Comparison of Four Different Methods for Epidemiologic Typing of Acinetobacter baumannii

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A set of 103 epidemiologically well-defined Acinetobacter baumannii isolates obtained from nine hospital outbreaks and 21 unrelated strains were characterized by pulsed-field gel electrophoresis (PFGE) of total genomic DNA digested with *Apal*. Among outbreak strains, eight different patterns and five possible variants were identified by PFGE. Results were compared with those from traditional typing methods such as plasmid profile analysis, antimicrobial susceptibility, and biotyping. Plasmid analysis revealed six different and two related patterns; one outbreak strain lacked plasmids. A total of 16 of the 21 unrelated strains harbored plasmids and exhibited unique patterns. Epidemiologically unrelated strains were placed into only two biotypes and had similar antimicrobial susceptibility patterns but were clearly distinguished by PFGE. PFGE of *A. baumannii* chromosomal DNA yielded reproducible and easily readable results and showed excellent discriminatory power. However, plasmid profile analysis may provide a cost-effective first step in epidemiologic typing of *A. baumannii* isolates obtained from well-defined hospital outbreaks.

Acinetobacter baumannii is a significant pathogen, usually in the context of serious underlying disease (3, 8, 10). Outbreaks of infections have been reported in neonatal intensive care units (ICUs) (19), medical and surgical wards (3, 13, 20), and burn units (24) and have been associated with medical equipment (3, 6, 24) and hands of personnel (17).

Epidemiological issues are often difficult to resolve because of the ubiquitous nature of *Acinetobacter* species in the environment (2) and as a commensal organism on human skin and mucous membranes (18, 25). Traditional techniques used for typing of *A. baumannii* are often based on phenotypic characters, including antibiotic analysis (14), biotyping (4, 5, 14), phage typing (5), serotyping (27), and cell envelope protein typing (5, 7). None of these techniques is capable of typing all strains. More recently, genotypic methods such as analysis of plasmid profiles (13, 17), ribotyping (7, 9), analysis of chromosomal DNA by pulsed-field gel electrophoresis (PFGE) (1, 11), and fingerprinting by arbitrarily primed PCR (12) have been introduced and have improved epidemiologic typing of *A. baumannii*.

In this study, the results of PFGE of chromosomal DNA from a collection of epidemiologically unrelated *A. baumannii* strains as well as from isolates obtained from several well-defined hospital outbreaks were compared with those obtained with established and commonly used methods for strain delineation including comparison of antibiotic profiles, biotyping, and analysis of plasmid patterns.

A total of 103 *A. baumannii* isolates recovered from different patients from nine hospital outbreaks and 21 epidemiologically unrelated strains were selected from more than 400 isolates that were collected prospectively from 275 patients during a 12-month survey (21). Representative isolates from each outbreak were chosen for their similarity as shown by biotyping and susceptibility testing. Isolates were identified according to the simplified identification scheme described by Bouvet and Grimont (4). Susceptibility to selected antimicrobial agents was determined by a microtiter broth dilution method (MicroScan MIC Plus Type MK Dried Panels; Baxter Healthcare Corp., West Sacramento, Calif.), as described previously (22). Biotyping was performed with utilization of six carbon sources (levulinate, citraconate, L-phenyllalanine, phenylacetate, 4-hydroxybenzoate, and L-tartrate) as described by Bouvet and Grimont (4).

Plasmid DNA was prepared as described by Hartstein et al. (13), with minor modifications (23). A plasmid type was defined as any plasmid pattern which varied from another pattern with regard to the number and size of plasmid bands. Two patterns were considered similar if one of the two compared patterns contained one or two additional bands. Identity and similarity were further confirmed by restriction endonuclease enzyme digestion of the preparations with HindIII and EcoRI (data not shown). Isolates were run in duplicate on different gels.

