ORIGINAL ARTICLE

PCR-based DNA fingerprinting (REP-PCR, AP-PCR) and pulsed-field gel electrophoresis characterization of a nosocomial outbreak caused by imipenem- and meropenem-resistant *Acinetobacter baumannii*

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Objective To demonstrate the usefulness of REP-PCR and AP-PCR on molecular typing of *A. baumannii* isolates.

Method From February to November 1997, 29 inpatients at Ramón y Cajal Hospital, Madrid—23 in five intensive care units (ICUs) and six at two different medical departments—were either colonized or infected with imipenem- and meropenem-resistant *Acinetobacter baumannii* (IMRAB) strains (MICs of 64–256 mg/L). A wide antibiotic multiresistance profile was observed with IMRAB strains, and only tobramycin, sulbactam and colistin displayed valuable activity. For typing IMRAB isolates, repetitive extragenic palindromic sequence-based polymerase chain reaction (REP-PCR) and arbitrary primer sequence-based polymerase chain reaction (AP-PCR) methods were used and compared with pulsed-field gel electrophoresis (PFGE) as reference technique. For comparative purposes, 30 imipenem- and meropenem-susceptible *A. baumannii* (IMSAB) strains isolated before, during and after the outbreak were included in this study.

Results The molecular typing results showed that the outbreak was caused by a single IMRAB strain (genotype 1). On the other hand, seven different genotypes were observed in the pre-, at- and post-outbreak strains tested by REP-PCR. Regarding AP-PCR, three of four at-outbreak IMSAB strains were indistinguishable from the IMRAB profile. Thus, with AP-PCR, only six genotypes were obtained, apart from the IMRAB genotype.

Conclusions Under our experimental conditions, REP-PCR had a higher discriminatory power than AP-PCR, with PFGE as reference technique. The REP-PCR technique is a useful and expeditious method for the epidemiologic characterization of *A. baumannii* nosocomial outbreaks, the results being comparable to those obtained with the PFGE technique.

Keywords REP-PCR, AP-PCR, *A. baumannii* multiresistant outbreak

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INTRODUCTION

Members of the genus *Acinetobacter* are non-fermentative, aerobic, Gram-negative coccobacilli that are ubiquitous in the environment [1] and commonly found as part of the normal human flora [2]. However, although *Acinetobacter* is known to be a pathogen of relatively low virulence, it is currently recognized as a relevant opportunistic nosocomial pathogen causing respiratory and urinary tract infections, but also bacteremia and secondary meningitis [3–5], particularly in immunocompromised patients and those admitted to intensive care units (ICUs) [6].

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Since 1986, DNA–DNA hybridization studies have shown thatthere are at least 19 *Acinetobacter* DNA groups [7,8]; *Acinetobacter baumannii* (DNA group 2 or genospecies 2) is recognized as the species most frequently associated with human infections and epidemic nosocomial outbreaks. On the other hand, although the *A. baumannii* susceptibility pattern is variable, many isolates exhibit resistance to multiple antibiotics, and the most recent trends indicate increasing antimicrobial resistance to old and new β-lactams, aminoglycosides, and fluoroquinolones. As a consequence, treatment of *A. baumannii* infections may be complicated, and, although imipenem and meropenem activity is almost universal among Gram-negative organisms, *A. baumannii* strains less susceptible or resistant to carbapenems causing nosocomial infections have recently been reported worldwide [9,10]. Therefore, the marked ability of *A. baumannii* to develop antimicrobial resistance rapidly in the clinical setting is probably the origin of its role as a nosocomial pathogen.

During the last decade, there have been reports of hospital-acquired infections involving multiresistant *A. baumannii* isolates, often in association with contamination of hospital equipment or cross-contamination by the colonized hands of patient-attending personnel [6,11–14]. In an epidemic situation, suitable identification of and appropriate discrimination between *A. baumannii* isolates provides a much better understanding of nosocomial outbreak delineation. Different genotypic and phenotypic methods have been successfully used for *Acinetobacter* strain typing, including plasmid profile, ribotyping, and DNA restriction fragment length polymorphisms determined by pulsed-field gel electrophoresis (PFGE), currently considered to be the standard method [12,13,15–17]. Repetitive extragenic palindromic sequence-based polymerase chain reaction (REP-PCR) and arbitrary primer sequence-based polymerase chain reaction (AP-PCR) are methods that generate DNA fingerprints that discriminate between bacterial strains, and have sporadically been used to characterize *A. baumanni* isolates from hospitalized patients [18–25].

