JOURNAL OF CLINICAL MICROBIOLOGY, Nov. 1999, p. 3654–3661 Vol. 37, No. 11
0095-1137/99/$04.00+0

Application of Different Genotyping Methods for *Pseudomonas aeruginosa* in a Setting of Endemicity in an Intensive Care Unit

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Received 25 March 1999/Returned for modification 26 May 1999/Accepted 31 July 1999

Colonization with *Pseudomonas aeruginosa* was studied by taking serial swab specimens from the oropharynges and anuses and tracheal and gastric aspirates from patients in an intensive care unit during a 10-month period in a setting of endemicity. Nineteen (10%) of the 192 patients included in the study were colonized on admission, while another 30 (16%) patients acquired *P. aeruginosa* while in the hospital. Typing of 353 isolates was performed by random amplified polymorphic DNA (RAPD) analysis, and 56 strains were selected for further typing by RAPD analysis, pulsed-field gel electrophoresis (PFGE), and amplified fragment length polymorphism (AFLP) analysis. By these methods, 42, 44, and 44 genotypes were found, respectively. Computer-aided cluster analysis indicated that similar groups of related isolates were obtained by each method. By taking admission periods into account, analysis of the typing results suggested cross-acquisition of *P. aeruginosa* for five patient pairs. The small number of transfers and the large number of genotypes found indicate that most *P. aeruginosa* strains were derived from the patients themselves. The numbers of observed typing patterns and band differences between related isolates were counted for each typing method. AFLP analysis with primers without a selective base proved to be the most discriminatory method, followed by PFGE, AFLP analysis (with one selective base), and RAPD analysis. On the basis of a comparison with established strain differentiation criteria for PFGE, the criteria for differentiation of *P. aeruginosa* by AFLP analysis are presented.

Genomic fingerprinting methods are now regarded as the most accurate methods for the typing of microorganisms for epidemiological purposes (10). These methods include pulsed-field gel electrophoresis (PFGE) (4), ribotyping (2), and PCR-based fingerprinting methods (3). Because *Pseudomonas aeruginosa* is one of the most common nosocomial pathogens and is often a major problem in intensive care units (ICUs), many studies have been directed at this microorganism. However, most studies concern outbreaks or studies between different hospitals for the occurrence of common types (5, 6, 9, 12). To date, no data on the value of these methods in a setting of endemicity have been available. That is a setting in which *P. aeruginosa* is frequently isolated from patients but in which the strains are not necessarily epidemiologically or genetically related. In the present study, *P. aeruginosa* strains collected from ICU patients were typed by different genotyping methods. These methods were PFGE, fluorescence analysis of random amplified polymorphic DNA (RAPD), and a relatively novel high-resolution genomic fingerprinting method, amplified fragment length polymorphism (AFLP) analysis (14). The results were compared in terms of discriminatory power and reproducibility.

MATERIALS AND METHODS

Strains. *P. aeruginosa* isolates were collected from patients admitted to the mixed medical-surgical ICU of the University Hospital Maastricht. The hospital is a 750-bed tertiary-care referral hospital with an ICU of 12 beds. As part of a study on transmission routes of respiratory tract colonization in a setting of endemicity (1), serial samples for surveillance cultures were taken on admission and twice weekly from patients consecutively admitted to the ICU during a 10-month study. These serial samples for surveillance cultures were tracheal and gastric aspirates and throat and anal swab specimens. Additional specimens were collected and analyzed as required for clinical reasons.

Typing. Typing was first performed by RAPD analysis of at least the first two *P. aeruginosa* isolates from each colonized body site. In case of a prolonged ICU stay, more isolates obtained until the time of discharge from the ICU were analyzed. The number of isolates typed per patient ranged from 2 to 30. Strain differentiation occurred according to published criteria, in which single band differences were ignored (8).

One isolate of each genotype from each patient (some patients were colonized with more than one genotype) was again typed by RAPD analysis in a single PCR run and was analyzed by automated laser fluorescence analysis in order to facilitate comparison of strains between patients. These isolates were also typed by PFGE. Strain differentiation by PFGE was based on the criteria of Tenover et al. (11). Strains were considered different types if there were more than six band differences and different subtypes if one to six band differences were observed. Finally, the same isolates were typed by AFLP analysis.

