Characterization of a Nosocomial Outbreak Caused by a Multiresistant Acinetobacter baumannii Strain with a Carbapenem-Hydrolyzing Enzyme: High-Level Carbapenem Resistance in A. baumannii Is Not Due Solely to the Presence of β-Lactamases

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From February to November 1997, 29 inpatients at Ramón y Cajal Hospital, Madrid, Spain, were determined to be either colonized or infected with imipenem- and meropenem-resistant Acinetobacter baumannii (IMRAB) strains (MICs, 128 to 256 µg/ml). A wide antibiotic multiresistance profile was observed with IMRAB strains. For typing IMRAB isolates, pulsed-field gel electrophoresis was used. For comparative purposes, 30 imipenem- and meropenem-susceptible A. baumannii (IMSAB) strains isolated before, during, and after the outbreak were included in this study. The molecular-typing results showed that the outbreak was caused by a single IMRAB strain (genotype A). By cloning experiments we identified a class D β-lactamase (OXA-24) encoded in the chromosomal DNA of this IMRAB strain which showed carbapenem hydrolysis. Moreover, the outer membrane profile of the IMRAB strain showed a reduction in the expression of two porins at 22 and 33 kDa when compared with genetically related IMSAB isolates. In addition no efflux mechanisms were identified in the IMRAB strains. In summary, we report here the molecular characterization of a nosocomial outbreak caused by one multiresistant A. baumannii epidemic strain that harbors a carbapenem-hydrolyzing enzyme. Although alterations in the penicillin-binding proteins cannot be ruled out, the reduction in the expression of two porins and the presence of this OXA-derived β-lactamase are involved in the carbapenem resistance of the epidemic nosocomial IMRAB strain.

Acinetobacter spp. are opportunistic pathogens with increasing relevance in nosocomial infections (6). They cause a wide range of clinical complications, such as pneumonia, septicemia, urinary tract infection, wound infection, and meningitis, especially in immunocompromised patients (6, 22). Antimicrobial treatment of these clinical infections, particularly those caused by Acinetobacter baumannii strains, may be compromised by the multiple-drug resistance of many isolates to β-lactams, aminoglycosides, and fluoroquinolones (4, 23).

During the last decade, hospital-acquired infections involving multiresistant A. baumannii isolates have been reported, often in association with contamination of the hospital equipment or cross-contamination by the colonized hands of patient-attending personnel (5, 6, 20, 37–39).

Regarding the resistance to β-lactam antibiotics of A. baumannii clinical strains, different mechanisms have been involved (2, 4). As in other gram-negative rods, the main mechanism of resistance to β-lactam antibiotics is the production of β-lactamases encoded either by the chromosome or by plasmids (19). In addition, a low permeability of the outer membrane of A. baumannii as well as alterations in the penicillin-binding protein (PBP) affinity has been involved in the resistance of A. baumannii to these antibiotics (2, 4, 11, 32).

In the last few years, carbapenem-resistant A. baumannii isolates have been reported worldwide (1, 12, 29). Loss of porins, PBP with reduced affinity, and different class B and D β-lactamases have been associated with resistance to carbapenems in A. baumannii clinical strains (2, 4, 7, 8, 9, 11, 12, 15, 18, 27, 29, 32).

The main objectives of this work were to characterize a nosocomial outbreak by antibiotyping and pulsed-field gel electrophoresis (PFGE) and to investigate the mechanisms of resistance to carbapenems in an epidemic multiresistant A. baumannii strain with a high level of resistance to carbapenems (i.e., the imipenem- and meropenem-resistant A. baumannii [IMRAB] strain), which caused a 10-month-long epidemic outbreak at our hospital in 1997, involving 29 patients.


