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Epidemiological Study of an *Acinetobacter baumannii* Outbreak by Using Polymerase Chain Reaction Fingerprinting

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A polymerase chain reaction (PCR) technique was applied to the fingerprinting of different strains of *Acinetobacter baumannii* from a cluster of patients infected or colonized with the incriminated pathogen. The DNA was extracted by boiling and was subjected to PCR amplification by using the core sequence of the M13 phage as a single primer. The amplified products were separated by agarose gel electrophoresis and were detected by staining with ethidium bromide. In 1990, 49 multiresistant *A. baumannii* strains were isolated from 13 patients from the same intensive care unit of the Charité Hospital; 45 of these outbreak isolates obtained from 12 patients showed the same PCR patterns, indicating an epidemiological relatedness of these strains. Four strains isolated from the same patient belonged to another genetic group, as revealed by a distinct amplification pattern. Another single subtype of *A. baumannii* was identified as the causative agent in patients during a second outbreak at a different intensive care unit in the same hospital. Seventeen isolates recovered from 10 immunocompromised patients had the same amplification patterns, which were distinct from all other PCR profiles. Five strains were obtained from two other hospitals; three isolates from the hospital of Magdeburg, Germany, had identical PCR patterns which, however, could be clearly distinguished from the patterns of all other strains. The remaining two isolates displayed individual patterns of amplified fragments. PCR fingerprinting may provide a useful and particularly rapid identification technique for epidemiological investigations of nosocomial infections.

During the last few years *Acinetobacter baumannii* strains have been found to be a frequent source of hospital-acquired infections, especially in patients with severe underlying diseases (2, 6, 7, 21, 22, 26). Although these microorganisms usually colonize normal skin, they may cause a wide range of infections including pneumonia, lung abscesses, meningitis, septicemia, urinary tract infections, as well as wound infections. To determine the origins of infection, the routes of transmission, and the duration of their persistence, discriminating and reproducible methods are required for the identification of pathogenic isolates. So far, methods which measure phenotypes, like serotyping, biotyping, phage typing, and analysis of outer membrane protein or enzyme profiles, have commonly been used to discriminate *A. baumannii* isolates (4, 5, 9, 16, 21).

The expression of phenotypic characters may vary and may be affected by many environmental factors, whereas methods of identification that are based on the genotype of an isolate are generally more stable. The analysis of restriction fragment length polymorphisms (RFLPs), ribotyping, and karyotyping by pulsed-field gel electrophoresis (PFGE) were successfully used for strain typing of different bacterial species, including *Acinetobacter* spp. (10, 11). These techniques, however, are often laborious and time-consuming. Recently, simpler methods for assessing DNA polymorphisms, by amplifying genomic DNA with single primers of arbitrary nucleotide sequences, have been reported (23–25). The aim of the study described here was to apply polymerase chain reaction (PCR) fingerprinting to the characterization of *A. baumannii* strains obtained from patients involved in outbreaks at the Charité Hospital.

MATERIALS AND METHODS

Bacteria. Forty-nine *A. baumannii* strains were isolated from 13 patients (patients B1 to B13) during an outbreak in the anesthesiological intensive care unit of the Charité Hospital between January and April 1990. Patients were infected or colonized with multiresistant and plasmidless strains of this pathogen. All patients were mechanically ventilated after neurosurgery. Seven of these patients had severe pneumonia; one of the patients developed a urinary tract infection. From February until March 1993, an additional 17 multiresistant *A. baumannii* isolates were obtained from 10 patients (patients B14 to B23) on different intensive care units in the same hospital. Three patients suffered from meningitis or pneumonia, one had a wound infection, and one developed a urinary tract infection. The remaining patients were colonized with the pathogen. Five isolates which were obtained from five patients in different intensive care units from hospitals in Magdeburg, Germany (patients M1 to M3), and Oschersleben, Germany (patients O1 and O2), were taken from a collection of the Robert Koch Institute, Wernigerode, Germany. The *A. baumannii* ATCC 19606 strain served as a control. The isolates were identified as *A. baumannii* by their sugar assimilation patterns (13) as well as by using the API 20 NE miniature system (API bioMérieux, Nürtingen, Germany).

Antibiotic susceptibilities. Susceptibilities to antibiotics were primarily assessed by the disk diffusion method on Mueller-Hinton agar and were later confirmed by the agar dilution test.