In the present study, the usefulness of REP-PCR and AP-PCR, two methods based on PCR-mediated detection of DNA polymorphisms, was evaluated and compared with PFGE as a reference technique for the characterization of a nosocomial outbreak caused by imipenem- and meropenem-resistant *A. baumannii* strains (IMRAB) in our hospital, which involved 29 patients and lasted for 10 months.

MATERIALS AND METHODS

Description of the outbreak

During the 10-month period from February to November 1997, multiresistant *A. baumanni* strains, resistant to imipenem and meropenem were isolated from 29 patients: 23 of them were hospitalized at five different medical and surgical ICUs in Ramón y Cajal Hospital, a 1200-bed tertiary-care teaching hospital. The original strain of the outbreak was simultaneously isolated from a bronchial aspirate and urine specimens of one patient admitted to the medical ICU. Afterwards, IMRAB isolates were obtained from four patients in the same ICU, one patient from a pediatric ICU, 17 patients in three different surgical ICUs, and six patients hospitalized in two different medical departments, Internal Medicine and Dermatology. Criteria of infection with IMRAB were documented by Infectious Diseases Unit physicians in 15 patients; the rest of the patients were considered to be colonized only. Infection control measures and the use of disposable gloves and aprons in the care of 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, the use of imipenem 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

In total, 226 *A. baumannii* clinical isolates were included in this study: 196 IMRAB isolates obtained from the 29 patients during the outbreak, and 30 imipenem- and meropenem-susceptible *A. baumannii* isolates (IMSAB) obtained from clinical specimens before the outbreak (November 1996 to January 1997), during the outbreak (February to November 1997), and after the outbreak (January to February 1998). Also, *A. baumannii* ATCC 189, ATCC 17978, ATCC 50853 and ATCC 9935 isolates were included in this study. The organisms were identified following the scheme by Kämpfer et al [26], adapted by Dijkshoorn [27], which includes bacterial growth at 37 °C, 41 °C and 44 °C. The phenotypic antimicrobial susceptibility pattern was studied in 196 IMRAB and 30 IMSAB isolates. For the genotypic characterization of isolates, the REP-PCR, AP-PCR and PFGE methods were performed with 30 IMRAB isolates, the first isolate from 28 patients and two isolates from the patient who had clinical strains susceptible and resistant to tobramycin, and 10 IMSAB isolates, obtained before, during and after the outbreak (Table 1). *A. baumannii* ATCC strains were used as controls.

Antibiotic susceptibility testing

Antibiotic susceptibility testing was performed by the standard NCCLS disk diffusion [28] and agar dilution [29] methods. Following bacterial isolation, disk diffusion tests were performed with Mueller–Hinton agar (Oxoid, UK), an inoculum of 10^5 CFU, and disks (Oxoid) of ticarcillin (75 μg), ampicillin/sulbactam (10/10 μg), ceftazidime (30 μg), imipenem and meropenem (10 μg), tobramycin (10 μg) and ciprofloxacin (5 μg). The MICs of the antimicrobial agents were determined by the agar dilution method with Mueller–Hinton agar (Oxoid), antibiotic-standard powders with stated potencies supplied by the drug manufacturers, and an inoculum of 10^4 CFU per spot. In both methods, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as internal controls in each susceptibility determination.