MATERIALS AND METHODS

Description of the outbreak. From February through November 1997, an IMRAB A. baumannii strain was isolated from 29 patients, 23 of whom were hospitalized in five different medical and surgical intensive care units (ICUs) at the Ramón y Cajal Hospital, a 1,200-bed tertiary-care teaching hospital. The original strain of the outbreak was simultaneously isolated from a bronchospi- rate and urine specimens of one patient admitted to the medical ICU. Afterwards, IMRAB isolates were obtained from 4 patients at the same ICU, 1 patient from a pediatric ICU, 17 patients from three different surgical ICUs, and 6 patients. Criteria for infection with IMRAB were documented by infectious diseases unit physicians for 15 patients; meanwhile, the rest of the patients were...
considered to be colonized only. Infection control measures and the use of disposable gloves and aprons while caring for IMRAB-infected and colonized patients were immediately implemented, the need for handwashing was reinforced, and, as far as possible, patients colonized or infected with IMRAB were isolated. Likewise, use of impirenem and meropenem, particularly in the areas involved in the outbreak, was restricted, and compliance with this antibiotic use policy was monitored by infectious diseases unit physicians.

Bacterial strains and plasmids. A total of 225 A. baumannii clinical isolates were included in this study: 196 IMRAB isolates obtained from these 29 patients during the outbreak and 30 impirenem- and meropenem-resistant A. baumannii (IMSAB) isolates, obtained before, during, and after the outbreak (Table 1), and had clinical strains susceptible and resistant to tobramycin. Also 10 IMSAB isolates, including the first isolate from 28 patients and 2 isolates from the patient who died during the outbreak (February through November, 1996 through January, 1997), during the outbreak (February through November 1997), and after the outbreak (January through February 1998). Also, A. baumannii ATCC 19600, ATCC 19798, ATCC 25953, and ATCC 9535-3 isolates were included in this study. The organisms were identified by the scheme by the Plasmid Isolation and Cloning of the TEM-1 Gene. A. baumannii RYC 52763/97 had only one plasmid, of about 22 kb, that was isolated by the alkaline lysis method (30). A blaTEM-1 gene was amplified from this plasmid by PCR using the specific blaTEM primers C1 5′-GGAATTCTTCGCGGAAGTTGCGCGG AAC and C2 5′-GGGATCCGTAACCTGTGCTGACG and cloned into the pBSG18 plasmid (pAB1).

Extraction of chromosomal DNA, cloning, and sequencing of the beta-lactamase genes. For the chromosomal-DNA purification, the strains were grown overnight on Mueller-Hinton agar plates supplemented with ampicillin (100 µg/ml) and sodium dodecyl sulfate (SDS) and 20 µl of proteinase K (1 mg/ml) were added. The mixture was incubated at 60°C for 90 min. The total genomic DNA obtained was added, mixed with gentle agitation, and centrifuged at 11,000 rpm with a microcentrifuge for 5 min. The supernatant was collected, and the DNA was precipitated after the addition of 0.5 volumes of 7.5 M ammonium acetate and 2 volumes of ethanol. The DNA was washed with 70% ethanol, dried, and resuspended in 100 µl of Tris-EDTA buffer.

The restriction enzymes were purchased from Boehringer GmbH (Mannheim, Germany) and were used according to the manufacturer’s directions. Cloning procedures were performed as described by Sambrook et al. (30). Bacterial cells were made competent by the calcium chloride method. For cloning experiments, pBSG18 and pUC18 plasmids were used. Transformants were selected on Luria-Bertani (LB) plates supplemented with kanamycin (10 µg/ml) and ampicillin (50 µg/ml). Kanamycin was omitted when bacteria were transformed with the pUC18 plasmid. For the pUC18 plasmid, 5-bromo-4-chloro-3-indolyl-b-D-galactosidase (X-Gal) and isopropyl-b-D-thiogalactosidase ( IPTG) were used for transformant selection. Sequencing was performed on both strands by the method of Sanger et al. (31). Sequencing was carried out with the Taq DyeDeoxy Terminator cycle sequencing kit, using specific primers to the coding sequence. The sequence was analyzed in an automated DNA sequencer (377 ABI-Prims; Perkin-Elmer).

