ESTUDI DELS MECANISMES DE RESISTÈNCIA MÚLTIPLE ALS ANTIBIÒTICS EN *Morganella morganii*

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Barcelona 2006

ANNEX

ELSEVIER

International Journal of Antimicrobial Agents xxx (2006) xxx–xxx

INTERNATIONAL JOURNAL OF Antimicrobial Agents

www.ischemo.org

Integron presence in a multiresistant *Morganella morganii* isolate

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Received 24 November 2005; accepted 12 January 2006

Abstract

A multiresistant strain of *Morganella morganii* was isolated from a patient affected by several severe pathologies. The isolate was found to be resistant to the following antimicrobials: ampicillin, nalidixic acid, cefalothin, cefoxitin, ceftriaxone, ciprofloxacin, chloramphenicol, streptomycin, erythromycin, gentamicin, novobiocin, penicillin, rifampicin, tetracycline and violet crystal. Mechanisms leading to this multiresistance were studied. Porins of *M. morganii* multiresistant and wild-type strains were analysed by sodium dodecylsulphate–polyacrylamide gel electrophoresis (SDS–PAGE) and were characterised by their ability to form channels in planar black lipid bilayers. The channels formed by porins from multiresistant and susceptible strains suggested that the porins of the multiresistant strain were not responsible for resistance. A 6.6 kb plasmid (pML2003) was detected, isolated and studied. PML2003 included two integrons. Direct sequencing revealed that one of the integrons contained two cassettes, aminoglycoside adenyltransferase (*aadB*) and chloramphenicol acetyltransferase (*catB3*) conferring resistance to aminoglycosides and chloramphenicol, respectively. The second integron contained carbenicillinase (*blaP1b*) and adenyltransferase (*aadA2*), which confer resistance to β-lactamases and streptomycin, respectively.

Keywords: *Morganella morganii*; SDS–PAGE; Multiresistance

1. Introduction

The opportunistic pathogen *Morganella morganii* is commonly isolated from human faeces and is frequently involved in urinary tract infections [1]. It is currently isolated from patients located in hospital Intensive Care Units [2]. Moreover, it has been involved in various infectious processes such as neonatal septicaemia [3], abdominal abscesses, biliary infections and primary bacteraemia [4–6]. In compromised patients, *M. morganii* has been involved in chorioamnionitis, diabetic foot infections, pyomyositis [7], pyoarthritis, pericarditis and meningitis [8]. Nosocomial infections have been also reported. It is assumed that this species is intrinsically susceptible to most of the antibiotics active against Gram-negative bacilli, such as aminoglycosides, carbenicillin, chloramphenicol, ciprofloxacin and acid nalidixic, but is resistant to fosfomycin, colistin and some β-lactams, usually owing to a chromosomal cephalosporinase [9–12]. The role of salicylate in decreasing the production of β-lactamase has been documented [13]. Other resistances to β-lactams related to mutational overproduction of the species-specific AmpC enzyme [14] and the occurrence of an extended-spectrum β-lactamase in *M. morganii* have been reported [15].

Integrons are site-specific recombination systems able to capture and mobilise antibiotic resistance genes and play a major role in the dissemination of antibiotic resistance genes in Gram-negative bacteria [16,17]. The essential components of the integron include the *intI* gene, a recombination site (*attI*) and a promoter (P_{ant}), which code for an integrase, insertion of gene cassettes and transcription of the inserted cassette, respectively. Gene cassettes usually contain a single open reading frame and a recognition site for the integrase

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doi:10.1016/j.ijantimicag.2006.01.006

known as the 59-base element [18]. These cassettes encode for resistance to antimicrobial agents. Integrons have been found in transposons and plasmids as well as in the chromosome [19] and are frequently found in Enterobacteriaceae [17]. A class 2 integron with *dfrA1-sat-aadA1* gene cassettes has been reported in *Morganella* spp. [20].