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TABLE 1. Antibiotic susceptibilities of *A. baumannii* strains of different origins

Patients	No. of isolates	No. of patients	Antibiotic susceptibilities[a]								PCR profile	PFGE profile
			OXY	STR	KAN	GEN	AMK	CTAZ	NAL	CIP		
B1–B12	45	12	R	R	R	R	R	I	S	S/I/R	I	1
B13	4	1	*S/I/R*	R	R	R	*S/I/R*	*S/I*	R	R	II	2
B14–B23	17	10	R	ND[b]	ND	R	*R/I*	*R/I*	ND	*R/I*	III	ND
M1–M3	3	3	R	S	*I/R*	*I/R*	S	I	S	I	IV	4
O1–O2	2	2	S	S	S	*S/R*	*S/I*	I	S	I	V, VI	5, 6

[a] All strains tested were susceptible to imipenem and exhibited resistance to chloramphenicol, nitrofurantoin, ampicillin, azlocillin, piperacillin, cefotiam, cefotaxime, and aztreonam. Abbreviations of the antibiotics are those recommended by the International Society of Chemotherapy, as follows: OXY, oxycillin; STR, streptomycin; KAN, kanamycin; GEN, gentamicin; AMK, amikacin; CTAZ, ceftazidime; NAL, nalidixic acid; CIP, ciprofloxacin. S, susceptible; I, intermediate; R, resistant. Italic type indicates the different resistance patterns of single strains.

[b] ND, not done.

DNA extraction. Bacteria were grown on Luria-Bertani agar overnight. Two to three colonies of each isolate were picked and suspended in 100 μl of sterile water. The suspension was overlaid with two drops of light mineral oil (Sigma Chemicals Co., St. Louis, Mo.) and was kept at 95°C for 15 min for cellular disruptions. Following brief centrifugation, 2.5 μl of the lysate was used for PCR.

PCR fingerprinting. The core sequence of phage M13 (5′-GAGGGTGGCGGTTCT-3′) was used as a single primer in the PCR experiments. This oligonucleotide was prepared commercially (Tib Molbiol Berlin GmbH). Amplification reactions were performed in a final volume of 50 μl containing 10 mmol of Tris-HCl (pH 8.3) per liter, 50 mmol of KCl per liter, 1.5 mmol of $MgCl_2$ per liter, 3 mmol of magnesium acetate per liter, 200 μmol each of dATP, dGTP, dCTP, and dTTP (Pharmacia LKB Biotechnology Inc., Piscataway, N.J.) per liter, 25 μmol of the primer per liter, 2.5 U of AmpliTaq DNA polymerase (Perkin Elmer/Cetus, Norwalk, Conn.), and 2.5 μl of crude DNA extract. The samples were overlaid with light mineral oil and were amplified in a thermocycler (Biometra Trioblock, Göttingen, Germany) for 27 cycles, as follows: 20 s at 95°C, 60 s at 50°C, and 20 s at 72°C. This was followed by a final extension of 6 min at 72°C. Reaction tubes were held at 4°C prior to analysis. Samples were concentrated to a volume of approximately 20 μl (Speed Vac; Savant, Hicksville, N.Y.) and were electrophoresed in 1.2% agarose gels. Amplified products were detected by staining with ethidium bromide.

RFLP analysis by PFGE. Genomic DNA was released as described previously (17) and was cleaved with the restriction endonuclease *Apa*I (20 U per sample). The DNA fragments were separated by PFGE in a 1.2% agarose gel (agarose type II; medium EEO; Sigma) with a CHEF-DR II apparatus (Bio-Rad Laboratories, Richmond, Calif.) at 200 V by using the following three intervals of ramped pulse times: 1 to 4 s for 5 h, 5 to 60 s for 18 h, and 60 to 90 s for 18 h. The DNA fragments were stained with ethidium bromide and photographed.

Densitometric evaluation of PCR and RFLP patterns. The negatives of photographs (type 667 Polaroid film) of PCR gels were scanned with a computer-integrated laser densitometer by using RFLPrint, version 1.1, software (SPARC station IRC from Sun Microsystems, Inc.). The scans were evaluated, and the percent similarity was calculated according to the neighbor-joining method (19).