Kinetin experiments and carbenem hydrolysis. For kinetic studies, cell-free lysate was obtained by sonication of the sediment of a 1-liter exponentially growing culture of A. baumannii RYC 52763/97 and E. coli harboring the OXA-24 beta-lactamase (plasmid pBBM-1) gene (at 37°C in LB broth medium containing 100 µg of ampicillin per ml). The sonicated extracts were dialyzed overnight at 4°C in 0.05 M phosphate buffer (pH 7.4) and then loaded into a 300-ml 75- by 2.5-cm Sephadex G100 column (Pharmacia Fine Chemicals AB, Uppsala, Sweden) previously equilibrated with the same buffer. The beta-lactamase activity eluted with 0.05 M phosphate buffer (pH 7.4) tested by the nitrocefin method. Fractions containing beta-lactamase activity were collected, concentrated with centricon (Amicon B15; W.R. Grace and Co., Uppsala, Sweden) previously equilibrated with the same buffer. The amount of the chromosomal DNA added to the reaction was 500 ng. Amplification reactions were carried out in a Progene thermal cycler (Techne, Cambridge, United Kingdom), with an initial denaturation (10 min at 94°C) followed by 30 cycles of denaturation (1 min at 94°C), annealing (1 min at 45°C), and extension (2 min at 72°C), with a single final extension of 16 min at 72°C. Aliquots (20 µl) of each sample were subjected to electrophoresis in 1.2% agarose gels. Amplified products were detected after staining with ethidium bromide (50 g/ml) and photographed with Polaroid type 665 film. Strains belonging to the same DNA group showed identical profiles or highly similar profiles (up to two bands different).

Analytical isoelectric focusing, beta-lactamasals were characterized by isoelectric focusing of ultrasonic bacterial extracts prepared by sonication (25). Beta-lactamasals were analyzed by isoelectric focusing of cell extracts on polyacrylamide gel containing amphyoles with a pH range of 3.5 to 9.5 (Ampholine PH 5-8; Pharmacia Biotech) in a Multithor II System (Pharmacia-LKB). The focused beta-lactamasals were detected by staining the gel with naphthol AS-BI-phosphate buffer (100 mM, pH 7.0). P values were determined by comparison with those of beta-lactamasals and proteins with known pl: TEM-1 (p 5.4), TEM-3 (p 6.3), SHV-1 (p 7.6), 6-lactamase (p 8.15), MER-1 (p 8.4), and trypsinogen (p 9.30), and A. baumannii RYC 52763/97 AmpC (p 5.4).

Conjugation experiments. Transfer of resistance by conjugation was attempted with E. coli BM21 and A. junnii MA RYC95 strains as recipients. Overnight filter mating experiments were performed at 30°C and 37°C, and the transconjugants were selected on MacConkey agar plates supplemented with ampicillin (25 µg/ml) and nalidixic acid (50 µg/ml) for E. coli and Columbia agar plates supplemented with 1-glucose (2% [w/vol]), neutral red, and ampicillin (25 µg/ml) for A. junnii.

isolate is part of outbreak; 2 to 3, isolate is probably part of the outbreak; 4 to 6, isolate is possibly part of the outbreak; and 7, isolate is not part of the outbreak (4)).
Ampicillin | >1,024 | 4 | 128 | >1,024 | 128
Ampicillin + clavulanic acid | 512 | 4 | 64 | 16 | 64
Ticarcillin | >1,024 | 4 | 32 | >1,024 | 256
Cefazolin | >256 | 8 | >256 | 16 | 32
Cefuroxime | >256 | 4 | >256 | 4 | 4
Cefoxitin | >256 | 4 | 4 | 0.06 | 0.06
Cefotaxime | >256 | 0.06 | 4 | 0.06 | 0.06
Ceftadizime | >256 | 0.125 | 16 | 0.125 | 0.25
Ceftepime | 256 | 0.25 | 0.06 | 0.06 | 0.125
Aztreonam | >256 | 0.06 | 1 | 0.06 | 0.125
Imipenem | 128 | 0.125 | 0.125 | 0.125 | 1
Meropenem | 256 | 0.03 | 0.03 | 0.03 | 0.125

All the β-lactamase genes were cloned into the same pBGS18- plasmid.