RAPD analysis. *P. aeruginosa* strains were grown on sheep blood agar. Crude bacterial lysates were prepared by suspending a 1-μl loopful of bacteria in 20 μl of 50 mM NaOH–0.25% sodium dodecyl sulfate (SDS) and heating for 15 min at 95°C. Lysates were diluted with 980 μl of water, and 2.5 μl was used for amplification in a 12-μl PCR mixture. PCR tubes further contained 0.5 U of Goldstar DNA polymerase (Eurogentec), a 400 μM concentration of each deoxynucleoside triphosphate, 12 pmol of Cy5-labeled ERIC2 primer (Cy5-AAGTAAGTG ACTGGGGTGAGCG-3′; Pharmacia), reaction buffer, and 2.5 mM $MgCl_2$. Amplification was performed as described previously (8). The PCR products were mixed with 12 μl of formamide containing 1% blue dextran, heated for 2 min at 95°C, and cooled on ice. Samples of 2 μl were electrophoresed on a 5% polyacrylamide gel containing 7 M urea in 0.5× TBE buffer (Tris-borate-EDTA) at 55°C and 8 W of constant power for 5 h on a Pharmacia ALFexpress electrophoresis system by using the thermoplate with an 8-cm separation distance. Molecular size markers (see "AFLP analysis") were used in every fifth lane.

PFGE. *P. aeruginosa* strains were grown overnight in brain heart infusion medium. The bacteria (1.5 ml) were pelleted with an Eppendorf centrifuge for 2 min, washed with 600 μl of EET buffer (100 mM EDTA, 10 mM EGTA, 10 mM Tris-HCl [pH 8.0]), and resuspended in 1 ml of the same buffer. Bacterial suspensions (150 μl) were mixed with 150 μl of low-melting-point agarose (1.6% in EET buffer, 45°C) and poured into a mold to form agarose plugs (20 min, 4°C). Plugs with immobilized bacteria were incubated overnight at 37°C with EET buffer containing 1 mg of proteinase K per ml and 1% SDS (fresh solution). Plugs were washed under gentle mixing at room temperature six times for 30 min

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each time with 600 μl of $T_{10}E_1$ buffer (10 mM Tris-HCl, 1 mM EDTA [pH 8.0]) and, finally, were washed for 30 min in 1 ml of $T_{10}E_{0.1}$ buffer (10 mM Tris-HCl, 0.1 mM EDTA [pH 8.0]). One-sixth of the plug was cut and washed two times for 30 min each time with 120 μl of restriction buffer H (Boehringer Mannheim) and was subsequently incubated with 10 U of *Spe*I (Boehringer Mannheim) in 120 μl of buffer H for 2 h at 37°C. The plugs were placed in a 1% agarose (Seakem GTG; FMC Bioproducts) gel in 0.5× TBE (45 mM Tris, 46 mM boric acid, 1 mM EDTA). PFGE was performed at 14°C with a CHEF-DR II system (Bio-Rad) at 200 V with switch times that ranged from 5 to 15 s for 10 h followed by switch times that ranged from 15 to 45 s for 10 h. Gels were stained with ethidium bromide, and DNA banding patterns were photographed after transillumination with UV light. To facilitate intergel comparison, one *P. aeruginosa* strain was used as a marker in all PFGE gels.

