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## Use of Interrepeat PCR Fingerprinting to Investigate an Acinetobacter baumannii Outbreak in an Intensive Care Unit

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An epidemiological investigation of an outbreak of Acinetobacter baumannii among patients on 2 closely related intensive care units (ICU) was performed by molecular typing with interrepeat polymerase chain reaction (interrepeat PCR). 31 A. baumannii isolates obtained from 15 ICU patients were characterized. All patients were infected or colonized with A. baumannii. After identification of the outbreak, 6 environmental isolates were collected from tap-water, sinks and cleaning detergents. PCR fingerprinting identified 3 genotypes among the outbreak-related strains. One predominant genotype was demonstrated in 14/15 patients and this genotype was also found among all environmental isolates. The cluster of A. baumannii represented an outbreak of 1 genotype, suggesting cross-contamination. The finding of the identical genotype among all environmental strains indicated a common environmental source causing the outbreak. The outbreak was controlled after reimplementation of an effective disinfection of workplace surfaces. This survey proved interrepeat PCR to be a rapid and reliable method to differentiate A. baumannii strains, thereby allowing epidemiological surveillance of large amounts of strains and early interventions to control outbreaks.

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#### INTRODUCTION

Acinetobacter spp. are widely found in nature and are also carried by humans. Acinetobacter spp. are generally not classified as particularly virulent, although they are increasingly involved in severe nosocomial infections, especially among seriously ill patients (1, 2). In recent years various outbreaks with these Gram-negative coccobacilli have been reported, most of them among intensive care and surgical patients (3-6). Transmission of Acinetobacter spp. may be due to cross-contamination via the hands of health care workers (HCW) (7, 8), but may also be attributed to contamination of environmental sources such as ventilators and respiratory care equipment (1-11) or (environmental) surfaces (5, 12), mattresses (13) and pressure transducers (14). Since Acinetobacter spp. are widely found in environmental and human sources, typing is essential to prove the identity of strains. Typing methods used to investigate the epidemic spread of Acinetobacter spp. in hospitals, included a wide variety of phenotypic and genotypic methods (15–22). With the exception of PCR fingerprinting most of these methods are either time and/or money consuming, or not discriminatory enough. We evaluated the use of interrepeat PCR fingerprinting to investigate an Acinetobacter baumannii outbreak in 2 intensive care units (ICU). The outbreak arose after changes in the cleaning/disinfection protocols of the unit and was controlled after indication of the source and reimplementation of an effective disinfection of workplace surfaces.

#### MATERIALS AND METHODS

#### Patients and hospital

The University Hospital, Nijmegen, is a 980-bed tertiary care facility. There are 5 intensive care units. Routine surveillance on the ICU includes culture and susceptibility testing on specimens of bronchial aspirate and urine twice a week. In case of infection additional cultures are taken. The epidemic A. baumannii strain was isolated from clinical specimens of 15 ventilated patients from 2 connected intensive care units (ICU I and II) and from 4 epidemiologically unrelated patients from the same ICUs 2 years before the outbreak period. A. baumannii was isolated from bronchial aspirates, urine, wounds, blood and intravascular devices. After detection of the outbreak a total number of 15 environmental samples were obtained. From 6/15 environmental samples, collected from tap-water, sinks, workplace surfaces and cleaning detergent, A. baumannii was isolated. The environmental samples were obtained in the month following the epidemic period.