Efflux mechanism. To determine the presence of an efflux mechanism involved in the resistance to carbapenems in the A. baumannii RYC 52763/97 strain, MIC assays were performed with Mueller-Hinton agar plates with (25 and 50 μg/ml) and without reserpine. A P. aeruginosa strain (RYC 44629/97) with a clear and defined efflux mechanism was used as a control. Meropenem and netilmicin were used as antibiotic controls to verify that reserpine inhibits the efflux mechanism.

Detection of the OXA-24 gene in the IMRAB A. baumannii strains. To determine the presence or absence of the OXA-24 gene in different A. baumannii strains and to study their putative role in carbapenem resistance, a PCR assay was performed. Six IMRAB and 10 IMSAB strains were used. Reactions were carried out with a 50-μl volume of a reaction mixture containing 20 mM Tris-HCl (pH 8.8), 100 mM potassium chloride, 2.0 mM magnesium chloride, 200 μM deoxynucleotide triphosphates, 50 pmol of each primer, 250 ng of the chromosomal DNA, and 2.5 U of Taq polymerase (Roche). The primers 5'-GTACTA ATCAAAGTTGTGGAA (P1) and 5'-TTCCTCTACGATTTGT (P2) adjacent to the OXA-24-coding region were used. Amplification reactions were submitted to the following program: initial denaturation (4 min at 94°C) followed by 30 cycles of denaturation (1 min at 94°C), annealing (1 min at 50°C), and extension (2 min at 72°C), with a single extension of 10 min at 72°C. The amplified 955-bp product was resolved by electrophoresis in a 1.5% (wt/vol) agarose gel containing ethidium bromide (50 μg/ml).

RESULTS

Antimicrobial susceptibility pattern. A. baumannii RYC 52763/97 (Table 2) and all IMRAB isolates exhibited a similar multiresistance profile, including resistance to semisynthetic penicillins, cephalosporins, cepoime, aztreonam, gentamicin, netilmicin, amikacin, and ciprofloxacin (MICs of >128 μg/ml for all antibiotics) and a high level of resistance to imipenem and meropenem (MICs, 128 to 256 μg/ml). Considering the critical concentrations of susceptibility, only tobramycin, sulfactam (MIC, 16 to 32 μg/ml), and colistin (MIC, 4 to 8 μg/ml) showed activity. Tobramycin MICs for the IMRAB isolates obtained from 23 patients were 4 to 8 μg/ml, while those for isolates from 5 patients were ≥128 μg/ml. One patient simultaneously harbored tobramycin-susceptible and -resistant IMRAB isolates. Regarding IMSAB isolates, apart from carbapenem susceptibility, several antimicrobial susceptibility patterns were obtained irrespective of the isolation time (Table 1).

PFGE and REP-PCR analysis. PFGE patterns of the representative A. baumannii IMRAB and IMSAB isolates are shown in Fig. 1. All IMRAB isolates analyzed had an identical band pattern and were classified in genotype A. In contrast, preoutbreak (strains 31 to 33), at-outbreak (strains 34 to 37), and postoutbreak (strains 38 to 40) IMSAB isolates belonging to a different genotype on the basis of the band profile and were assigned to genotypes B-C, D-E, and F-H, respectively.
A. baumannii ATCC strains showed a different band pattern than those of the IMRAB and IMSAB isolates (data not shown).

An interesting subject is at-outbreak carbapenem-susceptible A. baumannii strains 34 to 36. The genotypic data obtained with these strains led us to classify them into a different genotype (PFGE group D); however, comparing their profile with that of the IMRAB strains indicated that less than six different bands were obtained by PFGE. These results suggest the possibility of a genetic relationship between IMSAB strains 34 to 36 and the IMRAB isolates.