Outer membrane porins are essential for the uptake of hydrophilic nutrients into Gram-negative bacteria [21,22]. They are β-barrel channel-forming proteins of the outer membrane of Gram-negative bacteria, mitochondria and chloroplasts. The transmembrane pores produced by these proteins are formed by antiparallel amphipathic β-strands arranged in a barrel configuration. Many of these porins are either non-specific, such as OmpF or OmpC, or only moderately selective, such as PhoE of *Escherichia coli*. Their combined total number present in the membrane remains constant, whilst the amount of each type varies according to various external factors. In the past it has been demonstrated that mutations of porins could result in antibiotic resistance [23]. Mechanisms of multiresistance of the clinical isolate *M. morganii* HUB198351 were investigated.

2. Materials and methods

2.1. Bacterial strain and culture conditions

Morganella morganii HUB198351 was isolated from specimens of blood and urine obtained from a 73-year-old man affected by insulin-dependent type II diabetes mellitus and who was a smoker. Pulmonary obstructive disease and a squamous cell carcinoma of the trachea (T4 N3 M0) were diagnosed before his admittance to hospital. The patient was treated with chemotherapy and radiotherapy. He was admitted for respiratory alteration due to overinfection by *Aeromonas hydrophila*, urinary tract infection by *M. morganii* and finally bacteraemia by multiresistant *M. morganii*. Exitus laetalis occurred 24 h after admission. Identification and preliminary antibiotic susceptibility tests were accomplished using a Microscan system (Sacramento, CA). The strain was chosen for this study because such multiresistance is unusual in this species. *Morganella morganii* susceptible strain (CECT 173) was obtained from Spanish Type Culture Collection (corresponding to ATCC 25830). Bacteria were cultured in trypticase soy broth (TSB) or on trypticase soy agar. Antimicrobial susceptibility was determined in Muller–Hinton broth. Bacteriological media were purchased from Sharlau (Barcelona, Spain).

2.2. Antimicrobial agents and minimum inhibitory concentration (MIC) determinations

MICs of antimicrobial agents were determined by microdilution according to the guidelines of the National Committee for Clinical Laboratory Standards [24,25]. The following antimicrobials were tested: ampicillin, nalidixic acid, cefalothin, cefoxitin, ceftriaxone, ciprofloxacin, chloramphenicol, streptomycin, erythromycin, gentamicin, novobiocin, penicillin, rifampicin, tetracycline and violet crystal. All antibiotics were purchased from Sigma Chemical Co. (St. Louis, MO).

2.3. Ciprofloxacin accumulation

Ciprofloxacin accumulation was measured spectrofluorometrically using the method described by Mortimer and Piddock [26] with some modifications [27]. Isolates were incubated at 37 °C in TSB until $A_{600\ nm} = 0.5–0.7$. Bacteria were harvested by centrifugation (9000 × *g*) at room temperature, washed and concentrated 10-fold in phosphate buffer saline (PBS; pH 7.5). Bacteria were incubated for 10 min at 37 °C and ciprofloxacin was added at a final concentration of 10 mg/mL. After addition of ciprofloxacin, 1 mL samples were removed at various time intervals. Three minutes after addition of ciprofloxacin, the efflux pump inhibitor carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) (final concentration 100 μM) was added when necessary. The samples were immediately diluted in 1 mL of ice-cold PBS and centrifuged for 1 min at 11 000 rpm. Pellets were re-suspended in 1 mL of 0.1 M glycine buffer at pH 3.0 and finally incubated at room temperature overnight to allow bacterial lysis. Thereafter, the suspensions were centrifuged at 20 °C for 15 min to remove bacterial debris. Fluorescence of the supernatant was measured using a SLM Aminco 8100 spectrofluorometer (SLM-Aminco, Urbana, IL).

2.4. Plasmid detection and isolation

Plasmid presence was first detected by pulsed-field gel electrophoresis (PFGE) following the method reported by Barton et al. [28]. The plasmid was isolated by the procedure of Birnboim and Doly [29]. *Morganella morganii* HUB198351 was grown at 37 °C in TSB before separation in 0.7% agarose gel by PFGE at 85 V for 2 h. Gels were stained with ethidium bromide.