#### Microbiological methods

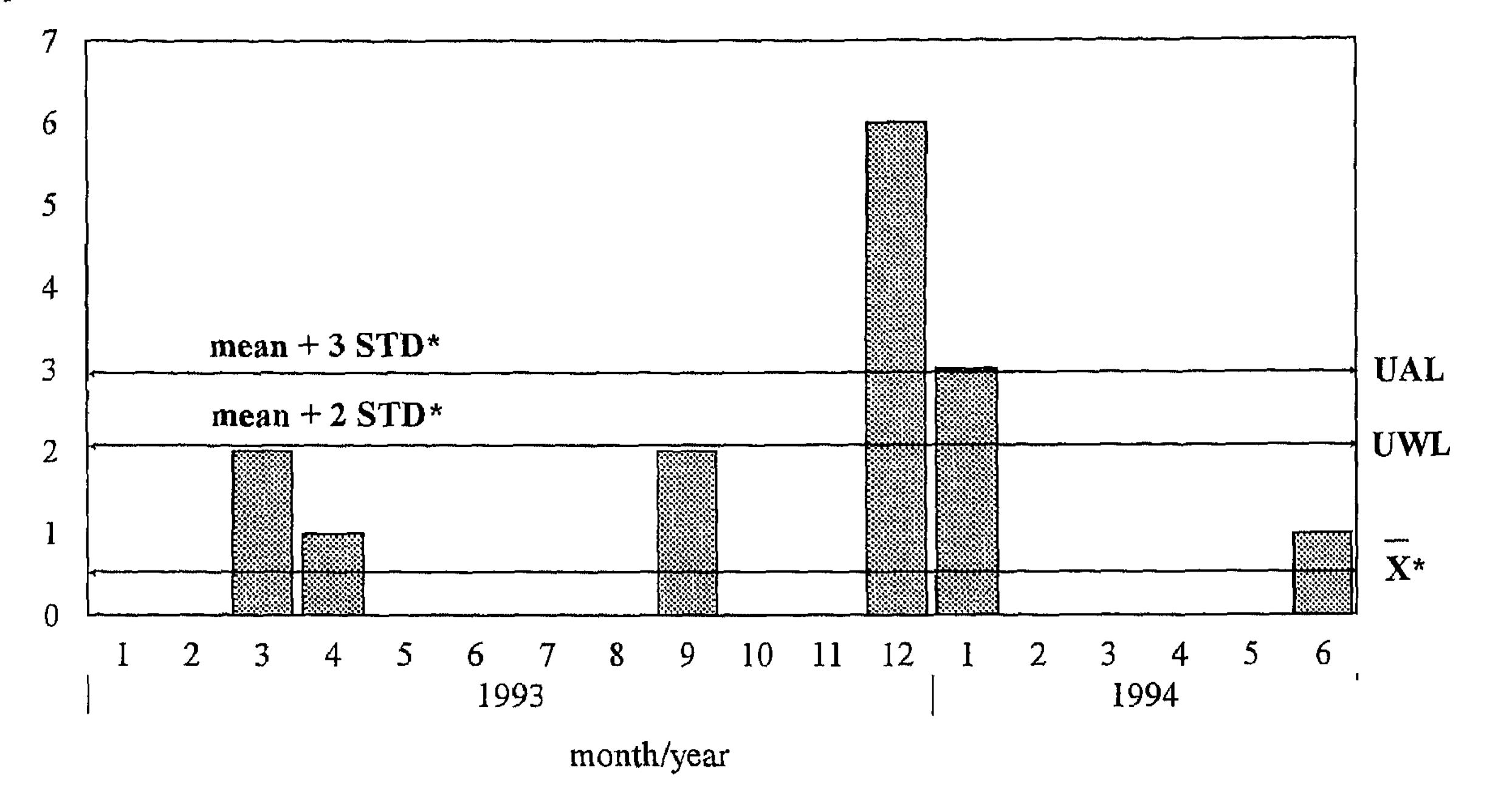
Isolates were identified by morphology, glucose fermentation and negative cytochrome oxidase. Identification to species level was performed using the API-50 system (API-Biomérieux, France) and by growth at 44°C (23).

#### Preparation of genomic DNA

After overnight growth on tryptic soy broth (TSB), 1 ml of the overnight culture was centrifuged and the cells were resuspended in 250  $\mu$ l STET buffer (233 mM sucrose, 50 mM Tris-HCl pH 8.0, 20 mM EDTA, 5% Triton X-100) and 50  $\mu$ l lysozyme (10 mg/ml) (Sigma, St Louis, USA). After 5 min incubation at room temperature, the solution was heated to 100°C for 1 min and put on ice for 2 min. Then, 10  $\mu$ l SDS (10%) and 15  $\mu$ l proteinase K (10 mg/ml) (Boehringer Mannheim, Germany) were added and the solution was incubated for 2 h at 55°C. Following phenol extraction, 1  $\mu$ l

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#### patients



#### \* mean and standard deviation (STD) are based on the pre-outbreak period 1/93-11/93

Fig. 1. Incidence of ICU patients colonized or infected with A. baumannii. The bars represent the number of colonized or infected patients. The mean number of patients (x = 0.5), the standard deviation (SD = 0.8), the upper warning limit (UWL = 2.0), and the upper action limit (UAL = 2.8), are indicated by horizontal lines.