The agarose gel electrophoresis of the amplified fragments by REP-PCR of representative A. baumannii IMRAB and IMSAB isolates is shown in Fig. 2. The patterns of eight IMRAB isolates (strains 1, 3, 6, 8, 24, 25, 26, and 28 in Table 1) obtained from different patients and wards of the hospital were identical, and strains were assigned to PCR group 1, thus confirming the PFGE results. In the same gel, for comparison, the pattern of four A. baumannii isolates susceptible to the carbapenems obtained pre- (strain 31), at (strains 34 to 35), and post- (strain 39) outbreak is shown. As observed in Fig. 2, strains 31 and 39 showed a very different band pattern than that of the IMRAB profile. However, in the case of strains 34 to 35, a similar band pattern was observed compared to those of the IMRAB strains (up to two different bands). Therefore, this result suggests the possibility of a genetic relationship between IMSAB strains 34 and 35 and the IMRAB isolates.

Isoelectric focusing analysis. The sonicated extract of the strain A. baumannii RYC 52763/97 contained three β-lactamase bands: one with a pl of 5.4 (TEM-1 like) was plasmid mediated because it was cloned as described in Materials and Methods; another focused at pl 9.0 and failed to be transferred by conjugation experiments; and the third, focused at pl 9.4, corresponded to the A. baumannii chromosomal cephaporphine previously described (7).

Cloning and sequencing the β-lactamase genes. In order to determine the presence of a carbapenemase responsible for the high levels of carbapenem resistance, we attempted to clone all of the β-lactamase genes of the A. baumannii RYC 52763/97 strain. Neither antibiotic resistance gene transfer was obtained by conjugation experiments with A. baumannii RYC 52763/97, although a blaTEM-1 gene was cloned from the plasmid of the A. baumannii RYC 52763/97 strain as described in Materials and Methods. Moreover, carbapenem MICs for the E. coli transforms harboring this blaTEM-1 gene were not affected (Table 2).

Chromosomal DNA of A. baumannii strain RYC 52763/97 was independently digested with HindIII and BglII as previously described (7, 8). The resulting fragments were ligated into the pBGS18 plasmid digested with HindIII and BglII, respectively, and transformants were selected on kanamycin (10 μg/ml) and ampicillin (25 μg/ml) plates. When chromosomal DNA was digested with HindIII and transformants were selected, an insert of 2.2 kb was obtained. Afterwards, the nucleotide sequencing revealed the presence of a bla gene, which showed a close homology with different chromosomal and plasmid-mediated AmpC β-lactamases and likely corresponds to the A. baumannii AmpC β-lactamase that has been previously reported (7). Carbapenem MICs for E. coli TG1 transformants harboring A. baumannii AmpC β-lactamase were identical to that for the E. coli host strain (Table 2).

When chromosomal DNA was digested with BglII, an insert of 4.2 kb harboring another bla gene was cloned. The bla gene of this plasmid was subcloned by enzymatic digestion with XbaI, yielding the plasmid pBMB-1 with an insert of 1.5 kb. After nucleotide sequencing, the amino acid sequence of this β-lactamase (OXA-24) showed a close homology with OXA-10 and OXA-7 β-lactamases (40% identity). The molecular characterization of this OXA-24 β-lactamase has recently been reported (8). Interestingly, the MICs of imipenem (1 μg/ml) and meropenem (0.125 μg/ml) were moderately increased for the E. coli transformants harboring the pBMB-1 plasmid with the OXA-24 enzyme (Table 2) with respect to that for the E. coli TG1 host strain.

Kinetic experiments and carbapenem inactivation. Imipenem and meropenem hydrolysis was detected spectrophotometrically with a sonicated extract of the strain A. baumannii RYC 52763/97. In addition, a positive Masuda test with imipenem was obtained (data not shown). This result strongly suggested the presence of a carbapenem-hydrolyzing enzyme in the epidemic IMRAB strain. Moreover, the addition of EDTA at different concentrations did not affect the imipenem hydrolysis, thus suggesting the absence of a class B β-lactamase.

Biochemical experiments performed with the semipurified OXA-24 enzyme showed imipenem and meropenem hydrolysis (Table 3). In addition, a positive Masuda test was also performed with imipenem and the semipurified OXA-24 β-lactamase (data not shown). These results correlated well with the increase in the carbapenem MICs observed with the E. coli TG1 strain harboring the OXA-24 enzyme (plasmid pBMB-1 in Table 2).