2.5. Polymerase chain reaction (PCR) amplification

The plasmid was analysed for the presence of gene cassettes associated with class 1 integron structures using a modified version of the PCR assay described by Lévesque et al. [30]. PCR was carried out in 100 μL volumes containing 10 μL of 10× PCR buffer (100 mM Tris–HCl pH 9.0, 500 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM each dNTP), 25 pmol each of the *int1* forward primer (5′-GGCATCCAAGCAGCAAGC-3′) and the *int1* reverse primer (5′-AAGCAGACTTGACCGAT-3′), 1 U of *Taq* DNA polymerase (Fermentas, Vilnius, Lithuania), 2 μL of plasmid and 28 μL of sterile distilled water. Thermal cycling reaction parameters included an initial denaturation at 94 °C for 5 min, followed by 35 cycles of 94 °C for 1 min, 55 °C for 1 min and 5 min of extension at 72 °C. Five seconds was added to the

extension time at each cycle. A final extension at 72 °C was carried out for 10 min and following this step all completed reactions were maintained at 4 °C. The PCR products were visualised by ethidium bromide staining after agarose gel electrophoresis as described elsewhere.

2.6. DNA sequence analysis

Any amplified PCR product of interest was initially gel extracted using a Qiagen gel extraction Kit (Qiagen, Crawley, UK). Each gel-purified product was sequenced using BigDie terminator (version 3.1; Applied Biosystems, Foster City, CA). Sequences were initially compared with the current GenBank sequence databases using the BLAST suite of programs [31].

2.7. Outer membrane protein (OMP) preparation

Whole bacterial proteins and OMPs were obtained as described elsewhere [32]. To visualise proteins, sodium dodecylsulphate–polyacrylamide gel electrophoresis (SDS–PAGE) was performed using a modification of the method of Laemmli [33]. Gels were stained with 0.25% Coomassie brilliant blue, de-stained and dried using Bio-Rad apparatus, namely Miniprotean II and Bio-Rad 543 (Bio-Rad Laboratories S.A., Madrid, Spain) for electrophoresis and gel drying, respectively.

2.8. Black lipid bilayer membrane experiments

Membranes were prepared from a 1% (w/v) solution of diphytanoyl phosphatidylcholine (Avanti Polar Lipids, Alabaster, AL) in *n*-decane in a Teflon cell consisting of two aqueous compartments connected by a circular hole with an area of ca. 0.4 mm^2 [34,35]. The aqueous salt solutions (analytical grade; Merck, Darmstadt, Germany) were used unbuffered at pH 6. The temperature was kept at 20 °C throughout the experiments. The single-channel measurements were performed with a pair of Ag/AgCl electrodes (with salt bridges) switched in series with a voltage source and a current amplifier (Keithley 367; Keithley Instruments, Cleveland, OH). The amplified signal was monitored with a storage oscilloscope and recorded with a strip chart recorder. Small amounts of the stock solutions containing pure *Morganella* porin were added after the lipid membrane turned optically black to reflected light.

For the zero current membrane potentials, the membranes were prepared in a 100 mM KCl solution and insertion of pores was monitored until a sufficient number of porin channels were reached in the membrane. The instrumentation was then switched to measure zero current potentials and a KCl gradient was established by adding 3 M KCl solution to one side of the membrane while stirring. The zero current membrane voltage reached its stationary value ca. 2–5 min after addition of the concentrated KCl solution and was analysed using the Goldman–Hodgkin–Katz equation [36].