Oligonucleotide primers

With the REP-PCR method, the primer pair REP1 5′-IIIGCGCCGICATCAGGC-3′ and REP2 5′-ACGTCTTAT-CAGGCCTAC-3′ [25] was used to amplify putative REP-like elements in the genomic bacterial DNA. The primer REP1 has the nucleotide inosine at ambiguous positions in the

Table 1 Imipenem- and meropenem-resistant (IMRAB) and imipenem- and meropenem-susceptible (IMSAB) *A. baumannii* isolates analyzed by REP-PCR, AP-PCR, and PFGE

Isolate no.[a]	Date of isolation (month/year)	Ward[b]	Susceptibility pattern CMP	CAZ	TOB	PFGE group	REP-PCR group	AP-PCR group
IMRAB 1–5	2–4/97	MICU	R	R	S/I	A	1	1
IMRAB 6–23	3–6/97	SICU	R	R	S/I	A	1	1
IMRAB 24	5–6/97	SICU	R	R	R	A	1	1
IMRAB 25–27	4–6/97	DERM	R	R	R	A	1	1
IMRAB 28–30	7–11/97	IMED	R	R	R	A	1	1
IMSAB-PRE 31	11–96	MICU	S	S	S	B	2	2
IMSAB-PRE 32	12/96	MUNI	S	I	R	C	3	3
IMSAB-PRE 33	1/97	MUNI	S	I	S	C	3	3
IMSAB-AT 34	2/97	SUNI	S	R	S	D	4	1
IMSAB-AT 35	7/97	SURG	S	R	S	D	4	1
IMSAB-AT 36	7/97	IMED	S	R	S	D	4	1
IMSAB-AT 37	4/97	SICU	S	S	S	E	5	4
IMSAB-PST 38	1/98	SUNI	S	R	R	F	6	5
IMSAB-PST 39	1/98	SICU	S	S	S	G	7	6
IMSAB-PST 40	2/98	SICU	S	I	R	H	8	7

[a]PRE, pre-outbreak; AT, at-outbreak; PST, post-outbreak. [b]MICU, Medical ICU; SICU, Surgical ICU; DERM, Dermatology; IMED, Internal Medicine; SUNI, surgical unit; MUNI, medical unit. CMP, carbapenem (imipenem and meropenem); CAZ, ceftazidime; TOB, tobramycin.

REP consensus sequence. Inosine contains the purine base hypoxanthine and is able to base-pair with A, C, G and T. With the AP-PCR technique, the primer used was 5′-TGGTCGCGG-3′. The oligonucleotides were prepared by Cruachen (Progenetic, S.L., Madrid, Spain).

Extraction of genomic DNA

Strains were grown overnight on MacConkey agar plates at 37 °C, and the growth from approximately one-quarter of a plate was resuspended in 180 μL of distilled water. Following this, 200 μL of buffer solution (0.01 M Tris-Cl, pH 7.8, 0.005 M EDTA, 0.5% SDS) and 20 μL of proteinase K (1 mg/mL) was added. The mixture was incubated for 2 h at 55 °C, and then 400 μL of phenol–chloroform solution was added, and mixed with gentle agitation; the mixture was then centrifuged at 11 000 rev/min 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 TE buffer.

REP-PCR

Amplification reactions were performed in a final volume of 50 μL. Mg^{2+}-free PCR buffer was purchased as a 10X concentrate consisting of 500 mM KCl–100 mM Tris-HCl (pH 9.0)–1% Triton X-100 (Perkin Elmer, Applied Biosystem Division). Each reaction mixture contained 5 μL of 10X PCR buffer, 2 U of AmpliTaq Gold (Perkin Elmer, Roche Molecular Systems, Inc., NJ, USA) and 200 μM (each) dATP, dCTP, dGTP and dTTP (Perkin Elmer, Roche Molecular Systems). The Mg^{2+} concentration was 3 mM and the primers were used at 0.5 μM concentration. The amount of chromosomal DNA added to the reaction was 500 ng. Amplification reactions were carried out in a Techne 'Progene' thermal cycler (Techne, Cambridge, UK), 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 mg/L) 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).