For PFGE, *A. baumannii* isolates were grown overnight on Mueller-Hinton agar plates at 30°C. Cells were harvested, suspended in 5 ml of 75 mM NaCl-25 mM EDTA (SE buffer; pH 7.4), pelleted by centrifugation, and washed twice in the same buffer. Bacterial cells were adjusted to 10⁶ CFU/ml in SE buffer. A total of 0.5 ml of the bacterial suspension was mixed with 0.7 ml of 2% low-gelling agarose (Amresco, Solon, Ohio) and poured into a plug mold. The agarose plugs were then incubated with a mixture of 50 mM Tris-50 mM EDTA (pH 8.0), 1% N-lauryl sarcosine, and 1 mg of proteinase K per ml (Merck, Darmstadt, Germany) for 18 h at 56°C in a water bath with gentle shaking. Genomic DNA was digested with 20 U of *Apal* (New England Biolabs) for 4 h. DNA fragments were separated by PFGE, with use of the Pulsaphor apparatus (Pharmacia-LKB, Bromma, Sweden). Plugs were placed into the slots of a 1.2% agarose gel and run in 0.5× TBE buffer (45 mM Tris [pH 8.0], 45 mM boric acid, 1 mM EDTA) for 24 h at 150 V. Pulse times ranged from 5 to 20 s. A ladder of bacteriophage lambda concatamers (New England Biolabs) was used as molecular weight markers. Gels were stained with ethidium bromide and photographed under UV light.

Epidemiological data on the different hospital outbreaks are shown in Table 1. All outbreaks occurred in ICUs. If multiple ICUs were involved, these were all located on the same floor. The duration of the outbreaks ranged from 2 months to more than 12 months, affecting from 9 patients on a single ward to

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more than 150 patients in multiple ICUs. The majority of isolates were recovered from patients who were also temporally clustered. In some instances, however, identical strains were recovered after an interval of 2 or 3 months, thus extending the duration of the outbreak.

Antibiotic susceptibility testing of *A. baumannii* isolates revealed similar patterns for any given cluster of isolates (Table 1). The following antibiotic resistance phenotypes were detected and arbitrarily designated A through E: A, susceptible to gentamicin, tobramycin, amikacin, ciprofloxacin, and imipenem; B, susceptible to imipenem only; C, susceptible to tobramycin and imipenem; D, susceptible to amikacin and imipenem; and E, susceptible to amoxicillin-clavulanate, piperacillin, and imipenem. Resistance patterns of epidemiologically unrelated strains mostly resembled pattern A.

Four different biotypes were observed among the outbreak isolates investigated. One outbreak each was due to *A. baumannii* biotypes 1 and 2, whereas *A. baumannii* biotypes 6 and 9 were involved in three and four outbreaks, respectively. Epidemiologically unrelated strains were placed into biotypes 6 (8 strains) and 9 (13 strains).

Six different plasmid types were observed among the 103 *A. baumannii* isolates recovered from hospital outbreaks, designated arbitrarily I to VI. Representative plasmid profiles from outbreak strains are shown in Fig. 1. A total of 16 of the 21 epidemiologically unrelated *A. baumannii* strains harbored plasmids, and plasmid patterns of these strains were clearly distinct from those of the outbreak strains and from each other (data not shown). Two plasmid profiles were considered similar (V/Va and VI/Vla; Fig. 1, lanes 1 to 8). Isolates representing biotype 1 were not typeable by plasmid profile analysis. Isolates within each of the other outbreaks exhibited identical plasmid profiles with two exceptions. Plasmid analysis of a blood culture isolate demonstrated profile VI, whereas the corresponding isolate recovered 2 days later from a central venous catheter had no detectable plasmid bands. Isolates representing plasmid profile VI were distributed among four ICUs of hospital C and were also responsible for the outbreak in a community hospital (hospital D), apparently due to transfer of a colonized patient from hospital C to hospital D. However, during the course of this outbreak two additional low-molecular-weight plasmid bands were observed in some of these isolates (profile Vla). All these isolates, however, had an identical banding pattern as shown by PFGE (data not shown).

PFGE of genomic DNA after digestion with Apal from outbreak strains revealed eight different patterns or PFGE types, named a through h, yielding 15 to 20 bands per strain (Fig. 2 and Table 1). There were marked restriction length polymorphisms among all these strains except between strains representing patterns f and g, which differed by only two bands (Fig. 2, lanes 10 to 16). Those two strains had identical susceptibility patterns and similar plasmid profiles (V and Vla) (Fig. 1, lanes 1 to 4) and were probably derived from a common ancestral strain. Among isolates demonstrating patterns d and b, one and two variants, respectively, were observed that showed differences of one band (Fig. 3, lanes 2 to 4 and 5 to 7). Among isolates representing PFGE type h, two variants were detected that differed in one band (data not shown).