3. Results

3.1. Antibiotic susceptibility testing

Morganella morganii HUB198351 was found to be resistant to several commonly used antibiotics. MIC values obtained in the presence of the efflux pump inhibitors CCCP, reserpine and potassium cyanide (KCN) (Table 1) were similar to those obtained without the inhibitors. Additionally, the kinetics of ciprofloxacin accumulation was determined to evaluate the actual role of the efflux pumps. Again, no significant differences in ciprofloxacin accumulation were observed in either of the strains with and without CCCP (Fig. 1). In contrast, significant differences between the ability to grow in the presence of one-quarter of the MIC for ciprofloxacin with and without CCCP were clear (Fig. 2).

3.2. Isolation of plasmid from M. morganii

PFGE demonstrated the presence of a single plasmid in the strain HUB198351, which was further characterised after purification by agarose gel electrophoresis and enzyme digestion. The plasmid (pML2003) had a size of approximately 6.6 kb.

3.3. Detection and analysis of integrons

The isolated plasmid was analysed by PCR for the presence of integrated gene cassettes with primers 3′CS and 5′CS for class 1 integrons. The amplicon size ranged from

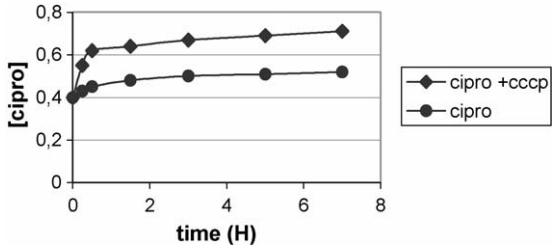

Fig. 1. Ciprofloxacin accumulation (μg/mL) by *Morganella morganii* HUB198351 either in the presence and absence of carbonyl cyanide *m*-chlorophenylhydrazone (CCCP).

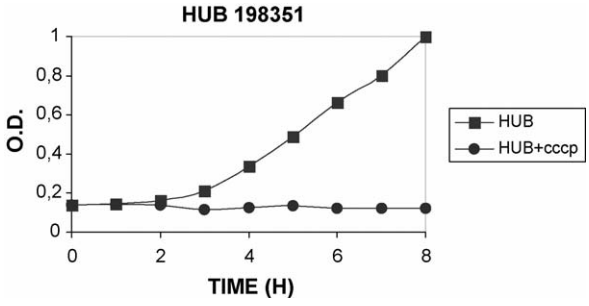

Fig. 2. Growth curves of *Morganella morganii* HUB198351 in the presence of ciprofloxacin (8 μg/mL) with and without carbonyl cyanide *m*-chlorophenylhydrazone (CCCP). OD, optical density.

Table 1
Minimum inhibitory concentrations (MICs) (μg/mL) of different antimicrobial agents against *Morganella morganii* HUB198351 and *M. morganii* CECT 173

Antimicrobial agent	*M. morganii* HUB198351	*M. morganii* HUB198351 with the efflux pump inhibitor			*M. morganii* CECT 173
		CCCP	Reserpine	KCN	
Ampicillin	800	400	800	200	1.5
Nalidixic acid	64	32	32	32	0.125
Cefalothin	3200	25	400	400	200
Cefoxitin	100	100	100	50	6.25
Ceftriaxone	1.6	0.025	0.4	0.8	0.007
Ciprofloxacin	32	8	32	8	0.0035
Chloramphenicol	64	32	64	16	4
Streptomycin	1600	1600	800	1600	6.25
Erythromycin	400	25	400	100	100
Gentamicin	50	6.25	12.5	50	0.4
Novobiocin	100	50	12.5	50	6.25
Penicillin	1600	1600	1600	1600	25
Rifampicin	12.5	0.8	6.25	12.5	0.2
Tetracycline	100	0.75	50	12.5	0.25
Violet crystal	100	100	100	200	1

CCCP, carbonyl cyanide *m*-chlorophenylhydrazone; KCN, potassium cyanide.

1.5 kb to 2.0 kb (Fig. 3) and included variable regions with two inserted gene cassettes each. Direct sequencing of the 1.5 kb amplicon demonstrated that this integron contained two cassettes, aminoglycoside adenyltransferase (*aadB*) and chloramphenicol acetyltransferase (*catB3*). The *aadB* gene confers resistance to aminoglycosides and the *catB3* gene confers resistance to chloramphenicol (accession number DQ237858).