AP-PCR

Amplification reactions were performed as described for REP-PCR. Each reaction mixture contained 5 μL of 10X PCR buffer, 2 U of AmpliTaq Gold (Perkin Elmer, Roche Molecular Systems, Inc.), and 400 μM (each) dATP, dCTP, dGTP and dTTP (Perkin Elmer, Roche Molecular Systems, Inc.). The Mg^{2+} concentration was 4 mM and the primer was used at 10 μM concentration. Amplification reactions were carried out in a Techne 'Progene' thermal cycler, with an initial denaturation (10 min at 94 °C) followed by two cycles (94 °C for 5 min, 20 °C for 5 min, 72 °C for 5 min) and 40 cycles of denaturation (1 min at 94 °C), annealing (1 min at 28 °C) and

extension (2 min at 72 °C), with a single final extension of 10 min at 72 °C. Aliquots (20 μL) of each sample were subjected to electrophoresis in 2.0% agarose gel in TBE buffer (1 × 0.09 M Tris-borate, 0.002 M EDTA). Amplified products were detected after staining with ethidium bromide (50 mg/L) and photographed with Polaroid type 665 film. Strains belonging to the same DNA group showed identical profiles.

Pulsed-field gel electrophoresis (PFGE)

Macrorestriction analysis of chromosomal DNA with *Sma*I and *Apa*I (New England Biolabs, Boston, MA, USA) was carried out by PFGE following published procedures [30].

PFGE was run in a CHEF-DRIII apparatus (Bio-Rad Laboratories, Richmond, CA, USA), with pulses ranging from 0.5 to 15 s at a voltage of 6 V/cm at 14 °C for 20 h. Products were detected after staining with ethidium bromide (50 mg/L) and photographed with Polaroid type 665 film. Criteria for interpreting PFGE patterns according to the number of differently sized fragments compared with the outbreak pattern were: 0, isolate is part of the outbreak; 2–3, isolate is probably part of the outbreak; 4–6, isolate is possibly part of the outbreak; and ≥7, isolate is not part of the outbreak [31].

Outer-membrane protein analysis

Outer-membrane proteins (OMPs) were analyzed from logarithmic cultures by previously described methods using sodium dodecyl sulfate–polyacrylamide gel electrophoresis [32]. All isolates were cultured in Luria–Bertani medium. Purified outer-membrane preparations were obtained by treatment of the cell envelopes with sodium *N*-lauryl sarcosinate. Samples of each preparation were applied to the gels and electrophoresed with an Electrophoresis Power Supply 500/400 (Pharmacia, Uppsala, Sweden).

Isoelectric focusing and carbapenem-hydrolysis analysis

β-Lactamases were analyzed by isolectric focusing, and pIs were determined by comparison with those of enzymes with known pIs [33].

Carbapenem hydrolysis was analyzed as previously described [34].

RESULTS

Antibiotic susceptibility pattern

All IMRAB isolates exhibited a similar multiresistance profile, including resistance to semisynthetic penicillins, ceftazidime, cefepime, cefpirome, aztreonam, gentamicin, netilmicin, and amikacin (MICs >128 mg/L for all antibiotics), and high-level resistance to imipenem and meropenem (MICs 64–256 mg/L). With regard to the critical concentrations for susceptibility, only tobramycin, ampicillin sulbactam (MIC 16–32 mg/L) and colistin (MIC 4–8 mg/L) showed valuable activity. The IMRAB isolates obtained from 23 patients displayed tobramycin MICs of 4–8 mg/L, while those from five patients showed tobramycin MICs of ≥128 mg/L. One patient simultaneously harbored tobramycin-susceptible and -resistant IMRAB isolates. With regard to IMSAB isolates, apart from carbapenem susceptibility, several antimicrobial susceptibility patterns were obtained, irrespective of isolation time.

REP-PCR

The agarose gel electrophoresis of the fragments amplified by REP-PCR of representative *A. baumannii* isolates is shown in Figure 1. The profiles generated with REP-PCR primers contained several bands, ranging in size from 0.1 to 5 kb. 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. In the same gel and for comparative purposes (Figure 1a), the patterns of four *A. baumannii* isolates susceptible to the carbapenems obtained before (strain 31), during (strains 34 and 35), and after (strain 39) the outbreak are shown. The resulting REP-PCR patterns of 10 IMSAB isolates (strains 31–40 in Table 1) obtained at different times from different patients and hospital wards are shown in Figure 1b. As shown in Figure 1b, different band profiles were obtained with the strains isolated before the outbreak (PCR group 2–3), during the outbreak (PCR group 4–5) and after the outbreak (PCR group 6–8) compared with the profile from the IMRAB strains. Strains 34–36 were assigned to the same genotype (PCR group 4) because of the high similarity band pattern. *A. baumannii* ATCC strains tested by REP-PCR showed a different band pattern to those of the IMRAB and IMSAB isolates (data not shown).

