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Use of Pulsed-Field Gel Electrophoresis for Investigation of Hospital Outbreaks of *Acinetobacter baumannii*

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Genomic DNAs from taxonomically and epidemiologically well-defined strains of *Acinetobacter baumannii* were digested with restriction endonucleases that cleave with low frequency, and the fragments were separated by pulse-field gel electrophoresis. Restriction fragment length polymorphisms were observed. Restriction fragment length polymorphism analysis can be used as an epidemiological tool to delineate outbreaks of nosocomial infections caused by *A. baumannii*.

Bacteria of the genus *Acinetobacter* are generally accepted as agents of nosocomial infections (3). This genus has recently undergone significant taxonomic reorganization, and our understanding of the epidemiology of members of the genus has, after years of uncertainty, been clarified by the taxonomic revision described by Bouvet and Grimont (4, 5). A total of 12 hybridization groups (genospecies) were identified, the descriptions of *A. calcoaceticus* and *A. lwoffii* were emended, and four new species—*A. baumannii*, *A. haemolyticus*, *A. johnsonii*, and *A. junii*— were proposed. The role of some species (*A. baumannii*, *Acinetobacter* genospecies 3 and 6, *A. haemolyticus*) as pathogenic agents was highlighted (6). The present distribution of species provides a much better understanding of the epidemiology of the genus. Discrimination among strains within a species is important for delineating nosocomial outbreaks. Several methods have been proposed for strain typing, including biotyping, phage typing, bacteriocin typing, serotyping, cell envelope protein profiles, enzyme profiles, plasmid profiles, and ribotyping (2, 3, 5, 9, 12, 14–16). DNA restriction fragment length polymorphisms (RFLPs) determined by pulsed-field gel electrophoresis (PFGE) have also been used to study strains of *Acinetobacter* isolated during an outbreak in a hospital (1). The results seemed promising, since all strains involved in the epidemic had the same restriction pattern, but further studies are needed to confirm the sensitivity of the method and its value as an epidemiological tool, especially for *A. baumannii*. We therefore determined the RFLPs of strains of *A. baumannii* of various biotypes obtained from hospitals and of strains of the same biotype (biotype 2) from different geographical origins.

Genomic DNA was prepared in agarose plugs as described previously (1). It was then incubated with a mixture of 0.5 M EDTA–1% (wt/vol) sodium dodecyl sulfate–1 mg of pronase (Calbiochem) per ml for 48 h at 37°C, washed, and digested with a suitable enzyme. Various enzymes were screened. *Hin*dIII and *Xba*I cut the chromosomal DNA into a large number of fragments of less than 50 kb (data not shown). *Apa*I and *Sma*I gave convenient numbers of fragments and were chosen for use in this study. One DNA insert was incubated for 2 h at 37°C with 60 U of *Apa*I (New England BioLabs, Inc.) in KGB buffer (100 mM potassium glutamate, 25 mM Tris acetate [pH 7.6], 10 mM magnesium acetate, 50 mg of bovine serum albumin per ml, 0.5 mM 2-mercaptoethanol [11]). Fragments of DNA were separated in a 1% agarose gel (Appligene) that was prepared and run in 0.5× Tris-borate-EDTA (TBE) buffer on a contour-clamped homogeneous field machine (CHEF-DR2; Bio-Rad). The pulse times were 10 s for 21 h and then 8 s for 12 h at 150 V. *Sma*I digests were prepared by incubating one DNA insert for 8 h at 25°C with 50 U of *Sma*I (Boehringer) by the recommendations of the manufacturer. The conditions for electrophoresis were 150 V for 27 h, with pulse times ranging from 20 to 5 s. Thereafter, the gels were stained with ethidium bromide and photographed.

The RFLPs produced with *Apa*I of 10 hospital strains of *A. baumannii* of various biotypes (Table 1) were determined. One strain of *A. baumannii* biotype 2, three strains of biotype 9, and three strains of biotype 6 were isolated from patients at a hospital in Rennes, France. One strain of biotype 6 came from a hospital in Dieppe, France, and two strains of biotype 2 were from a hospital in Nîmes, France. The results obtained are shown in Fig. 1. Strains belonging to different biotypes showed different restriction profiles, and even within strains of the same biotype, strains that were not epidemiologically related exhibited DNA polymorphisms. Two of the four strains of *A. baumannii* biotype 6 were isolated from the same patient and had identical patterns (Fig. 1, lanes 6 and 7); the other two strains had different patterns (Fig. 1, lanes 4 and 5). The pattern of the biotype 2 strain from Rennes (Fig. 1, lane 11) was quite different from those of the biotype 2 strains from Nîmes (Fig. 1, lanes 12 and 13). Two of the biotype 9 strains differed by one band at 65 kb (Fig. 1, lanes 8 and 10). The third strain (Fig. 1, lane 9) was completely different. The genomic DNAs of seven of these strains were also cut by *Sma*I. The results are shown in Fig. 2. The genomic DNAs of biotype 6 strains were partially cut.