![Figure 1](http://jcm.asm.org/)

**Table 1. Epidemiological data and summary of typing results of 103 *A. baumannii* strains from nine hospital outbreaks.**

<table>
<thead>
<tr>
<th>Location of hospital outbreak</th>
<th>Ward type</th>
<th>No. of patients involved</th>
<th>Duration of outbreak (mo)</th>
<th>Body site or tract</th>
<th>Biotype</th>
<th>Antibiogram type</th>
<th>Plasmid type</th>
<th>PFGE type</th>
</tr>
</thead>
<tbody>
<tr>
<td>University hospital (A)</td>
<td>NICU</td>
<td>10</td>
<td>6</td>
<td>Respiratory</td>
<td>1</td>
<td>A</td>
<td>NTa</td>
<td>a</td>
</tr>
<tr>
<td>University hospital (A)</td>
<td>RU</td>
<td>12</td>
<td>2</td>
<td>Urine</td>
<td>6</td>
<td>B</td>
<td>c</td>
<td>b</td>
</tr>
<tr>
<td>Children's hospital (B)</td>
<td>2 ICUs</td>
<td>9</td>
<td>6</td>
<td>Blood cultures</td>
<td>6</td>
<td>A</td>
<td>II</td>
<td>c</td>
</tr>
<tr>
<td>Teaching hospital (C)</td>
<td>BICU</td>
<td>12</td>
<td>4</td>
<td>Various</td>
<td>6</td>
<td>C</td>
<td>III</td>
<td>d</td>
</tr>
<tr>
<td>Teaching hospital (C)</td>
<td>BICU</td>
<td>8</td>
<td>3</td>
<td>Various</td>
<td>2</td>
<td>B</td>
<td>IV</td>
<td>e</td>
</tr>
<tr>
<td>Teaching hospital (C)</td>
<td>4 ICUs</td>
<td>12</td>
<td>5</td>
<td>Various</td>
<td>9</td>
<td>D</td>
<td>V</td>
<td>f</td>
</tr>
<tr>
<td>Teaching hospital (C)</td>
<td>4 ICUs</td>
<td>56</td>
<td>10</td>
<td>Various</td>
<td>4</td>
<td>E</td>
<td>VI, VIa</td>
<td>h</td>
</tr>
<tr>
<td>Teaching hospital (C)</td>
<td>4 ICUs</td>
<td>154</td>
<td>&gt;12</td>
<td>Various</td>
<td>9</td>
<td>E</td>
<td>VI</td>
<td>h</td>
</tr>
<tr>
<td>Community hospital (D)</td>
<td>MICU</td>
<td>7</td>
<td>8</td>
<td>Various</td>
<td>9</td>
<td>E</td>
<td>VI</td>
<td>h</td>
</tr>
</tbody>
</table>

a NICU, neurological ICU; RU, rehabilitation unit; 2 ICUs, neonatal ICU and preterm neonate ICU; BICU, burn unit; 4 ICUs, surgical, neurological, and neurosurgical ICUs and burn unit; MICU, medical ICU.

b Total number of patients involved in any given outbreak.

c Biotype as determined by Bouvet and Grimont (4).

d NT, not typeable.
Isolates from the community hospital (D) and those responsible for the prolonged outbreak in hospital C gave virtually indistinguishable patterns (PFGE type h). Nonrelated strains gave unique PFGE patterns (Fig. 3, lanes 12 to 20) that were easily distinguishable from one another and from those of outbreak isolates. In contrast, isolates obtained from a given outbreak demonstrated identical or very similar banding patterns. Isolates demonstrating identical PFGE patterns were all placed in the same biotype. Conversely, there was considerable restriction length polymorphism among A. bau mannii strains that belong to the same biotype.

For comparison and reproducibility testing, one strain was retested on each gel. In addition, five A. bau mannii strains representing different biotypes and different PFGE types were maintained at room temperature for up to 8 months and subcultured every 2 weeks. Longitudinal reproducibility of plasmid profiles and PFGE patterns was studied by running these isolates side by side on the same gel with corresponding isolates that were kept frozen. Plasmid profiles and PFGE patterns were identical; no loss of bands was observed.