AP-PCR

The agarose gel electrophoresis of the AP-PCR-amplified fragments of representative *A. baumannii* isolates is shown in Figure 2. Different band patterns with fragments ranging in size from 0.3 to 3.5 kb were obtained. AP-PCR profiles of the eight IMRAB strains (1, 3, 6, 8, 24, 25, 26 and 28 in Table 1) involved in the outbreak showed the same band pattern and were assigned to PCR group 1 (Figure 2a). In the same gel and for comparative purposes, the patterns of four *A. baumannii* isolates susceptible to the carbapenems obtained before (strain 31), during (strains 34 and 35) and after (strain 39) the outbreak are shown. The resulting AP-PCR pattern of 10 IMSAB isolates (strains 31–40 in Table 1) is shown in Figure 2b.

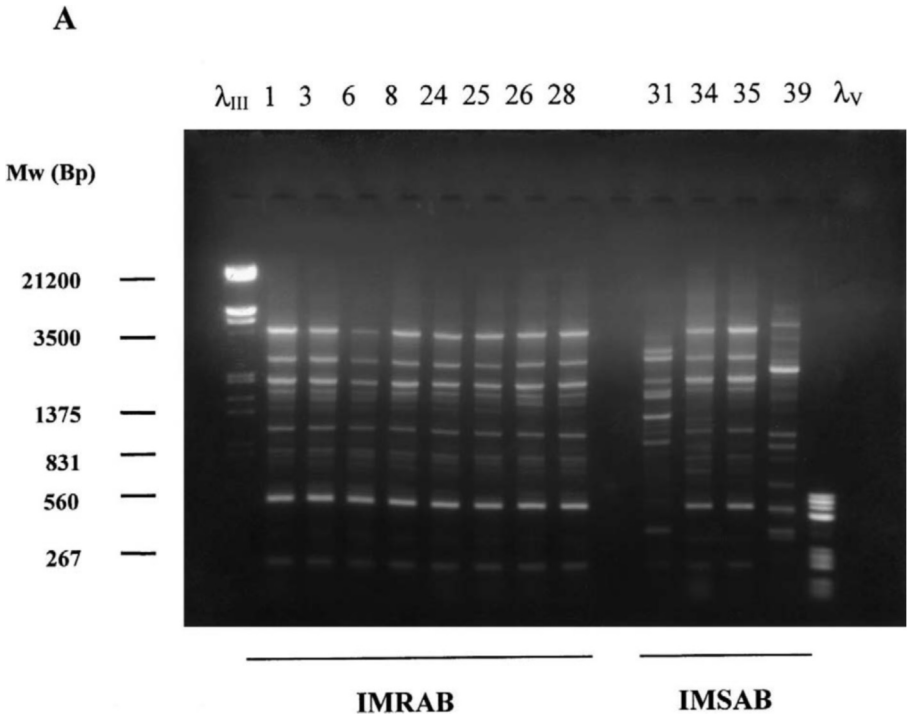

Figure 1 Patterns obtained by REP-PCR. The numbers above the figure indicate the corresponding strain. Lanes $_{III}$ and $_{V}$, DNA Molecular Weight Markers III and V, respectively (Boehringer GmbH, Mannheim, Germany). (a) IMRAB: *Acinetobacter baumannii,* imipenem and meropenem resistant; IMSAB: *Acinetobacter baumannii,* imipenem and meropenem susceptible. (b) IMSAB PRE: IMSAB strains before the outbreak (November 1996 to January 1997). IMSAB AT: IMSAB strains during the outbreak (February to July 1997). IMSAB POST: IMSAB strains after the outbreak (January to February 1998).