RESULTS AND DISCUSSION

PCR fingerprinting has successfully been applied to the detection of polymorphisms in DNAs of viral, bacterial, fungal, plant, and animal origin (8, 15, 23, 24). The applicability of this technique has been demonstrated for epidemiological subtyping of bacterial and fungal isolates (18, 20). Single primers which were originally applied as hybridization probes in conventional DNA fingerprinting to detect minisatellite and microsatellite DNAs such as the core sequence of phage M13 (14) and the simple repeat sequence $(GACA)_4$ (1) were used to amplify polymorphic regions in the *A. baumannii* DNA. Besides, in our experiments, primers which had already been used in other laboratories for PCR fingerprinting, like the universal sequence of M13 (23) or the 10mer oligonucleotide AP 3 (25), were tested. Comparable results were obtained with all primers used, but the M13 core sequence primer usually generated a higher number of amplification products. Since the discriminating power of the core sequence of M13 was found to be greater than those of all other primers, this oligonucleotide was used to fingerprint the clinical isolates of *A. baumannii*. The PCR protocol was optimized with regard to template and primer concentrations, annealing temperature, and cycle number (12). Stable and reproducible fingerprints were yielded if primer annealing was carried out at the melting temperature of the oligonucleotide sequence. The reduction of the PCR cycle number to 27 cycles resulted in the appearance of sharper bands in the amplification patterns. If more than 30 cycles were performed, artifactual bands occurred in the control samples without DNA; however, these were clearly distinguishable from all the bands obtained with various DNA samples. It is assumed that these artifacts may result from the contamination of *Taq* polymerase with bacterial DNA (3). By using the M13 primer and the optimized PCR program, 13 to 18 fragments of between 0.2 and 3 kb could be amplified; this is sufficiently distinct to discriminate all *Acinetobacter* strains tested.

The 49 *A. baumannii* strains isolated during the first outbreak in 1990 revealed similar susceptibility patterns, being resistant to the majority of antibiotics tested except for a heterogeneous susceptibility to ciprofloxacin (Table 1). The PCR patterns of 45 isolates obtained from patients B1 to B12 that displayed similar antibiotic susceptibilities were found to be identical (Fig. 1; PCR profile I). Variations in the intensities of the amplified bands were occasionally observed. Those differences may be attributed to slight variations in the temperatures of individual wells in the thermocycler or to different amounts of template DNA in the crude extracts, but they did not influence the evaluation of the PCR patterns. Accordingly, the four isolates from patient B13 which had a distinct amplification pattern (Fig. 1; PCR profile II) had antimicrobial susceptibilities different from those of all other isolates (Table 1). These findings suggest

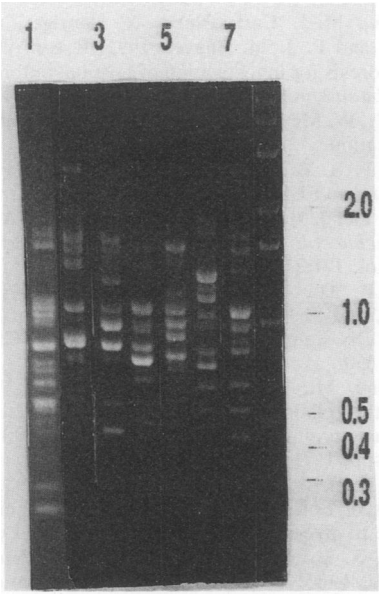

FIG. 1. Fingerprints obtained by PCR with the M13 core sequence primer for seven different groups of *A. baumannii* strains. Lane 1, strains from patients B14 to B23, 17 isolates (PCR profile III); lane 2, ATCC 19606 (PCR profile VII); lane 3, strains from patients B1 to B12, 45 isolates (PCR profile I); lane 4, strains from patient B13, 4 isolates (PCR profile II); lane 5, strains from patients M1 to M3, 3 isolates (PCR profile IV); lane 6, a strain from patient O1, 1 isolate (PCR profile V); lane 7, a strain from patient O2, 1 isolate (PCR profile VI); lane 8, molecular size markers (in kilobases).

