (5). To date, studies have focused mainly on the sequenced strains Acinetobacter baylyi ADP1 and Acinetobacter genomic DNA group 3 (GDG 3), a species frequently associated with hospital-acquired infections in Southeast Asia.

AdeXYZ. An efflux system, AdeXYZ, which shares more than 97% identity with AdeIJK (14) has been found in Acinetobacter GDG 3 (10). This pump is present in ca. 90% of the strains of the species. Attempts to disrupt the adeY gene failed but the homology with AdeIJK suggests that it could have a similar function, i.e. intrinsic resistance and probable acquisition of higher levels of resistance by overexpression. AdeX, Y, and Z share 80, 89, and 87% amino acid identity, respectively, with the MFP, RND, and OMF proteins of an efflux system from A. baylyi ADP1 (10) which has been shown to contribute to intrinsic resistance of the species to β-lactams, ciprofloxacin, tetracycline, rifampin, and chloramphenicol (20), a
substrate range consistent with that of an AdeIJK-like system. The adeY gene has also been found in an isolate of Acinetobacter GDG 13TU and of Acinetobacter GDG17 (10).

**AdeDE.** Using degenerate primers to detect putative adeB-like genes, Chau et al. have characterized the AdeDE system in Acinetobacter GDG3 (8). No genes for an OMF were found downstream from the adeDE gene cluster, suggesting that another outer membrane protein could be recruited to form a tripartite efflux pump. The AdeDE system, which displays less than 45% identity with AdeAB, extrudes aminoglycosides, carbapenems, ceftazidime, fluoroquinolones, erythromycin, tetracycline, rifampin, and chloramphenicol (8). Inactivation of adeE in a clinical isolate leads to a greater than four-fold increased susceptibility to these antibiotics. The adeE gene was found in ca. 70% of Acinetobacter GDG 3 strains, in one Acinetobacter GDG 13TU, and one Acinetobacter GDG 17 (10).

**ACQUIRED EFFLUX SYSTEMS IN ACINETOBACTER SPP.**

In addition to the above chromosomally-encoded efflux systems, several reports have described the presence of acquired efflux pumps in Acinetobacter spp. isolated in hospital settings or from the environment (66). The corresponding structural genes are part of plasmids, transposons, or resistance islands. In the A. baumannii AYE strain, eight efflux genes are carried by resistance island AbaR1 (18). There is evidence for horizontal transfer of a floR-tet(G) region among A. baumannii, Salmonella enterica, and P. aeruginosa, to form the AbaR1 island, the Salmonella Genomic Island 1, and the Tn6061 transposon, respectively (11).

**Tetracycline.** Several Tet efflux pumps of the MF superfamily conferring tetracycline resistance have been acquired by clinical isolates of A. baumannii (66). TetA and TetB are the most prevalent, TetA conferring resistance to tetracycline only, TetB extruding in addition minocycline. The tet(B) gene was found in at least 50% of tetracycline-resistant A. baumannii
isolates, and *tet(A)* between 14% and 46% (22, 31, 39, 66). The genetic basis for these genes remains mostly unknown. A partially characterized Tn1721-like transposon contains the *tetR* and *tet(A)* genes, encoding, respectively, a regulatory and a resistance protein (56), and *tet(B)* is carried by 5- to 9-kb plasmids in MDR *A. baumannii* (63). The Tet39 determinant, conferring resistance to tetracycline but not to minocycline, was characterized in a clinical and in eleven environmental isolates of *Acinetobacter* spp. (2). The *tet(39)* and *tetR* genes, for the pump and its regulator, are located on ca. 25- to 50-kb transferable plasmids. Tet39 was recovered in 75% of a collection of 222 oxytetracycline-resistant *Acinetobacter* spp. isolated from fish farms in Thailand (2). Among 80 tetracycline-resistant isolates belonging to the *A. baumannii*-*A. calcoaceticus* complex, *tet(A)* was found in 17 strains, *tet(B)* in 15, and *tet(39)* in 34, suggesting that Tet39 is a relevant tetracycline resistance mechanisms in clinical strains (4). To date, Tet39 remains closely associated with *Acinetobacter* spp., but its plasmid location should enable dissemination to other species. A strain of *A. radioresistens* harboring a *tet(H)* gene was isolated from a fish farm (42). The gene, associated with an IS1597 copy, is located on a plasmid but transfer attempts to *E. coli* were unsuccessful. Finally, the *tet(G)* and *tetR* genes are part of the AbaR1 resistance island, which also carries a Tn1721-like transposon encoding TetA and TetR in *A. baumannii* strain AYE (18). The hypothetical presence of resistance islands in ca. two thirds of *A. baumannii* suggests a potential wider distribution of TetG in *A. baumannii*.