AFLP analysis. AFLP analysis was based on the method described by Vos et al. (14) and Koeleman et al. (7), with slight modifications. The following modifications to the method described by Koeleman et al. (7) were made. *P. aeruginosa* strains were grown overnight in brain heart infusion medium. The bacteria (1.5 ml) were pelleted with an Eppendorf centrifuge for 2 min and were resuspended in 100 μl of $T_{10}E_1$ buffer. A total of 100 ng instead of 50 ng of DNA was used as input. Adapters were as described by Vos et al. (14). Furthermore, 5 pmol instead of 50 pmol of *Eco*RI adapter was used in the ligation reaction. For amplification, 5 μl of the diluted ligation mixture was added to a final volume of 10 μl of the reaction mixture, which contained 200 μM deoxynucleoside triphosphates, 1 U of ampli-therm DNA polymerase (ITK Diagnostics), reaction buffer containing 1.5 mM $MgCl_2$ (ITK), 4 pmol of Cy5-labeled *Eco*RI+0 primer (Cy5-GACTGCGTACCAATTC-3′; Pharmacia), and 12 pmol of the *Mse*I-Ad1 primer (5′-GACGATGAGTCCTGAG) or the *Mse*I+C primer (5′-GATGAGTCCTG AGTAAC-3′; one selective C base). Amplification was performed as described previously (7).

The PCR products were mixed with 10 μl of formamide containing 1% blue dextran, heated for 2 min at 95°C, and cooled on ice. Samples of 5 μl were electrophoresed on a 6% polyacrylamide gel containing 7 M urea in 0.5× TBE buffer at 55°C and 20 W of constant power for 8 h on a Pharmacia ALFexpress electrophoresis system by using the thermoplate with a 20-cm separation distance.

Phage λ DNA processed by the same AFLP protocol with *Eco*RI+0 and *Mse*I-Ad1 primers resulted in fragments in the size range of 58 to 609 bp. The phage λ DNA processed in this way was used as a marker in every fifth lane.

Computer analysis. Scanned photographs from PFGE gels and banding patterns from RAPD and AFLP analyses obtained after conversion of the peak patterns generated by ALFexpress gel electrophoresis (ALFexpress software, Windows 95 version) were stored in tagged image file format and were processed with GelCompar 3.1 software (Applied Maths, Kortrijk, Belgium) as described previously (7). Complete PFGE patterns were used for analysis. For AFLP analysis, only fragments in the range of 40 to 600 bp were considered. The RAPD patterns were analyzed up to a fragment that was common to all *P. aeruginosa* strains analyzed and that was about 1,000 bp. Similarity between fingerprints was calculated with the Pearson product moment correlation coefficient (*r*). Cluster analysis was performed by the unweighted pair group method with average linkages (UPGMA).

RESULTS

During the 10-month study, 297 patients were admitted to the ICU. Samples for culture were available from 192 (65%) of these patients. A total of 1,089 surveillance samples for culture and 2,393 clinical samples for culture were analyzed. Forty-nine (26%) of the 192 patients included in the study were colonized with *P. aeruginosa*. Of these, 19 (39%) were colonized on admission to the ICU and 30 (61%) acquired *P. aeruginosa* while in the ICU. Genotyping by RAPD analysis was performed for 353 isolates from 44 patients (isolates from 5 patients were lost). Typing resulted in roughly 10 major bands per pattern. Several patients were colonized with more than one genotype. Selection of one isolate of each genotype from each patient resulted in 56 isolates, which were retyped by RAPD analysis, PFGE, and AFLP analysis.

Figures 1, 2, and 3 show the different banding patterns obtained by PFGE, AFLP analysis with the *Eco*RI+0–*Mse*I+C (AFLP *Mse*I+C) primer combination, and RAPD analysis, respectively. The number of bands per pattern was about 20 for PFGE and 50 for AFLP analysis. The three typing methods were analyzed in more detail by comparing the clusters generated by GelCompar 3.1 software (Fig. 1 to 3). Before clusters of strains with high degrees of homology can be identified and labeled, a cutoff value for each method was defined. PFGE is regarded as the "gold standard" for typing, and the results obtained by this method were taken as a starting point to define cutoff values for the three typing methods. By applying the criteria of Tenover et al. (11), based on the numbers of band differences (see Materials and Methods), six clusters of related isolates were identified (Fig. 1, clusters I to VI). This corresponded to a cutoff value of 80% identity for PFGE. However, a seventh cluster (cluster VII) also identified strains with >80% homology and contained two isolates that were not epidemiologically related and that differed by eight bands. These two isolates (isolates 9 and 58) were part of a cluster of three isolates (isolates 9, 56, and 58), of which two (isolates 56 and 58) and all three were clustered by AFLP analysis and RAPD analysis, respectively. To obtain comparable clusters by AFLP and RAPD analyses, the cutoffs were set at 80 and 90% identity, respectively. Compared with PFGE, AFLP analysis generated two additional clusters (Fig. 2) (clusters VIII and IX), whereas RAPD analysis clustered the strains in clusters V and VI into two groups but with a distribution different from those obtained by AFLP analysis and PFGE (Fig. 3). In addition, cluster IV obtained by PFGE and AFLP analysis was not identified by RAPD analysis (82% similarity), and an extra cluster (cluster X) was found by RAPD analysis.