OMP analysis. OMP analysis of the A. baumannii RYC 52763/97 strain and one IMSAB isolate (number 34 in Table 1) showed a reduction in the expression of two porins at 22 and 33

![FIG. 2. Patterns obtained by REP-PCR. The numbers above the figure indicate the corresponding strains (Table 1). Lanes λIII and λV, DNA molecular weight markers III and V, respectively (Boehringer GmbH, Mannheim, Germany).](http://jcm.asm.org/.../1763/97)
kDa in the IMRAB strain (Fig. 3A). In the same figure, the densitometry pattern of the gel is shown (Fig. 3B).

**Efflux mechanism.** No differences in the MICs for the *A. baumannii* RYC 52763/97 strains was observed when reserpine was added, thus suggesting that a putative efflux mechanism was not present in this strain.

**Detection of the OXA-24 gene in the epidemic IMRAB strains but not in carbapenem-susceptible *A. baumannii* strains.** Six epidemic IMRAB strains (strains 1, 3, 6, 24, 25, and 28 in Table 1) and 10 genetically unrelated *A. baumannii* strains (strains 31 to 40 in Table 1) with different levels of susceptibility to carbapenems (imipenem MIC range, 0.1 to 4 mg/liter) were used to investigate the presence of the OXA-24 gene by using specific OXA-24 primers. A positive amplification band was observed in all carbapenem-resistant epidemic IMRAB strains, while no amplification was observed in all carbapenem-susceptible *A. baumannii* strains included in this study. Moreover, the OXA-24 gene can be used as a marker of the epidemic outbreak strain.

**DISCUSSION**

In the last 2 decades, a significant number of *Acinetobacter* nosocomial infection outbreaks, caused mainly by *A. baumannii* strains, have been reported, causing increasing concern in hospitals (5, 6). In order to investigate the origin of infection, the route of spread, and the prevalence of isolates in a bacterial population, several phenotypic and molecular typing methods have been described. Although antibiotyping may alert us to the emergence of a multiresistant *A. baumannii* outbreak, distinguishing between strains with slight differences in their resistance profiles may be difficult. Therefore, genotypic methods including plasmid typing, ribotyping, PFGE of chromosomal DNA restriction fragments, and PCR fingerprinting have been used to investigate nosocomial *A. baumannii* outbreaks (13, 17, 28, 33, 34, 39; Bou et al., 38th ICAAC). We report here the molecular typing of a nosocomial outbreak caused by a multiresistant *A. baumannii* strain. By using PFGE and REP-PCR, we demonstrated the spread of one epidemic strain between 29 patients during a period of 10-months. On the other hand, seven different genotypes (B through H) were observed in the preoutbreak, at-outbreak, and postoutbreak *A. baumannii* strains included in this study. Regarding the resistance to carbapenems in *A. baumannii*, different mechanisms have been involved. PBP with reduced affinity and loss of porins, besides several class D and class B \(\beta\)-lactamases, have been associated with resistance to carbapenems in *A. baumannii* clinical strains (2, 4, 7, 8, 9, 11, 12, 15, 18, 27, 29, 32). In addition, we describe here the molecular mechanism associated with resistance to the carbapenems in an epidemic *A. baumannii* strain. Three different \(\beta\)-lactamases have been characterized in the epidemic IMRAB strain, a TEM-1-type plasmid-mediated enzyme, the *A. baumannii* cephalosporinase AmpC-type enzyme, and a novel OXA-derived chromosomally mediated enzyme (7, 8).