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TABLE 1. Characteristics of strains examined in this study

Strain no.[a]	Date of isolation (mo/yr)	Geographical origin (France)	Ward	Source	Biotype	Phage type	Reference
87624	12/1988	Rennes	Neurosurgery	Cerebrospinal fluid	2		
AGN 2		Nîmes	Urology	Urinary tract	2	97	1
AGN 3		Nîmes	Urology	Urinary tract	2	97	1
AGN 20		Nîmes	Orthopedic surgery	Pus	ND[b]		
9999		Dieppe			6		
83381	11/1988	Rennes	Geriatrics	Urinary tract	6		
93257[c]	12/1988	Rennes	Medical intensive care unit	Pus	6		
A 4175[c]	1/1989	Rennes	Medical intensive care unit	Drain	6		
A 279[c]	1/1989	Rennes	Medical intensive care unit	Pus	9		
93034	12/1988	Rennes	Cardiac surgery	Respiratory tract	9		
78465	10/1988	Rennes	Surgery	Bile	9		

[a] Reference strains of *A. baumannii* from the collection of the Institut Pasteur, Paris, France. Strain IP 53-79 is ri CIP strain "Gaillard" Whitlow 1951; strain IP 70-34^T is ATCC 19606.
[b] ND, not determined.
[c] The strains were isolated from the same patient.

The value of the RFLP data for differentiating strains within the same biotype was examined by cleaving DNAs from 11 *A. baumannii* biotype 2 strains isolated in different parts of the world (Table 2) with *Apa*I and analyzing them by PFGE (Fig. 3). Four strains came from Paris; two strains came from Palermo, Italy; two strains came from Rotterdam, The Netherlands; one strain came from Hamburg Germany; one strain came from Brazil; and one strain came from Abidjan, Côte d'Ivoire. Identical patterns were found only for strains of the same geographical origin.

The results of this study indicate that there is considerable DNA polymorphism within the species *A. baumannii* and even among strains from different geographical regions that belong to the same biotype. Thus, RFLPs can be used as an epidemiological tool to delineate outbreaks of nosocomial infections. Strains can be compared rapidly, and any hospital outbreak can be confirmed or negated with a minimum of delay. For instance, a previous study (1) confirmed the spread of an epidemic strain of *A. baumannii* in a urology unit. At the same time, infections involving multiresistant *Acinetobacter* species occurred in an orthopedic unit, where bacteria of this genus were rarely isolated. Multiresistant

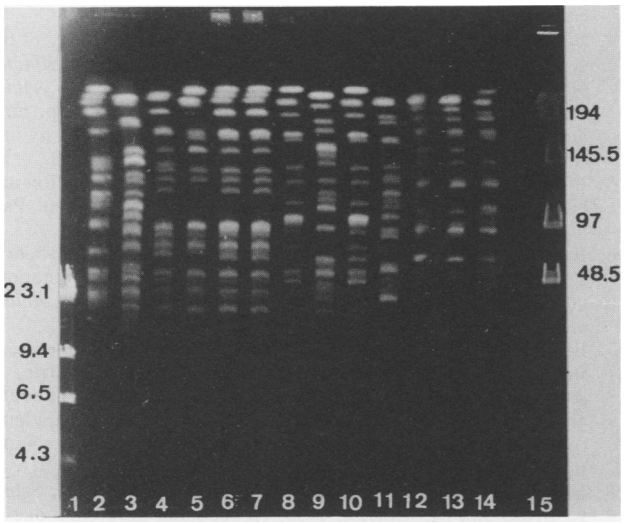

FIG. 1. *Apa*I digestion of DNA from *A. baumannii* of different biotypes. Lane 1, lambda DNA cut by *Hin*dIII (expressed in kilobases); lanes 2 to 13, the *A. baumannii* strains described in Table 1; lanes 2 and 3, reference strains IP 53.79 and IP 70.34 T, respectively; lanes 4 to 7, biotype 6 strains (strains 9999, 83381, A 4175, and 93257, respectively); lanes 8 to 10, biotype 9 strains (strains A 279, 78465, and 93034, respectively); lanes 11 to 13, biotype 2 strains (87624, AGN 2 and AGN 3, respectively); lane 14, biotype not determined (strain AGN 20); lane 15, lambda DNA concatemers (expressed in kilobases).

