Acinetobacter baumannii Outbreak Isolates Characterized by Three Typing Methods

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Forty-two strains of *Acinetobacter baumannii* were isolated from 15 patients hospitalized in a French intensive care unit. An epidemiological study based on the typing of these isolates was carried out using biotyping, antibiotyping, and ribotyping to recognize the transmission of multiresistant strains by transfer of a patient from one hospital to another. Fifteen strains from the outbreak (1 strain for each patient), five *Acinetobacter baumannii* strains isolated before the outbreak in Bellevue Hospital (St. Etienne), and five strains isolated in Cochin Hospital (Paris) were included. The three methods gave a good correlation: the epidemic strains had the same antibiotic resistance pattern, the same biotype, and the same ribotypes obtained with three different endonucleases.

Three years ago *Acinetobacter baumannii* strains were not commonly isolated in Bellevue Hospital (St. Etienne, France), representing only 0.85 % of isolates in 1992. However, from November 1992 to March 1993 an outbreak of *Acinetobacter baumannii* strains occurred in the intensive care unit (ICU) of this hospital. Forty-two multiresistant *Acinetobacter baumannii* strains were isolated from 15 hospital patients. A case-control study was conducted, as cross-contamination from colonized patients was strongly suspected. Spread of the outbreak was controlled by emphasis on hand hygiene, review of care of intubated patients, and treatment of colonized patients. A patient transferred from Cochin Hospital (Paris) to our hospital (St. Etienne) was suspected to be the index case.

The aims of this study were to determine, using several typing methods, whether all *Acinetobacter baumannii* isolates were identical and to identify routes of transmission as well as the index case.

Materials and Methods. Bellevue Hospital in St. Etienne is a 470-bed medico-surgical teaching hospital with a 15-bed ICU. From November 1992 to March 1993, 42 *Acinetobacter baumannii* strains were isolated from 15 patients in the ICU. An investigation of the outbreak was carried out, and biotyping, antibiotyping, iodometric detection of β-lactamase activity, and ribotyping were applied on the following strains:

(i) One strain for each patient (S1 to S15): 13 strains from sputum and pharyngeal swabs and two from suppuration. The numerous isolates from the same patient all had the same antibiogramme.

(ii) Five *Acinetobacter baumannii* isolates (S16 to S20) from Cochin Hospital (November 1992 to June 1993): one from sputum, three from suppuration, and one from blood.

(iii) Five *Acinetobacter baumannii* (S21 to S25) isolates from the Bellevue Hospital ICU before the outbreak (November 1992 to June 1993): one from sputum, two from urine, and two from suppuration.

Bacteria were identified as belonging to the genus *Acinetobacter* by morphology and negative cytochrome oxidase as well as by the API 20 NE system (API bioMérieux, France). Identification was confirmed by the transformation assay of Juni (1). Identification at the species level of *Acinetobacter* and biotyping of *Acinetobacter baumannii* isolates were done according to Bouvet and Grimont (2).

Antibiotic susceptibility profiles of the strains were investigated by determining the MICs by the agar dilution method on Mueller-Hinton agar (Diagnostics Pasteur, France) containing a serial twofold dilution of antibiotics following the NCCLS guidelines (3). Plates were inoculated with 10^4 cfu per spot using a multi-inoculator device and incubated at 37°C for 18 h. The antibiotics tested were ticarcillin (SmithKline Beecham, UK), piperacillin (Lederle, France), cefotaxime and cefpirome (Roussel, France), ceftazidime (Glaxo, UK), aztreonam, amikacin, and cefepime (Bristol-Myers Squibb, USA), imipenem (Merck Sharp & Dohme, USA), meropenem (Zeneca-Pharma, UK), gentamicin (Schering Plough,

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France), tobramycin (Lilly, USA), ciprofloxacin (Bayer-Pharma, Germany), colimycin (Roger Bellon, France), and rifampicin (Marion Merrell Dow, France). Beta-lactamase inhibitors tested were clavulanic acid (SmithKline Beecham, UK), tazobactam (Lederle, France), and sulbactam (Pfizer, France).

Detection of β-lactamase activity was determined in cell-free extracts of the 25 strains using an iodometric procedure (3). It was performed in agar (bioMérieux) at 37°C with benzylpenicillin (1 mM) as the substrate. This allows differentiation between cephalosporinase-producing strains (β-lactamase inhibition by cloxacillin 0.1 mM) and penicillinase-producing strains (β-lactamase inhibition by clavulanic acid 0.1 mM). The method of Bouvet and Grimont (2) based on the utilization of levulinate, citraconate, L-phenylacetate, 4-hydroxybenzoate, and L-tartrate was used for biotyping.