To compare the discriminatory powers of the typing methods and to establish strain differentiation criteria for AFLP analysis on the basis of band differences, strains that differed by less than seven bands by PFGE were investigated in more detail. Table 1 shows the results for 18 isolates, which gave 24 pairs of isolates with similar patterns. These isolates were considered to be similar or to be of closely related genotypes with less than or equal to six band differences by PFGE. These isolates were additionally typed by AFLP analysis with the *Eco*RI+0–*Mse*I-Ad1 primer (AFLP *Mse*I-Ad1), which resulted in approximately 150 bands per pattern. The sum of the number of band differences found between these pairs of isolates is useful for overall comparison of the discriminatory powers of the different methods. It increased in the order RAPD analysis < AFLP analysis with *Mse*I+C < PFGE < AFLP analysis with *Mse*I-Ad1. The number of strain (sub)types also increased in this order. The same results were obtained when strains with ≤10 PFGE band differences were analyzed. To obtain interpretive criteria for AFLP analysis, the criteria for PFGE were multiplied by a factor obtained by dividing the sum of the number of band differences by AFLP analysis by that of PFGE. The number of band differences, which should than be applied to the identification of different genotypes, is >4 bands and >10 to 12 bands by AFLP analysis with *Mse*I+C and AFLP analysis with *Mse*I-Ad1, respectively.

By use of these criteria, analysis by eye did not identify clusters VII, VIII, IX, and X as such and would not have clustered isolate 15 in cluster VI, as too many band differences were obtained by all three methods. In addition, the ICU stays of most of the patients whose isolates were in these clusters did not overlap.

Table 2 shows the number of patterns and genotypes obtained by each method if the numbers of band differences were more than one, more than six, and more than four for RAPD analysis, PFGE, and AFLP analysis with *Mse*I+C, respectively. The large number of genotypes suggests that most *P. aeruginosa* strains were derived from the patients themselves.

During the 10-month follow-up, five pairs of patients whose ICU stays overlapped were identified, and the patient pairs were found to harbor similar *P. aeruginosa* strains, as determined by all three typing methods (Table 1; Fig. 4). One of these pairs of patients even shared two different *P. aeruginosa* genotypes. These results are very suggestive of cross-acquisi-