Different band patterns were obtained with pre- and post-outbreak strains, and these strains were assigned to PCR groups 2–3 and 5–7, respectively. With at-outbreak IMSAB strains (34–36), a band pattern was obtained identical to that of IMRAB strains, and these strains were assigned to PCR group 1. The at-outbreak IMSAB strain 37 was assigned to PCR group 4. *A. baumannii* ATCC strains tested by AP-PCR showed a different band pattern to those of the IMRAB and IMSAB isolates (data not shown).

PFGE

The PFGE reference technique was performed with the same IMRAB and IMSAB strains used in PCR assays (Figure 3). All IMRAB isolates analyzed had an identical band pattern and were classified as genotype A. In contrast, pre-outbreak IMSAB isolates (strains 31–33), at-outbreak IMSAB isolates (strains 34–37) and post-outbreak IMSAB isolates (strains 38–40) belonged to a different genotype on the basis of the band pattern and were assigned to genotypes B–C, D–E and F–H, respectively (Table 1). As in the PCR techniques, *A. baumannii* ATCC strains tested by PFGE showed a different band pattern to those of the IMRAB and IMSAB isolates (data not shown).

It is interesting to consider the at-outbreak carbapenem-susceptible *A. baumannii* strains 34–36. The genotypic data obtained with these strains led us to place them in a different genotype (REP-PCR group 4 or PFGE group D); however, compared with the profile of the IMRAB strains, less than three and six different bands by REP-PCR and PFGE were obtained, respectively; and by AP-PCR these strains showed a band pattern identical to that of the IMRAB profile. These results suggest the possibility of a close relationship between the IMSAB 34–36 strains and the IMRAB isolates. OMP analysis performed with both strains, one IMRAB and the other IMSAB, showed a reduction in the expression of two porins of 22 and 33 kDa in the carbapenem-resistant *A. baumannii* isolate when compared with the at-outbreak carbapenem-susceptible *A. baumannii* isolate (data not shown). Moreover, a pI 9.0 β-lactamase with carbapenemase activity was detected in the IMRAB strain which was absent in the IMSAB 34–36 strains.

DISCUSSION

In the last two decades, a significant number of *Acinetobacter* nosocomial infection outbreaks, caused mainly by *A. baumannii* strains, have been reported, causing increasing concern in