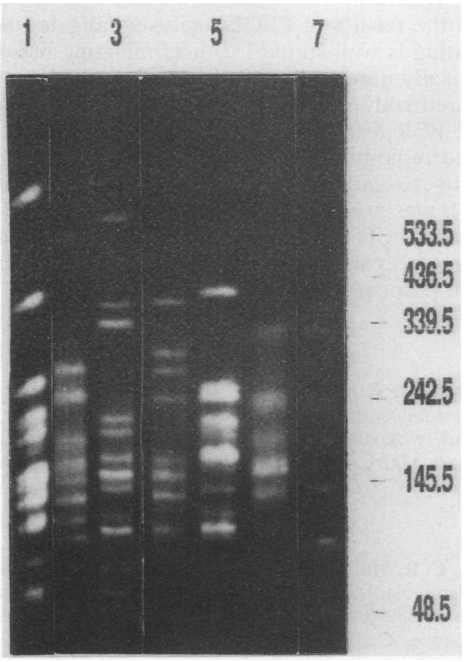

FIG. 2. PFGE patterns of *Apa*I-cleaved DNAs obtained from six different groups of *A. baumannii* strains. Lane 1, ATCC 19606 (PFGE profile 7); lane 2, strains from patients B1 to B12, 45 isolates (PFGE profile 1); lane 3, strains from patient B13, 4 isolates (PFGE profile 2); lane 4, strains from patients M1 to M3, 3 isolates (PFGE profile 4); lane 5, a strain from patient O1, 1 isolate (PFGE profile 5); lane 6, a strain from patient O2, 1 isolate (PFGE profile 6); lane 7, molecular size markers (in kilobases).

that 45 strains isolated during the outbreak in 1990 belonged to the same subtype (PCR profile I) of *A. baumannii* and should be epidemiologically related. A densitometric analysis of the PCR fingerprints revealed 100% homology for these *Acinetobacter* isolates. Our attempts to identify a common source or mode of transmission of the epidemic strain were not successful. The remaining four strains, however, represented a different subtype (PCR profile II). A densitometric analysis of the PCR fingerprints revealed a similarity of 69% for the *Acinetobacter* isolates of PCR profiles I and II.

The 17 strains isolated at the Charité Hospital in 1993 were also identical, as indicated by the same amplification pattern (Fig. 1; PCR profile III), but they could be differentiated from all other *Acinetobacter* strains investigated. It was suggested that a single subtype of *A. baumannii* was the causative agent in patients during this new outbreak at a different intensive care unit. The computer-assisted analysis of the genetic relationship showed a low degree of similarity (44%) of *A. baumannii* strains isolated during these two outbreaks at the Charité Hospital (PCR profiles I and III).

The three *Acinetobacter* isolates from the hospital of Magdeburg were originally not expected to be epidemiologically related because these isolates varied in their susceptibilities to 2 of the 17 antibiotics tested. Identical fingerprints, however, were generated by the M13-primed PCR (Fig. 1; PCR profile IV), suggesting a genetic relatedness of these strains. The amplification pattern differed clearly from those of all other strains.

Individual PCR fingerprints were yielded for both strains from the hospital of Oschersleben (Fig. 1; PCR profile V and VI) as well as for the reference strain ATCC 19606 (PCR profile VII). The two strains from Oschersleben, however, showed differences only in their susceptibilities to 2 of 17 antibiotics tested (Table 1).

The results of PCR fingerprinting agreed well with those of RFLP analysis by PFGE (Fig. 2). The 45 strains from patients B1 to B12 had similar PFGE patterns (PFGE profile 1), as did the three *A. baumannii* isolates from the hospital of Magdeburg (PFGE profile 4). Within PFGE profile 1, the homology was 91 to 100%. The variations observed for one high-molecular-weight fragment could be explained by methylation of *Apa*I restriction sites in different isolates. Strains with distinct PCR profiles were also characterized by clearly distinguishable RFLP patterns (Table 1). For example, the similarity between strains with PFGE profiles 1 and 2 was 50%; strains with all other profiles showed a lower degree of homology.

The antibiotics resistance data alone did not suffice for the epidemiological identification of nosocomial *Acinetobacter* strains, since strains with identical PCR fingerprints differed slightly in their susceptibilities. Conversely, strains with distinct amplification patterns had very similar resistance properties (strains from patients O1 and O2). Variations in the antibiotic resistance patterns of strains belonging to the same PCR group might be due to the different plasmid contents of these strains; for example, one of three strains from patients M1 to M3 harbored a high-molecular-weight plasmid. But it must be considered that, by using single arbitrary primers, DNA fragments were amplified at random, no sequence information about these fragments is available, and point mutations within amplified fragments might remain undetected. Furthermore, sequences coding for antibiotic resistances might possibly not be amplified by the PCR method described here. Nevertheless, the compar-

ison with the results of PFGE analysis indicated that PCR fingerprinting is well-suited for discriminating between epidemiologically unrelated *A. baumannii* strains.

Compared with other methods of DNA-based strain identification, PCR fingerprinting offers the advantages of simplicity and rapidity. The use of a simple boiling procedure allowed us to extract DNA from many strains simultaneously. It was possible to differentiate about 40 strains in 2 days. Hence, PCR fingerprinting may prove to be especially useful for surveying large numbers of individuals for epidemiological purposes.

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