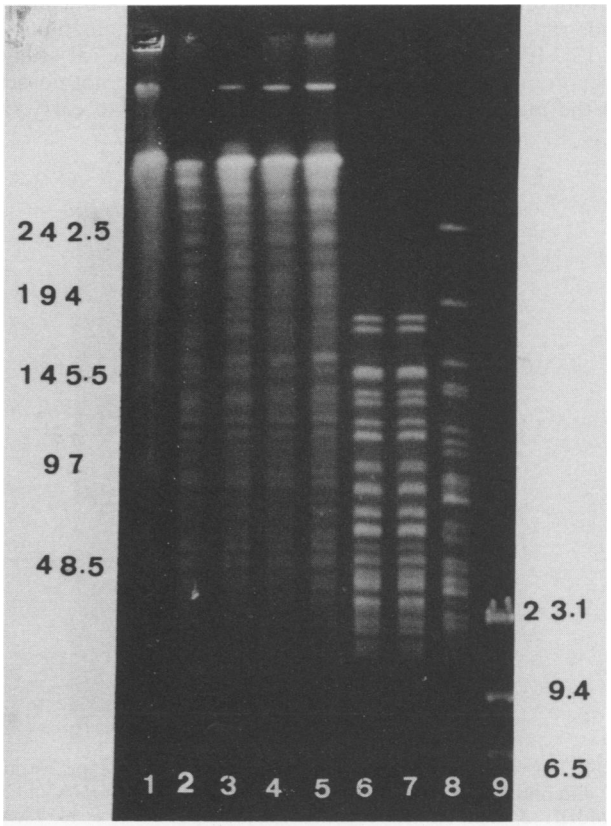

FIG. 2. *Sma*I digestion of DNA from *A. baumannii* of different biotypes. Lane 1, lambda DNA concatemers (expressed in kilobases); lanes 2 to 5, biotype strains 6 (strains 9999, 83381, A 4175, and 93257, respectively); lanes 6 to 8, biotype 9 strains (strains A 279, 93034, and 78465, respectively); lane 9, lambda DNA cut by *Hin*dIII (expressed in kilobases).

TABLE 2. Geographical origins of strains examined in this study

Strain no.	Date of isolation (mo/yr)	Geographical origin	Ward	Source	Biotype	Phage		CEPP[a]	Reference
						Type	Subtype		
PB 429	5/1985	Val-de-Grâce, Paris, France	Surgery	Urinary tract	2	75			8
PB 433	9/1985	Val-de-Grâce, Paris, France	Medical	Pus	2	Atypical			8
PB 597		Bichat, Paris, France		Skin lesion	2	46			
PB 598		Bichat, Paris, France		Pus	2	Atypical			
PB 675		Palermo, Italy		Urinary tract	2	75			10
PB 676		Palermo, Italy		Respiratory tract	2	75			
PB 372		Brazil		Cerebrospinal fluid	2				
PB 1072		Abidjan, Côte d'Ivoire		Unknown	2				
PB 1602		Hamburg, Germany		Unknown	2				
PB 1254		Rotterdam, The Netherlands		Clinical material	2	NS[b]	17	A	6
PB 1257		Rotterdam, The Netherlands		Clinical material	2	NS	17	A	6

[a] CEPP, cell envelope protein profile.
[b] NS, not susceptible to the phages of the phage-typing system considered.

Acinetobacter species were also found in the environment. The restriction patterns of the DNAs from these bacteria were completely different, proving that it was not a true outbreak (data not shown). Identification and typing of the strains by M. L. Joly-Guillou (Centre de Réference des *Acinetobacter*) confirmed that the strains belonged to species other than *A. baumannii*. If a laboratory possesses the PFGE equipment and a set of convenient restriction enzymes, RFLP analysis is a versatile method that can be used to differentiate not only *Acinetobacter* species but also a wide range of bacteria involved in nosocomial infections (1, 7, 13). It allows precise and rapid epidemiological studies even in moderately sized laboratories which do not possess all the media, phages, and antisera necessary to carry out conventional epidemiological analyses for all the bacteria responsible for nosocomial infections.

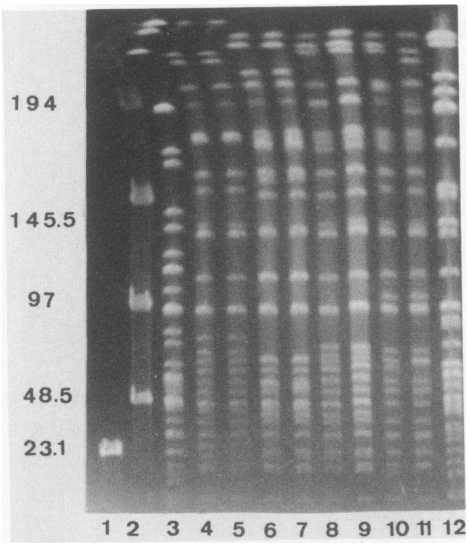

FIG. 3. *Apa*I digestion of DNA of *A. baumannii* biotype 2 strains of different geographical origins. Lanes: 1, lambda DNA cut by *Hin*dIII; 2, lambda DNA concatemers (kilobases); 3 to 12, *A. baumannii* strains (strain 372 from Brazil [lane 3]; strains 429 [lane 4] and 433 [lane 5] from Hôpital du Val-de-Grâce, Paris, France; strains 597 [lane 6] and 598 [lane 7] from Hôpital Bichat, Paris, France; strains 675 [lane 8] and 676 [lane 9] from Palermo, Italy; strains 1257 [lane 10] and 1254 [lane 11] from Rotterdam, The Netherlands, strain 1602 [lane 12] from Hamburg, Germany).

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