The 59-base element core site necessary for recombination between gene cassettes and integrons was also detected. Direct sequencing of the 2.0 kb amplicon allowed the identification of two cassettes, carbenicillinase (*blaP1b*) and adenyltransferase (*aadA2*). The *blaP1b* gene confers resistance to β-lactamases and the *aadA2* confers resistance to streptomycin (accession number DQ237857).

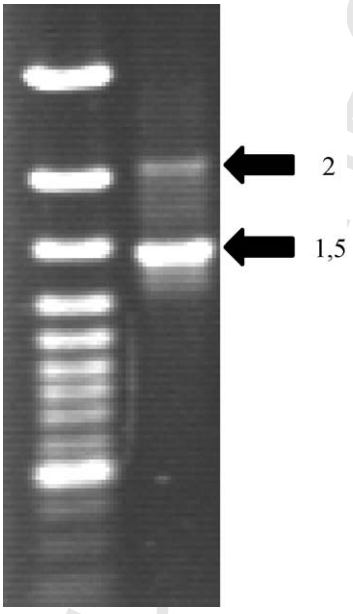

Fig. 3. Polymerase chain reaction amplification products obtained with primers 3′CS and 5′CS. Fragments were separated by electrophoresis through a 0.7% agarose gel. Lane 1: marker GeneRuler 1 kb DNA ladder (Fermentas, Vilnius, Lithuania); lane 2: integrons of 1.5 kb and 2.0 kb.

To our knowledge, this is the first report of the presence of *aadB*, *catB3*, *blaP1b* and *aadA2* genes in *M. morganii*. Deduced amino acid sequences from all four gene cassettes of *M. morganii* were compared with *Pseudomonas aeruginosa* (*blaP1b*, *aadA2*, *aadB*) and *E. coli* (*catB3*). The alignment showed a high level of amino acid identity (94%).

3.4. Purification of porin from M. morganii

Antibiotic resistance of the multiresistant *M. morganii* strain could be partly due to a change in outer membrane permeability, as reported previously [23]. This could be caused by either a change in the number of porins in the outer membrane or by a mutation of the major porin. To check these possibilities, porins were isolated from both *M. morganii* strains. Extraction of porins from *M. morganii* was easy using the detergent genapol. A porin of 36 kDa molecular mass was detected in both *Morganella* strains when the porin samples were boiled (Fig. 4). The oligomer, which is presumably a trimer analogous to the situation in other enteric bacteria, has an apparent molecular mass of 76.5 kDa. The isolated porin appeared to be pure and homogeneous as judged by regular SDS–PAGE and urea–SDS–PAGE. Expression of this 36 kDa protein was very similar in wild-type and antibiotic-resistant mutants. General characteristics of this porin were similar to those described for major porins of *E. coli*. It should be noted that we were unable to demonstrate the presence of LamB protein in *Morganella*. In the peptidoglycan–protein complexes, another protein was detected firmly attached to the peptidoglycan, which is presumably the OmpA analogue of *M. morganii.*

Pores formed by Omp36 of wild-type and antibiotic-resistant *M. morganii* strains were analysed. Omp36 forms

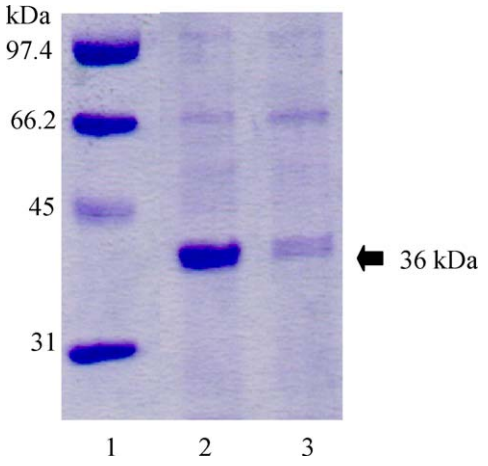

Fig. 4. Characterisation of outer membrane proteins. Lane 1: molecular weight standards; lane 2: partially purified 36 kDa protein from *Morganella morganii* HUB198531; lane 3: partially purified 36 kDa protein from *M. morganii* CECT 173.