RNAse A (10 mg/ml) (Sigma) was added, and the mixture was incubated at 37°C for 20 min. Phenol-chloroform-isoamylalcohol (25:24:1) and chloroform-isoamylalcohol (24:1) were used for further extraction of the DNA. Subsequently, the DNA was precipitated using ethanol and resuspended in 100  $\mu$ l of distilled water (24). An aliquot was electrophoresed in a 1% agarose gel containing 0.1  $\mu$ g/ml ethidium bromide to estimate the DNA yield and verify its integrity.

was initiated, including observation of HCW and environmental sampling. Five A. baumannii isolates were collected from tap-water, sinks and workplace surfaces. Another isolate was detected in a diluted cleaning detergent. A new cleaning team had (unnoticed) switched from a surface disinfectant to a non-medicated cleaning detergent to clean the workplace surfaces. Consequently the outbreak was controlled by reimplementation of an effective surface disinfectant, supported by constant feedback of results of the epidemiological investigation and stressing the importance of hand-disinfection to prevent crossinfection. Results of PCR-fingerprinting of 31 strains isolated from 15 patients from the connected ICUs I and II, 5 epidemiologically unrelated strains are shown in Table I and Fig. 2. Three different genotypes were identified among the outbreak related strains. Genotype A was predominantly present (27/31 strains isolated from 14/15 patients). Genotype B was identified in 3 strains isolated from 1 patient. In 2 strains isolated from patient 15, 2 different genotypes were identified: genotypes A and C.

#### Amplification and PCR fingerprinting

Amplification of bacterial DNA was performed according to a protocol described earlier (25). Negative controls, reaction mixture without template DNA, were included in all reactions. A Perkin-Elmer thermal cycler was used for amplification. The cycling program for the use of primers ERICI and ERIC2, consisted of 5 min incubation at 94°C, 35 cycles at 94°C for 1 min, 25°C for 1 min, 74°C for 2 min, followed by a final extension step at 74°C for 10 min. Amplified DNA was separated by gel electrophoresis in an 1.5% agarose gel and visualized by ethidium bromide staining. One hundred base pair ladders were used as molecular size makers. Gels were photographed and visually inspected by 3 independent observers.

#### RESULTS

The incidence of ICU patients colonized or infected with PCR-fingerprinting of 4 epidemiologically unrelated A. baumannii between January 1993 and June 1994 is strains, isolated from 4 different patients at the same ICUs shown in a modified statistical process control (SPC) chart 2 years previously, distinguished 3 other genotypes and 1 (Fig. 1). The December value of newly colonized and genotype identical to the outbreak-related strains (genotype infected patients exceeded the upper action limit, and it A). Among the environmental isolates, collected from tapwas therefore statistically unlikely that the distribution water, sinks, workplace surfaces and the cleaning detergent, occurred by chance alone (26). An outbreak investigation only genotype A was found (data not shown).

Table I. Characterization of A. baumannii strains isolated during the outbreak. Epidemiologically unrelated patients are marked by an asterix

Patient	Date 1993–1994		Source ICU	PCR lane <sup>a</sup>	Genotype <sup>b</sup>
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environment and human skin being the most likely source. Therefore, outbreaks are frequently found in intensive care units (ICU), where ventilator equipment, humidifiers and nebulizers are in use (9-11).

We described an outbreak of A. baumannii among patients on 2 closely related ICUs. During this outbreak, 1 predominant genotype was identified by PCR-fingerprinting (genotype A). This genotype was found in 14/15 patients colonized or infected with A. baumannii, suggesting crosscontamination. Having found the identical genotype among the environmental isolates, tap-water sinks and including the dilution of a non-disinfecting cleaning detergent, a common environmental source causing the outbreak seemed likely, especially since the outbreak occurred after switching from a disinfectant to a non-disinfecting cleaning detergent. Therefore changes in the old cleaning-disinfection protocols of the unit and effective disinfection of workplace surfaces were reinforced. Soon thereafter the outbreak was controlled, as demonstrated by the decline of the number of patients colonized or infected with A. baumannii (Fig. 1). Similarly, Tankovic et al. (27) performed extensive environmental and personnel sampling to control an outbreak of A. baumannii. Outbreak strains were found on surfaces. furniture, sinks, patient care items and in soil. Despite the use of multiple infection control measures, such as cohort isolation and room decontamination, the outbreak was controlled only after closing the unit. In contrast we were able to control the outbreak by complete cleaning and disinfection of the unclosed unit. Others also showed the importance of hand-washing and glove use to control outbreaks with Acinetobacter spp. (28, 29). Due to the wide environmental spread of Acinetobacter spp., it is important to prove the identity of strains involved in a possible outbreak. Since typing results are furthermore extremely valuable in search for the outbreak source, a reliable and fast available typing method is essential. Typing methods used so far to investigate the epidemic spread of Acinetobacter spp., included phenotypic and genotypic methods. Phenotypic typing systems such as serotyping (15, 30), bacteriocin typing (16), biotyping (17, 18), whole cell and cell envelope protein electrophoresis using SDS-PAGE (19, 31), and susceptibility patterns (32) have been used to investigate Acinetobacter spp. outbreaks. In general these methods appear less reliable and stable than genotypic methods and have shown to be less discriminatory (19).