The purpose of this study was to evaluate PFGE analysis of A. bau mannii genomic DNA from a collection of epidemiologically unrelated strains and from isolates obtained from multiple hospital outbreaks. The results obtained were compared with those of biotyping, antimicrobial resistance patterns, and plasmid profile analysis to further assess the usefulness of these traditional methods in relation to modern molecular techniques for epidemiological typing of A. bau mannii.

The biotyping system proposed by Bouvet and Grimont (4) allows the differentiation of 19 different biotypes. However, the discriminatory power of biotyping is poor, especially because only a few biotypes have been involved in hospital outbreaks—namely, biotypes 1, 2, 6, and 9 (7, 14). These biotypes were also observed among the isolates investigated in the present study. With the exception of the large outbreak in hospital C with multiple biotypes involved, isolates obtained from each of the hospital outbreaks were placed into a single biotype which proved to be a stable marker during the course of the outbreak.

Antibiotic susceptibility patterns were less helpful in the epidemiological study of A. bau mannii in our study. Minor variations were frequently observed among outbreak isolates and were difficult to interpret without the help of a complementary typing system. In addition, strains exhibiting different PFGE patterns were placed into the same susceptibility type (Table 1). Thus, both biotyping and antimicrobial susceptibility patterns may be suitable as screening methods in epidemiological investigations but require confirmation of results by complementary techniques.

Plasmid profile analysis has proved useful for the study of outbreaks of A. bau mannii infections (3, 13, 17). The technique is simple, requires a minimum of equipment and expense, and is accessible for most diagnostic laboratories. Plasmid profile analysis in our hands demonstrated acceptable typeability. With one exception, all outbreak strains could be differentiated by this method; among unrelated strains, 16 of 21 were typeable. Plasmid profiles were highly reproducible for all isolates that had been stored at room temperature for prolonged periods of time and subcultured at regular intervals. Plasmid profiles were also stable among most strains within each outbreak. However, during the extended outbreak due to A. bau mannii biotype 9 observed in hospital C, the outbreak strain apparently acquired two additional plasmids of low molecular weight. Isolates representing both plasmid types—designated VI and Vla—were recovered concomitantly from patients in the same ward and in two cases even from the same patient. The genotypic results obtained by PFGE for all these isolates were identical.

Analysis of genomic DNA by PFGE has proved to be highly discriminatory and comparable or often superior to other available techniques (16, 26). This method was used by Allar-
det-Servent et al. (1) to investigate an outbreak of A. baumannii in a urological ward. In a subsequent study, Gouby et al. (11) demonstrated considerable DNA polymorphism among A. baumannii strains isolated in different parts of the world, even among strains that belong to the same biotype. In our study, genetic fingerprinting of 21 unrelated A. baumannii strains demonstrated a considerable number of restriction fragment length polymorphisms. Each strain exhibited a unique banding pattern. The presence of such diversity is the basis for the assumption that isolates with identical or almost identical patterns represent a single clone. Restriction fragment patterns of chromosomal DNA were virtually identical for most temporally or epidemiologically related isolates. Comparison of profiles f and g, however, revealed minor differences suggesting that both strains may have derived from a common ancestral strain. In addition, five possible variants were observed among the isolates investigated. However, it has been suggested that detection of a single band difference is not a reliable basis for concluding that two isolates that are epidemiologically related represent different strains (16). The stability of the typing system was further demonstrated by the identification of identical patterns in serially passaged strains. Numerous studies have evaluated multiple typing methods for the epidemiological investigation of A. baumannii isolates (5, 7, 14, 15). Unfortunately, controls showing dissimilarity of unrelated isolates and similarity of serially passaged isolates have not always been included. Overall, there was a remarkable degree of uniformity in typing results obtained by plasmid profile analysis and PFGE. Both methods allowed differentiation among sets of outbreak-associated isolates and separation of unrelated strains. Plasmid profiling is well suited for the analysis of outbreaks of A. baumannii infections that are restricted in terms of time and place (those involving acute outbreaks within a single hospital). PFGE may be reserved for situations in which clinical and plasmid data conflict or are inconclusive. The usefulness of PFGE as an epidemiological tool in comparison with other modern molecular typing methods such as ribotyping (9) and fingerprinting by arbitrarily primed PCR (12) remains to be determined.

REFERENCES