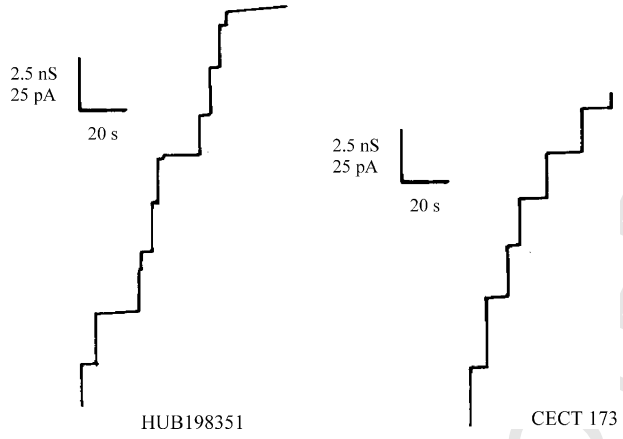

Fig. 5. Single-channel recordings of an artificial lipid bilayer membrane in the presence of 36 kDa protein from the cell wall of *Morganella morganii* HUB198351 and *M. morganii* CECT 173.

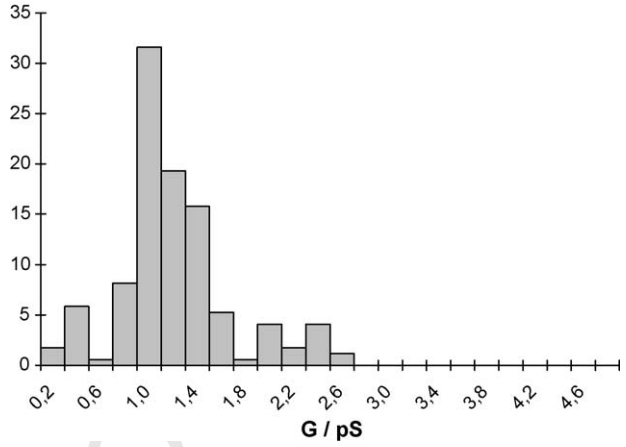

Fig. 6. Histogram of all conductance steps observed with diphytanoyl phosphatidylcholine/*n*-decane membranes in the presence of 10 ng/mL Omp36 from *Morganella morganii* HUB198531 in 1 M potassium acetate.

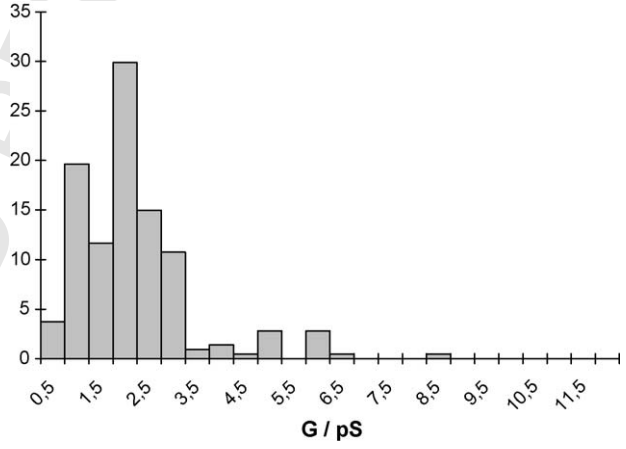

Fig. 7. Histogram of all conductance steps observed with diphytanoyl phosphatidylcholine/*n*-decane membranes in the presence of 10 ng/mL Omp36 protein from *Morganella morganii* HUB198531 in 1 M potassium chloride.

channels in the lipid bilayer when it is in the oligomeric form. This ability is completely lost for heat-dissociated monomers. Fig. 5 shows single-channel recordings of Omp36 derived from the two different strains. The histograms (Figs. 6 and 7) derived for the porins of both strains showed that their single-channel conductance was very similar, therefore it is rather unlikely that the Omp36 of the multiresistant strain is mutated. This result was supported by single-channel experiments performed with salts other than KCl, which indeed showed that there was no difference between Omp36 obtained from the two strains (Table 2).