**Florfenicol/chloramphenicol.** The structural genes for the MFS CmlA and FloR efflux systems conferring resistance to phenicols have been found in *A. baumannii* AYE as part of the AbaR1 resistance island (18). This is, to the best of our knowledge, the only report of acquired chloramphenicol efflux genes in this species.

**Quartenary ammonium.** Four copies of the *qacE* gene are part of the AbaR1 resistance island (18) and the gene was detected in 41% of 86 *A. baumannii* (55); no other *qac*...
alleles were detected. The qacE gene, encoding a SMR efflux pump, is typically carried by type 1 integrons where it forms, together with the sulfonamide resistance gene sul1, the 3’ conserved region. Presence of the gene in A. baumannii could thus reflect, in addition to quartenary ammonium resistance, a type 1 integron frequently associated with multidrug resistance.

CONCLUSION

Comparison of the resistance levels of a clinical A. baumannii MDR strain and of mutants overexpressing one of three RND pumps (Table 1) confirms that efflux is a major factor for resistance to various drug classes including β-lactams, chloramphenicol, macrolides, tetracyclines, and the aminoglycosides, high-level resistance to fluoroquinolones requiring additional mechanisms such as alteration of DNA type II isomerases.

Most of the systems possess physiological functions and are believed to play a role in the homeostasis of the bacterial cell. Increased knowledge of these systems, and particularly of the RND family, could help us to decipher their natural functions, the mechanism of substrate recognition and extrusion, and provide clues to block or alter these machineries. Comprehension of the regulation of their expression is another goal. Much more is known about efflux systems in P. aeruginosa or in E. coli than in Acinetobacter spp. and it becomes evident that a complex and intricate network of regulation governs the efflux capacity of bacteria. Interplay or compensatory mechanisms between efflux systems could exist, such as the influence of MexAB-OprM on the expression of MexCD-OprJ and MexEF-OprN in P. aeruginosa (35), and complex and multi-entry pathways must regulate these functions, AcrAB-TolC in E. coli being the best example (21).

A. baumannii is a typical opportunistic pathogen of hospital settings. Combining acquisition of resistance genes and overexpression of efflux pumps provides a successful strategy to survive, adapt, and be selected in this environment. As new antibiotics active
against gram-negative bacteria are scarce, improved hygiene procedures and optimal drug use are necessary to limit the selection and dissemination of such microorganisms.
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FIG. 1. Schematic representation of operons for RND efflux systems in A. baumannii. Open arrows represent coding sequences and indicate direction of transcription.
Table 1. Antibiotic susceptibility of MDR or non-MDR A. baumannii and derivatives deleted for or overexpressing an RND efflux pump

<table>
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<tr>
<th>Antimicrobial agent</th>
<th>BM4454&lt;sup&gt;a&lt;/sup&gt;</th>
<th>BM4555&lt;sup&gt;b&lt;/sup&gt; (adeABC)&lt;sup&gt;c&lt;/sup&gt;</th>
<th>BM4652&lt;sup&gt;d&lt;/sup&gt; (adeABCΔIJK)</th>
<th>BM4664&lt;sup&gt;e&lt;/sup&gt; (adeABCΔIJK adeFGH)&lt;sup&gt;f&lt;/sup&gt;</th>
<th>BM4669&lt;sup&gt;g&lt;/sup&gt; (adeABCΔIJK adeFGH)</th>
<th>BM4587&lt;sup&gt;h&lt;/sup&gt; adeABC&lt;sup&gt;i&lt;/sup&gt;</th>
<th>BM4665 adeIJK&lt;sup&gt;j&lt;/sup&gt;</th>
<th>BM4666 adeFGH&lt;sup&gt;k&lt;/sup&gt;</th>
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<sup>a</sup> MICs were determined by the E-test procedure (bioMérieux, Marcy l’Etoile, France).
<sup>b</sup> MDR clinical isolate overexpressing adeABC
<sup>c</sup> Derivative of BM4454 deleted for adeABC
<sup>d</sup> Derivative of BM4454 deleted for adeABC and adeIJK
<sup>e</sup> Derivative of BM4652 overexpressing adeFGH
<sup>f</sup> Derivative of BM4454 deleted for adeABC, adeIJK, and adeFGH
<sup>g</sup> Non-MDR clinical isolate
<sup>h</sup> Mutant of BM4587 overexpressing adeABC
<sup>i</sup> Mutant of BM4587 overexpressing adeIJK
<sup>j</sup> Mutant of BM4587 overexpressing adeFGH
<sup>k</sup> NA, not assessed because of the presence of an apramycin resistance cassette.