For rRNA gene restriction patterns (ribotyping), strains were grown in 5 ml of trypto casein soy (TCS) broth and incubated at 37°C for 16 h with shaking. The cells were pelleted at 10,000 x *g* for 15 min and lysed. High molecular weight DNA was extracted and was purified according to Grimont and Grimont (4). Purified bacterial DNA (3 to 5 μg) was cleaved by restriction endonucleases *Cla*I, *Eco*RI, and *Pvu*II, following the supplier's instructions (Amersham International, UK; Boehringer, Germany; Pharmacia, Sweden) at 37°C for 4 h. Restriction DNA fragments were separated electrophoretically through 0.8 % (w/v) horizontal agarose gel (Apligène, France) in Tris-acetate buffer (40 mM Tris-acetate, 2 mM EDTA, pH 8.1) for 16 h at 1.5 V/cm. Restriction fragments of *Xenorhabdus* sp. strain 278 obtained after cleavage by *Eco*RI was used as a molecular weight marker. This marker was directly visualized with the same probe for rRNA genes. The DNA fragments were transferred to a nylon membrane (Hybond-N, Amersham) using a VacuGene system (Pharmacia). The probe was constituted of 16+23S rRNA from *Escherichia coli* labelled with acetylaminofluorene (Eurogentec, Belgium) (5). The nylon membranes were hybridized with the acetylaminofluorene-labelled ribosomal RNA probe (Eurogentec, Belgium) as described previously (4).

Prehybridization and hybridization were carried out at 60°C for 16 h, followed by three washes only in 2 x SSC/0.1 % SDS at 60°C (4, 5). These nonstringent conditions were required when bacteria phylogenetically remote from *Escherichia coli* were hybridized with an *Escherichia coli* rRNA probe. With the radioactive probe, the membranes were autoradiographed as described elsewhere (5). Before the immunoenzymatic detection of the AAF-probe, the membranes were saturated for 30 min at room temperature with a blocking solution containing 1 % casein (Merck, ref. 2242, or BDH, ref. 44020) in maleic acid buffer (0.1 M maleic acid, 0.15 M NaCl, pH 7.5) and then incubated for 2 to 3 h with anti-AAF monoclonal antibody (1 μg/ml in blocking solution) and incubated for 1 h with anti-mouse IgG alkaline phosphatase-conjugated antibodies. After the addition of substrate (nitroblue tetrazolium + 5-bromo-4-chloro-3-indolyl phosphate), purplish blue bands appeared within a few minutes to 1 h. The membranes were scanned using an Apple One-Scanner. Detection of lanes and bands was performed by interpolation of fragment sizes using the method of Schaffer and Sederoff (6). Clustering by single linkage and schematic representation of patterns were obtained using the Taxotron package (Institut Pasteur Taxolab, France).

Results and Discussion. All 15 clinical strains (S1 to S15) isolated during the outbreak gave an identical susceptibility pattern (pattern A). Table 1 presents the five phenotypes corresponding to the susceptibility patterns. Strains S16 and S18 (Paris) showed the same antibiotype (pattern A). With the other isolates, four different antibiotypes were characterised: pattern B for strains S19 and S20 (Paris); pattern C for strains S17 (Paris), S23, S24, and S25 (St. Etienne); pattern D for strain S22 (St. Etienne); and pattern E for strain S21 (St. Etienne). Using iodometric testing of β-lactamase activity, all strains except strain S22 produced only a cephalosporinase (strain S22 produced penicillinase as well as cephalosporinase). This method does not offer sufficient discrimination. Figure 1 gives a standardized representation showing migration patterns of rRNA gene-restriction fragments after cleavage by *Pvu*II with the 25 clinical isolates of *Acinetobacter baumannii.* Strains S1 to S15 (St. Etienne outbreak) and S16 and S18 (Paris) gave identical ribotypes obtained with the three endonucleases. Other strains yielded different patterns. Table 2 summarizes the antibiotypes, biotypes, and ribotypes obtained with the three endonucleases. All outbreak isolates shared the same biotype 9. This biotype was found in both Cochin Hospital and Bellevue Hospital.

Acinetobacter baumannii is an aetiological agent of nosocomial infections acquired in ICUs (7).

Table 1: MICs of antibiotics and inhibitors for the epidemic strain and the strains isolated in Paris and St. Etienne.