Table 2
Average single-channel conductance of porin channels obtained from *Morganella morganii* strains CECT 173 and HUB198351

Salt	Concentration (M)	Strain CECT 173 single-channel conductance, *G* (nS)	Strain HUB198351 single–channel conductance, *G* (nS)
LiCl	1.0	1.00	1.50
KCl	0.1	0.20	0.10
KCl	0.3	0.50	0.60
KCl	1.0	2.00	2.00
KCl	3.0	6.00	6.00
Potassium acetate (KCH_3COO) (pH 7)	1.0	1.00	1.00

Note: The membranes were formed of diphytanoyl phosphatidylcholine dissolved in *n*-decane. The aqueous solutions were unbuffered and had a pH of 6 unless otherwise indicated. The porin concentration was ca. 10 ng mL. The applied voltage was 20 mV and the temperature was 20 °C. The average single-channel conductance, *G*, was calculated from at least 80 single events.

The selectivity of Omp36 was also studied by zero current membrane potential measurements in the presence of KCl gradients. Five-fold KCl gradients (100 mM versus 500 mM) were established across lipid bilayer membranes in which ca. 100–1000 channels were reconstituted. The gradients resulted in an asymmetry potential of ca. 80 mV at the more dilute side for Omp36 from the two sources (mean of three measurements each). This result indicated little preferential movement of potassium ions over chloride ions through the channel at neutral pH. The zero current membrane potentials were analysed using the Goldman–Hodgkin–Katz equation [34,35]. The ratio of the potassium permeability, P_K, divided by the chloride permeability, P_{Cl}, was ca. 2, irrespective of whether Omp36 of the wild-type or the resistant strain was studied (see also Section 4).

4. Discussion

The results of the susceptibility of *M. morganii* HUB198351 to a wide variety of antimicrobial agents clearly showed that this was a multiresistant clinical isolate. The strain was resistant to all antibiotics tested as well as to violet crystal. Resistance to such a wide variety of antimicrobials is unusual in *Morganella* and this was the reason why we focused this study on *M. morganii* HUB198351. Resistance to antimicrobials is due to the expression of a variety of different molecular mechanisms, including enzymes able to degrade antibiotics, changes in the target affinity for antimicrobials, reduced permeability and active efflux. Thus, we have explored the role of each of these mechanisms in the multiresistance of *M. morganii* HUB198351. Active efflux of antimicrobials can be explored by means of several methodologies. In principle, it is possible to obtain evidence of extrusion when metabolic inhibitors able to block efflux pumps have a direct influence on MIC values. However, taking into account that the MIC is read after long periods of incubation, it is feasible that antimetabolites have no effect on MIC values [27]. In fact, when CCCP, reserpine and KCN were added, none or slight modifications (less than 4-fold) in MICs were detected for most antibiotics such as ampicillin, nalidixic acid, cefoxitin, ciprofloxacin, chloramphenicol, streptomycin, erythromycin, gentamicin, novobiocin, penicillin and violet crystal. In contrast, the presence of some antimetabolites in some cases caused a dramatic increase in susceptibility. This is the case for rifampicin with CCCP (8-fold) or tetracycline (64-fold). This suggests that some efflux mechanism operates in the bacterium, although there was no evidence that a broad spectrum of substrates susceptible to extrusion should be expected. Thus, the actual role of active efflux cannot be measured by comparing MICs. This is also confirmed by the resistance of the bacterium to violet crystal, which can be only explained by active efflux, since no other mechanism has been proposed to explain bacterial resistance to this stain. In fact, the data should be confirmed by experiments in which efflux can be measured over short periods of time. When ciprofloxacin accumulation was measured in the first 10 min either in the presence or absence of an efflux pump inhibitor such as CCCP, slight differences were detected (Fig. 1). Moreover, additional experiments of growth curves in the presence of one-quarter of the MIC of ciprofloxacin were carried out. It seems clear that when efflux is inhibited, the susceptibility of the multiresistant strain increased dramatically (Fig. 2). Thus, it can be assumed that when analysed properly, active efflux appeared to play an important role in determining resistance in this strain.