The three \(\beta\)-lactamase genes were cloned into pBGS18 plasmid, and the protein products were expressed in *E. coli* TG1 cells. As shown in Table 2, the MICs of the carbapenems conferred by each \(\beta\)-lactamase did not reach the carbapenem MICs for the original *A. baumannii* RYC 52763/97 strain, suggesting that other mechanisms are involved in the carbapenem resistance of *A. baumannii* strains, in addition to the \(\beta\)-lactamases. An increase in the carbapenem MICs was observed only for OXA-24 \(\beta\)-lactamase; in addition, *E. coli* extracts expressing the OXA-24 enzyme yielded a carbapenem hydrolysis that was very similar to that of the IMRAB strain (data not shown). Also, it is important to mention the imi-
penem and meropenem hydrolysis detected in the spectrophotometer with the semipurified OXA-24 enzyme ($V_{\text{max}}$ 4% and 75% that of benzylpenicillin, respectively). By cloning and kinetic experiments, no other β-lactamase with carbapenem hydrolysis was detected in the A. baumannii RYC 52763/97 strain (index case), suggesting that carbapenem hydrolysis is associated only with the presence of this OXA enzyme. Moreover, protein extracts of the RYC 52763/97 strain showed imipenem hydrolysis that was not inhibited by the addition of EDTA, thus suggesting that a class B β-lactamase was not present in the epidemic IMRAB strain.

Therefore, the high levels of carbapenem resistance were not due solely to the presence of β-lactamases. This result prevents the use of β-lactamase inhibitors (MIC of sulbactam, 16 to 32 μg/ml) in the treatment of the infections caused by IMRAB strains and leaves as a unique alternative the use of colistin and polymyxin B, nephrotoxic and neurotoxic drugs used at the beginning of the 1950s (35).

In general, the emergence of carbapenem-hydrolyzing enzymes in A. baumannii has been limited compared to the prevalence of other β-lactamases. Recently the production of carbapenem-hydrolyzing enzymes in different A. baumannii strains has been reported (12, 15, 18, 29). Several of these β-lactamases showed characteristics of metalloenzymes (class B β-lactamases by the classification method of Bush et al. [10]) because their enzymatic activity was inhibited in the presence of 1 mM EDTA and activated in the presence of 1 mM ZnCl₂ solution. In contrast, we report here the presence of an OXA carapenem-hydrolyzing activity in an epidemic outbreak strain. The fact that this OXA enzyme is chromosomally mediated makes the spread of the OXA gene to other microorganisms or A. baumannii strains difficult. Thus, the PCR assay using the OXA primers with different IMSAB strains isolated before, during, and after outbreak did not show a positive band in either of the A. baumannii strains. In addition, a transfer of the imipenem resistance was not detected in filter mating experiments by using A. junii and E. coli BM21 as the recipient cells.

On the other hand, three at-outbreak IMSAB isolates (34 to 36 [Table 1]) obtained from different patients at different wards displayed a different genotype (PFGE group D) than that of IMRAB isolates. Following the criteria of Tenover et al. (40), these isolates are possibly part of the outbreak, as revealed by the band pattern (less than six different bands by PFGE when compared with the IMRAB pattern). In addition, a REP-PCR assay performed with the same strains (Fig. 2) yielded a very similar band pattern to that of the IMRAB strains, thus suggesting a genetic relationship. The first at-outbreak IMSAB isolate (isolate 34 [Table 1]) was isolated in a surgical ICU when the outbreak started. Supporting this view, the results obtained with the OMP analysis between the IMRAB strain and this strain 34 showed that a single loss of porins might be involved in addition to other mechanisms in the resistance to carbapenems (Fig. 3); moreover, by PCR assay, the OXA-24 gene was not detected in the IMSAB 34 to 36 strains. These results strongly suggest that both carbapenem hydrolysis by the OXA-enzyme and loss of OMPs are involved in the carbapenem resistance of the epidemic IMRAB strain. It is also important to note that reiseprine experiments failed to detect any efflux mechanism in the IMRAB strains. The data obtained between the 34 to 36 isolates and the IMRAB strains may suggest an evolutionary relationship between these strains. Also, it is important to emphasize that the amount of carbapenem consumed remained practically unchanged during the months previous to the outbreak in our hospital.

In summary, we report here the characterization of a nosocomial outbreak caused by an A. baumannii multiresistant strain harboring a novel class D enzyme (OXA-24) with carbapenem-hydrolyzing activity besides a reduction in the expression of two outer membrane proteins at 22 and 33 kDa. A correct antibiotic policy should be addressed at the hospitals to avoid the dissemination of this class of strains.

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REFERENCES