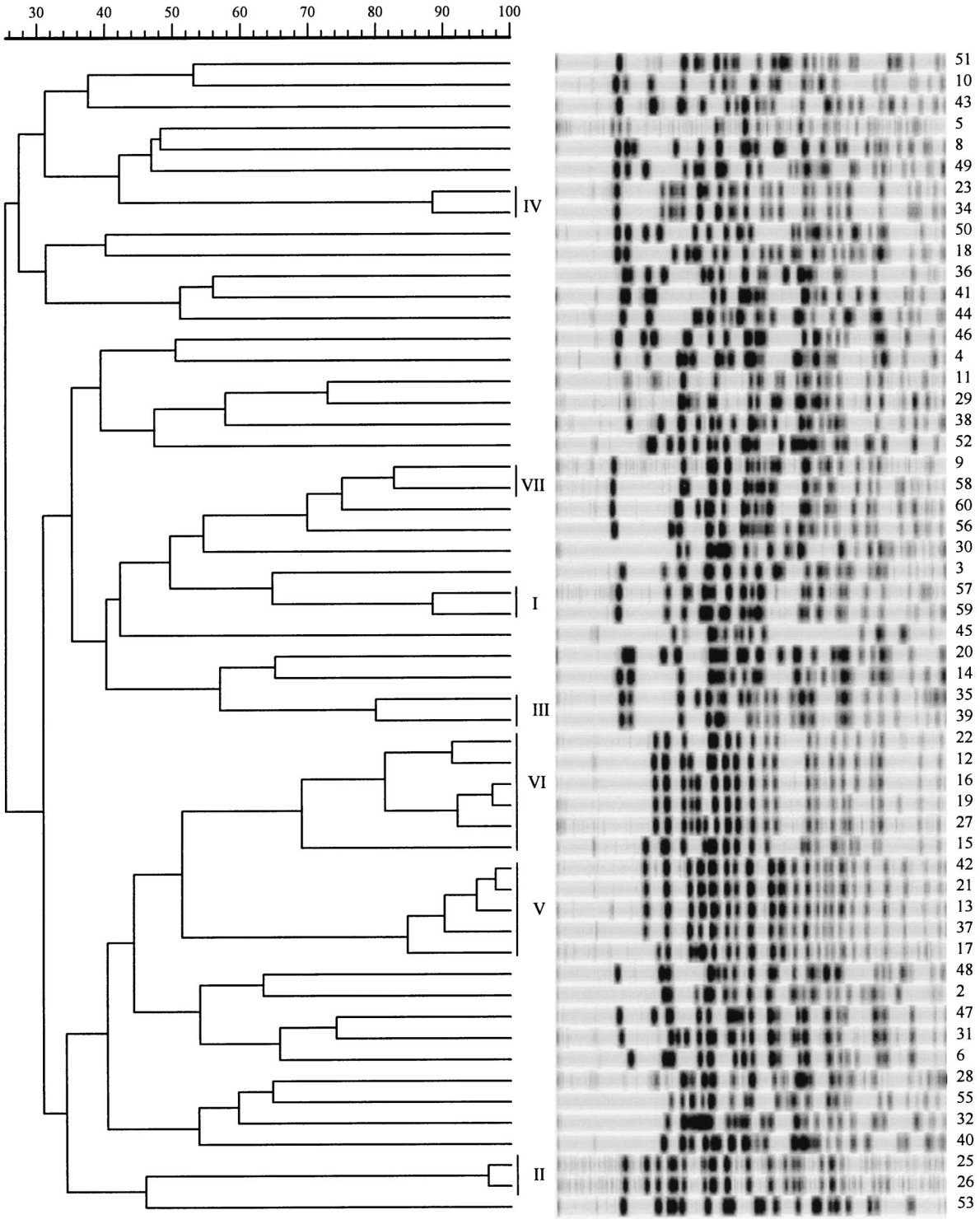

FIG. 1. Digitized PFGE patterns and dendrogram for 56 *P. aeruginosa* isolates cut with *Spe*I obtained after selection of 1 isolate of each genotype from each of 44 patients colonized with *P. aeruginosa* during a 10-month period of study in an ICU. The dendrogram was constructed by cluster analysis by UPGMA with GelCompar 3.1 software (Applied Maths). Percentages of similarity are shown above the dendrogram. Roman numerals indicate clusters with ≥80% homology, which corresponds to the criteria of Tenover et al. (11) except for cluster VII, with isolates with eight or more band differences, and cluster III, with isolates with slightly less than 80% homology with three band differences.

tion. Isolates in clusters IV, V, and VI were obtained from patients whose ICU stays did not overlap (Fig. 4). Two isolates in cluster IV were isolated 4 months apart from different patients. Isolates in cluster V appeared in September 1994 in two patients whose ICU stays overlapped and reappeared in another patient together with an isolate in cluster VI >1 month after the two patients from September 1994 were dismissed, and isolates in both clusters V and VI reappeared in two new