Antibiotic or inhibitor	MIC (mg/l)				
	Phenotype A (S16, S18, S*)	Phenotype B (S19, S20)	Phenotype C (S17, S23, S24, S25)	Phenotype D (S22)	Phenotype E (S21)
Clavulanic acid	40	80	20	20	10
Tazobactam	16	128	4	16	4
Sulbactam	4	32	1	16	1
Ticarcillin	64	128	16	1,024	16
Piperacillin	128	256	32	256	16
Cefotaxime	128	256	8	256	4
Ceftazidime	32	128	4	32	4
Cefepime	32	64	2	32	2
Cefpirome	128	256	2	64	2
Aztreonam	64	256	16	64	16
Imipenem	1	8	0.25	1	1
Meropenem	2	32	0.5	1	1
Gentamicin	512	64	1	512	1
Tobramycin	256	512	1	1,024	1
Netilmicin	16	128	< 1	128	1
Amikacin	64	64	4	128	2
Ciprofloxacin	128	64	< 0.5	128	8
Colimycin	0.8	0.8	0.8	0.8	0.8
Rifampicin	16	4	8	4	8

S*: Epidemic strain; antibiotype corresponding to all 15 clinical strains (S1 to S15) isolated during the outbreak.

This microorganism may cause a wide range of infections including pneumonia, lung abscesses, septicaemia, meningitis, and urinary tract infections, as well as wound infections (8). To determine the origins of infection, the routes of transmission, and the duration of the isolates' presence in a population, discriminating and reproducible methods are required for the identification of the isolates. Several methods have been described for typing *Acinetobacter baumannii* strains: biotyping, antibiotyping, cell-envelope protein SDS-PAGE, plasmid typing, pulsed-field gel electrophoresis of chromosomal DNA restriction fragments, ribotyping, and polymerase chain reaction fingerprinting (8–11). In our study there was good correlation between the three methods used: antibiotyping, biotyping, and ribotyping.

This study demonstrated that (i) all *Acinetobacter baumannii* isolates from the Bellevue outbreak showed a single pattern, (ii) the epidemic strain was unrelated to the other *Acinetobacter baumannii* strains found earlier in this hospital, and (iii) the epidemic strain was identical to the isolates from Cochin Hospital observed in November 1992 and June 1993. We confirmed that the epidemic index case was a patient transferred from Cochin Hospital to Bellevue Hospital in November 1992. This patient was hospitalized in Cochin Hospital from June to November 1992 and subsequently transferred to the ICU at Bel-

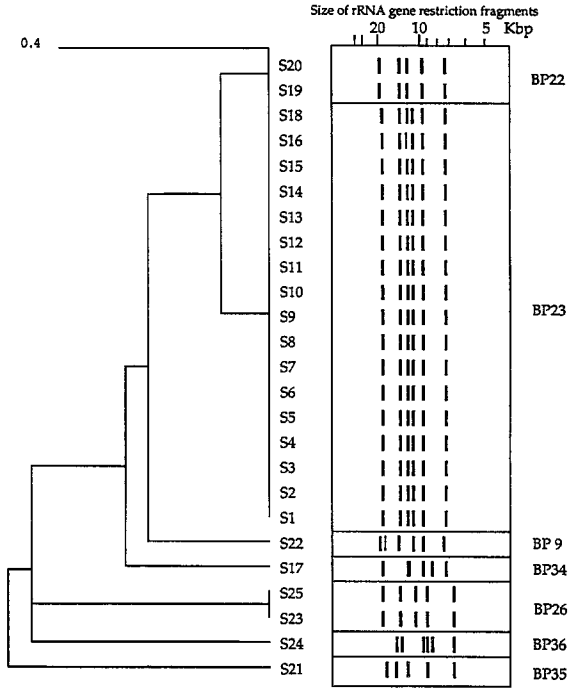

Figure 1: Schematic graph showing patterns of rRNA gene restriction fragments after cleavage of *Acinetobacter baumannii* DNA by *Pvu*II restriction enzyme. Clustering was by single linkage using Taxotron software.

Table 2: Epidemiological study of 25 *Acinetobacter baumannii* strains.

Strain	Location	Date of isolation	Ribotype *ClaI*	Ribotype *PvuII*	Ribotype *EcoRI*	Biotype	Antibiotype
S1	Bellevue outbreak	12.02.92	BC2a	BP23	BE18	9	A
S2	Bellevue outbreak	12.21.92	BC2a	BP23	BE18	9	A
S3	Bellevue outbreak	12.22.92	BC2a	BP23	BE18	9	A
S4	Bellevue outbreak	01.12.92	BC2a	BP23	BE18	9	A
S5	Bellevue outbreak	01.18.93	BC2a	BP23	BE18	9	A
S6	Bellevue outbreak	02.02.93	BC2a	BP23	BE18	9	A
S7	Bellevue outbreak	02.08.93	BC2a	BP23	BE18	9	A
S8	Bellevue outbreak	02.15.93	BC2a	BP23	BE18	9	A
S9	Bellevue outbreak	02.18.93	BC2a	BP23	BE18	9	A
S10	Bellevue outbreak	02.22.93	BC2a	BP23	BE18	9	A
S11	Bellevue outbreak	03.01.93	BC2a	BP23	BE18	9	A
S12	Bellevue outbreak	03.01.93	BC2a	BP23	BE18	9	A
S13	Bellevue outbreak	03.09.93	BC2a	BP23	BE18	9	A
S14	Bellevue outbreak	03.09.93	BC2a	BP23	BE18	9	A
S15	Bellevue outbreak	03.18.93	BC2a	BP23	BE18	9	A
S16	Cochin, Paris	11.15.93	BC2a	BP23	BE18	9	A
S17	Cochin, Paris	06.09.93	BC21	BP34	BE31	6	C
S18	Cochin, Paris	06.13.93	BC2a	BP23	BE18	9	A
S19	Cochin, Paris	06.19.93	BC2a	BP22	BE29	2	B
S20	Cochin, Paris	06.30.93	BC2a	BP22	BE29	2	B
S21	Bellevue, St. Etienne	01.07.92	BC19	BP35	BE32	9	D
S22	Bellevue, St. Etienne	09.08.93	BC2a	BP9	BE33	6	D
S23	Bellevue, St. Etienne	11.16.92	BC20	BP26	BE34	9	C
S24	Bellevue, St. Etienne	12.16.92	BC20	BP36	BE35	9	C
S25	Bellevue, St. Etienne	11.06.92	BC20	BP26	Noncleaved	8	C