Morganella morganii HUB198351 carries a plasmid (pML2003) that contains two integrons (2 kb and 1.5 kb). Direct sequencing of both the 1.5 kb and 2 kb amplicons validated the preliminary evidence for the presence of antibiotic resistance gene cassettes. BLAST searches closely matched similar sequences in *Proteus mirabilis*, *E. coli*, *Salmonella typhimurium* and *P. aeruginosa*. As expected, antibiotics to which the gene cassette conferred resistance were coincident with the phenotypic resistances determined by susceptibility testing.

To prove the pore-forming ability of Omp36, we performed conductance measurements in black lipid bilayers. After a delay of 1–2 min, probably owing to the slow aqueous diffusion of the protein, the current increased in a stepwise fashion similar to that observed for other Gram-negative bacterial porins [22,34]. The current increase was gradual under these conditions and the conductance of these steps was ca. 2 nS when the porin was freshly added to the pre-existing membrane. We would like to emphasise that 2 nS is close to the conductance of other Gram-negative bacterial porins, e.g. OmpC or OmpF of *E. coli*, which show conductance values of 1.5–2.0 nS under the same conditions [22,35,37,38].

It is obvious that the 2 nS channel represents the sum of the conductance of three individual channels in a trimer. However, this could not be shown here as it was not possible to close the channels even at voltages as high as 150 mV, which means that the Omp36 channels of *M. morganii* were not voltage-gated like the OmpF of *E. coli* and the conductance of a single channel in a trimer could not be evaluated [39]. The Omp1 channel conductance of 1.5 nS in 1 M KCl suggests that the diameters of the three Omp36 channels in a trimer are presumably very similar to those of the OmpF trimers, which are close to 1 nm [22,35,38]. The results presented here clearly indicate that Omp36 is not responsible for the antibiotic resistance of the clinical isolate of *M. morganii*.

Morganella morganii HUB198351 porin was only moderately cation selective, as inferred from single-channel experiments in which KCl was replaced by LiCl or potassium acetate (KAc), i.e. the mobile K^+ and Cl^- ions were replaced by the less mobile Li^+ and acetate$^-$ ions (Table 2). The single-channel conductance in 1 M LiCl and 1 M KAc decreased on average by a factor of ca. two compared with the conductance in 1 M KCl when the reconstitution of monomeric and dimeric conductive units is considered. However, the single-channel conductance was lower in KAc than in LiCl, suggesting that the channel was cation selective ($P_{cation}/P_{anion} = 2$).

This result also indicated that the single-channel conductance followed the conductivity of the bulk aqueous salt solutions of KAc and LiCl, which was ca. one-half that of 1 M KCl [40]. These results are expected if the outer membrane porin of *M. morganii* HUB198351 is wide and water-filled. It is noteworthy that similar results were obtained for the general diffusion porin of *E. coli* and other enteric bacteria [35,41]. Measurements at various KCl concentrations support this hypothesis, as the single-channel conductance was a linear function of the bulk aqueous conductance (Table 2), which also characterises enteric general diffusion porins.

Acknowledgments

This work was supported by grant SAF2002-00698 (Spanish Ministry of Science and Technology/FEDER) to Miguel Viñas and by a grant of the Deutsche Forschungsgemeinschaft (Be 865/9-5) and the Fonds der Chemischen Industrie to Roland Benz.

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