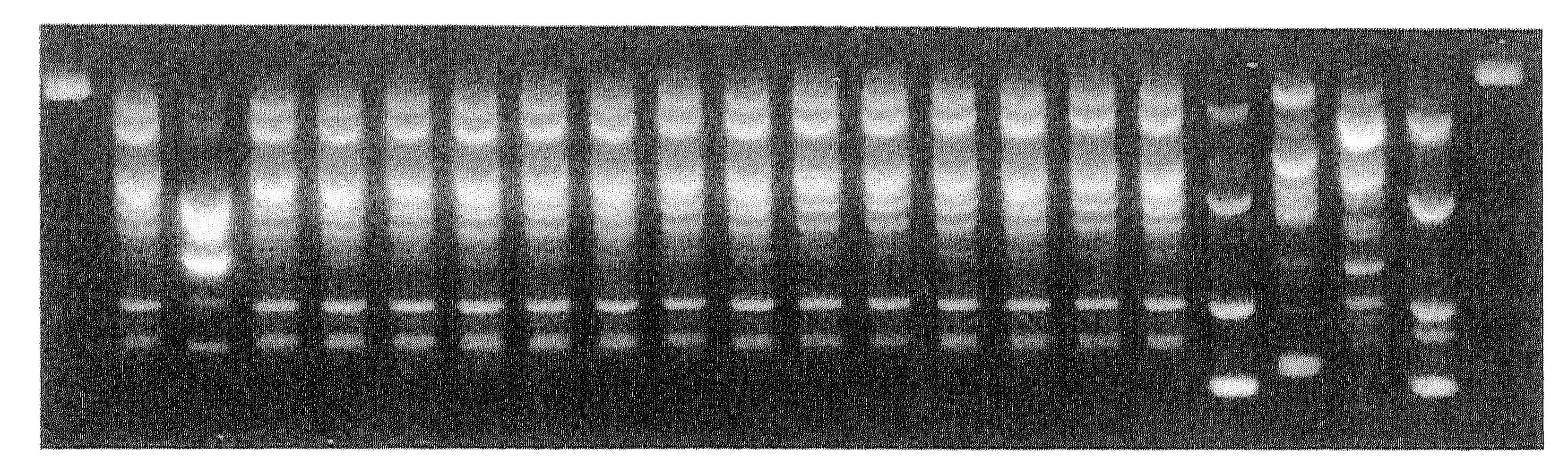
<sup>a</sup> Numbers refer to lanes of PCR-fingerprinting as shown in Fig. 2.

<sup>b</sup> Letters refer to different PCR banding patterns. A. baumannii strains with identical genotypes are designated by the same letter.
BA = bronchial aspirate, IVD = intravascular device, U = urine, B = blood, W = wound.

### DISCUSSION

Acinetobacter strains are increasingly involved in outbreaks of nosocomial infections (3), with moist, inanimate hospital

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*Fig. 2.* PCR-fingerprinting of A. baumannii strains isolated during the outbreak. The numbers of PCR lanes refer to the A. baumannii strains listed in Table I. A molecular size marker (M) is shown on the left.

Among the genotypic typing systems, ribotyping (18, 19), DNA macrorestriction analysis (33), pulsed-field gel electrophoresis (22, 27) and PCR fingerprinting (21, 33, 34) have been used to distinguish outbreak strains. Reboli et al. (34) found repetitive element PCR-mediated DNA fingerprinting clearly superior to biotyping, antibiogram and plasmid analysis, methods which did not allow to discriminate between epidemic and sporadic strains.

PCR fingerprinting was shown to be useful in the epidemiological investigation of outbreaks with bacterial species, others than Acinetobacter spp. (35-39). The method is fairly easy to perform and the results are rapidly available. Furthermore, this technique offers the ability to be adopted to other microorganisms which we feel is very important in

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daily clinical microbiological infection control practice.

In accordance with previous results, our study indicates that interrepeat PCR is a rapid and reliable method to differentiate A. baumannii strains allowing epidemiological surveillance of large amounts of strains and for early interventions to control outbreaks.

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