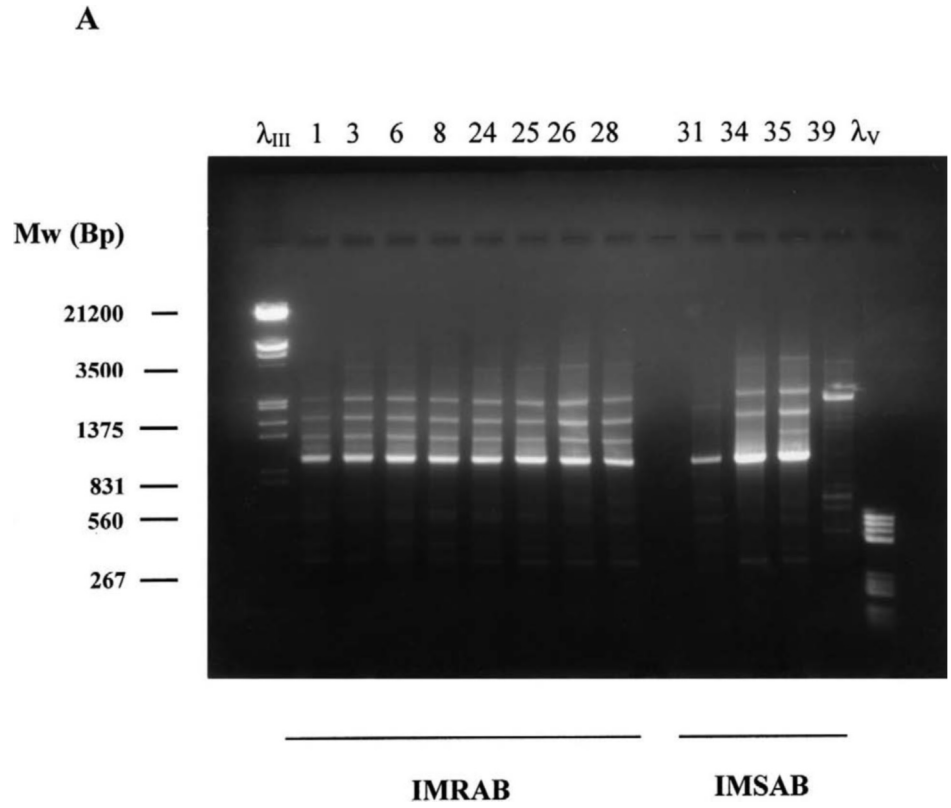

Figure 2 Patterns obtained by AP-PCR. The numbers above the figure indicate the corresponding strain. Lanes $_{III}$ and $_{V}$, DNA Molecular Weight Markers III and V, respectively (Boehringer Mannheim). (a) IMRAB: *Acinetobacter baumannii,* imipenem and meropenem resistant; IMSAB: *Acinetobacter baumannii,* imipenem and meropenem susceptible. (b) IMSAB PRE: IMSAB strains before the outbreak (November 1996 to January 1997). IMSAB AT: IMSAB strains during the outbreak (February to July 1997). IMSAB POST: IMSAB strains after the outbreak (January to February 1998).

hospitals [24,35,36]. 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, distinction between strains with slight differences in resistance profile may be difficult. Therefore, genotypic methods, including plasmid typing, ribotyping, PFGE of chromosomal DNA restriction fragments, and polymerase chain reaction fingerprinting, have been used to investigate nosocomial *A. baumannii* outbreaks [12,13,15–21].

REP-PCR and AP-PCR have previously been used for typing strains belonging to the *A. calcoaceticus–A. baumannii* complex [22–25]. Likewise, the use of PFGE as a technique for typing *A. baumannii* isolates has also been reported [16,17]. That all these techniques are useful for *A. baumannii* outbreak characterization is beyond question, but considering the time required (results with REP-PCR and AP-PCR were obtained in less than 10 h after colonies were grown on a solid medium, while PFGE results were obtained after 90 h), and the costly equipment necessary to perform the epidemiologic studies, it would be pertinent to demonstrate the utility and speed of the PCR techniques compared with the PFGE technique. In addition, only two reports comparing REP-PCR, AP-PCR and PFGE techniques have been published. Liu et al [20], using different PCR-based DNA fingerprinting techniques and PFGE, and investigating the epidemiology of *A. calcoaceticus–A. baumannii* complex, concluded that both techniques are useful for molecular typing. Also, Grundmann et al [19], investigating the nosocomial transmission of *A. baumannii*, concluded that PCR-fingerprinting results were consistent with macrorestriction fragment patterns obtained by PFGE.

We evaluated the REP-PCR and AP-PCR methods, compared with the PFGE technique, for the characterization of a nosocomial outbreak caused by IMRAB strains involving 29 patients. For comparative purposes, IMSAB isolates obtained before, during and after the outbreak were included in the study. All IMRAB isolates showed a similar antibiotic multiresistance pattern and, irrespective of tobramycin resistance level, showed an identical genotypic pattern (PCR group 1) by REP-PCR and AP-PCR. In contrast, with regard to IMSAB isolates, six or seven different genotypes were established with