levue Hospital. A strain of *Acinetobacter baumannii* was isolated in a bedsore on his head soon after admission to Bellevue. The pattern of the strain from this patient was the same as that of the epidemic strain.

Antibiotyping was a useful tool for recognizing the outbreak in our hospital because our isolates usually showed a low level of resistance to antimicrobial agents. On the basis of other data, phenotypic methods such as biotyping and antibiotyping used alone were of limited value. Although a combination of several typing methods is usually required, genotypic methods such as ribotyping are the most discriminating method for *Acinetobacter baumannii* and allowed the identification of cross-contamination with *Acinetobacter baumannii* in an ICU.

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Evaluation of Pulsed-Field Gel Electrophoresis and rep-PCR for the Epidemiological Analysis of *Ochrobactrum anthropi* Strains

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Pulsed-field gel electrophoresis and polymerase chain reaction genome fingerprinting based on repetitive chromosomal sequences (rep-PCR) were used for typing 14 strains of *Ochrobactrum anthropi*. Six strains isolated during an outbreak of bacteraemia in patients who had received a contaminated rabbit anti-thymocyte globulin gave identical patterns by both techniques. Different patterns were found in sporadic and reference strains, except for one clinical isolate received from another hospital that showed the same pattern as the epidemic clone. This patient had also received rabbit anti-thymocyte globulin from the same source at the time of the outbreak. This study illustrates the advantages of genetic typing methods in terms of high typeability and discriminating power, even for rare pathogens. Furthermore, it highlights the need for interhospital communication for effective identification of common sources of outbreaks related to intrinsic drug contamination.

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Ochrobactrum anthropi, previously *Achromobacter* CDC group Vd, is a gram-negative, nonfermentative bacillus. This ubiquitous environmental bacterium is considered a rare, opportunistic pathogen and has been found to cause catheter-associated sepsis (1).

Our institution previously reported an outbreak of *Ochrobactrum anthropi* in patients who had received rabbit anti-thymocyte globulin (RATG), an anti-rejection drug (2). Extensive epidemiological investigations established intrinsically contaminated RATG as the source of this outbreak. These outbreak strains, together with one probably related strain and several unrelated strains, were used to evaluate molecular typing methods, such as pulsed-field gel electrophoresis (PFGE) and PCR genome fingerprinting based on repetitive chromosomal sequences (rep-PCR), for this species.

Materials and Methods. A total of 14 *Ochrobactrum anthropi* isolates were genotyped by PFGE and rep-PCR. Five *Ochrobactrum anthropi* (strains no. 2–6) were isolated from blood cultures and one (strain no. 7) from an RATG vial during an outbreak that occurred in our institution from 29 April to 21 May 1992 (2). Briefly, five organ transplant recipients admitted to two different units became bacteraemic. Four of them were symptomatic. They all had received RATG. Bacteriological analysis of the RATG confirmed the contamination of some of the vials, both used and unused, by *Ochrobactrum anthropi*. All of the vials had the same batch number.

One *Ochrobactrum anthropi* (strain no. 8) was isolated in the same month at another university hospital in Brussels: Hôpital Erasme. The patient from whom the bacterium was recovered had undergone a heart transplantation on 12 May 1992. He had received prophylactic anti-rejection treatment, including RATG. Four days after treatment he developed fever as high as 39°C, without any apparent source of infection. *Ochrobactrum anthropi* was isolated from several blood cultures as well as from a catheter culture. As soon as the catheter was withdrawn and RATG administration stopped, the patient's temperature returned to normal.

The other *Ochrobactrum anthropi* strains (n = 6) were selected as epidemiologically unrelated strains isolated at different times and/or hospitals.