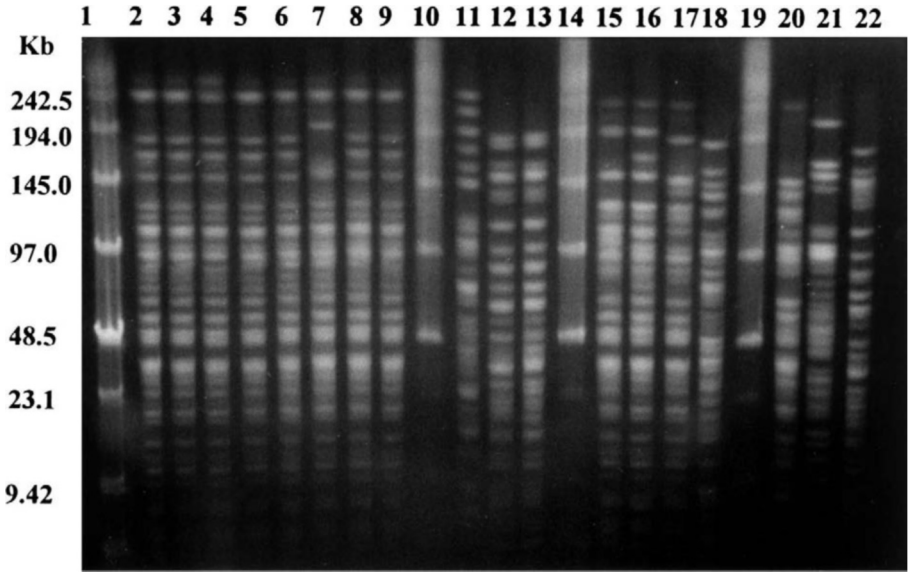

Figure 3 Patterns obtained by PFGE with *Sma*I. Lanes 1, 10, 14 and 19: Low Range PFG DNA Marker (New England Biolabs, Hitchin, Hertfordshire, UK). Lanes 2–9: IMRAB strains 1, 3, 6, 8, 24, 25, 26 and 28, respectively. Lanes 11–13: IMSAB PRE 31–33, respectively. Lanes 15–18: IMSAB AT 34–37, respectively. Lanes 20–22: IMSAB POST 38–40, respectively.

both techniques, irrespective of the antibiotic susceptibility pattern of the strains. Comparing the band pattern and the genotypes of the IMRAB and IMSAB strains, a good correlation was obtained with the REP-PCR-based DNA fingerprinting technique compared with the PFGE method. Thus, in our experience, REP-PCR had a higher discriminatory power than the AP-PCR technique, as demonstrated with at-outbreak IMSAB strains (34–36), showing a band pattern virtually similar to that of the IMRAB strains with the AP-PCR technique (AP-PCR group 1), while with the REP-PCR, a few different bands were observed with these strains compared with the IMRAB pattern (REP-PCR group 4). Identical results were obtained with the PFGE technique, thus confirming the excellent performance of the REP-PCR technique for the *A. baumannii* outbreak characterization.

Concerning the origin of the outbreak, different hypotheses are suggested. The original IMRAB strain of the outbreak was isolated from one patient admitted to the medical ICU, and, afterwards, IMRAB isolates were obtained from patients at the same unit, three different surgical ICUs, and two medical departments. Retrospectively, and with the epidemiologic data obtained with the pre-outbreak strains, the inference was made that the original strain of the outbreak might have been introduced into the hospital by a patient who was previously treated at another institution. By then, IMRAB isolates were a concern in several Spanish hospitals (J. Vila, personal communication). This hypothesis is supported in part by the fact that the hospital outbreak started after admission of the patient.

On the other hand, three at-outbreak IMSAB isolates obtained from different patients in different wards displayed a different genotype (PFGE group D) to that of IMRAB isolates; however, and following the criteria of Tenover et al [31], 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). The first at-outbreak IMSAB strain (34 in Table 1) was isolated in a surgical ICU when the outbreak started. A putative hypothesis is that the IMSAB isolate might have acquired an additional mechanism of resistance to imipenem, which gave it a selective advantage, thus allowing its spread at the beginning of the outbreak. Supporting this view, the results obtained with the OMP analysis showed that a single loss of porins might be involved in the carbapenem resistance, in addition to the presence of a pI 9.0 β-lactamase with carbapenemase activity in the IMRAB strain. However, the opposite hypothesis is also possible, that antibiotic resistance may revert from the IMRAB strains when selective pressure of carbapenems diminishes or no longer exists (strains 35 and 36 were isolated in July 1997). Therefore, according to this hypothesis, these strains could be considered as the ancestors or the descendants of the IMRAB strains. Also, it is important to emphasize that the amount of carbapenem consumed remained practically unchanged during the months previous to the outbreak.

In summary, REP-PCR is a useful and expeditious method for the epidemiologic characterization of *A. baumannii* isolates, either susceptible or resistant to carbapenems, and its results are comparable to those obtained with the PFGE technique.

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