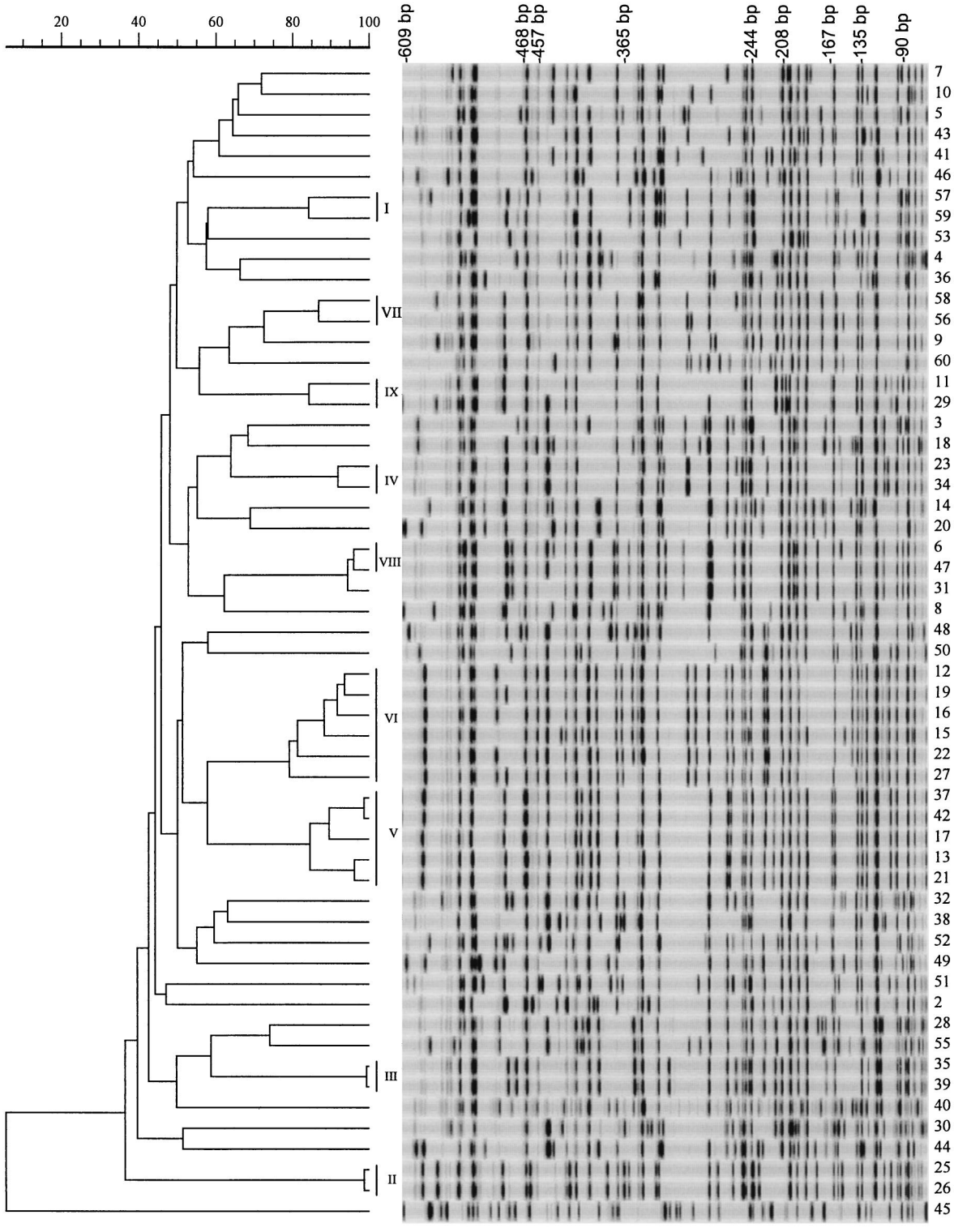

FIG. 2. Digitized AFLP (*Mse*I+C primer) patterns and dendrogram for 56 *P. aeruginosa* isolates obtained after selection of 1 isolate of each genotype from each of 44 patients colonized with *P. aeruginosa* during a 10-month period of study in an ICU. The dendrogram was constructed by cluster analysis by UPGMA with GelCompar 3.1 software (Applied Maths). Percentages of similarity and molecular sizes are shown above the dendrogram. Roman numerals indicate clusters with ≥80% homology by PFGE; these correspond to ≥80% homology by AFLP analysis, except that two extra clusters (clusters VIII and IX) were obtained by cluster analysis.

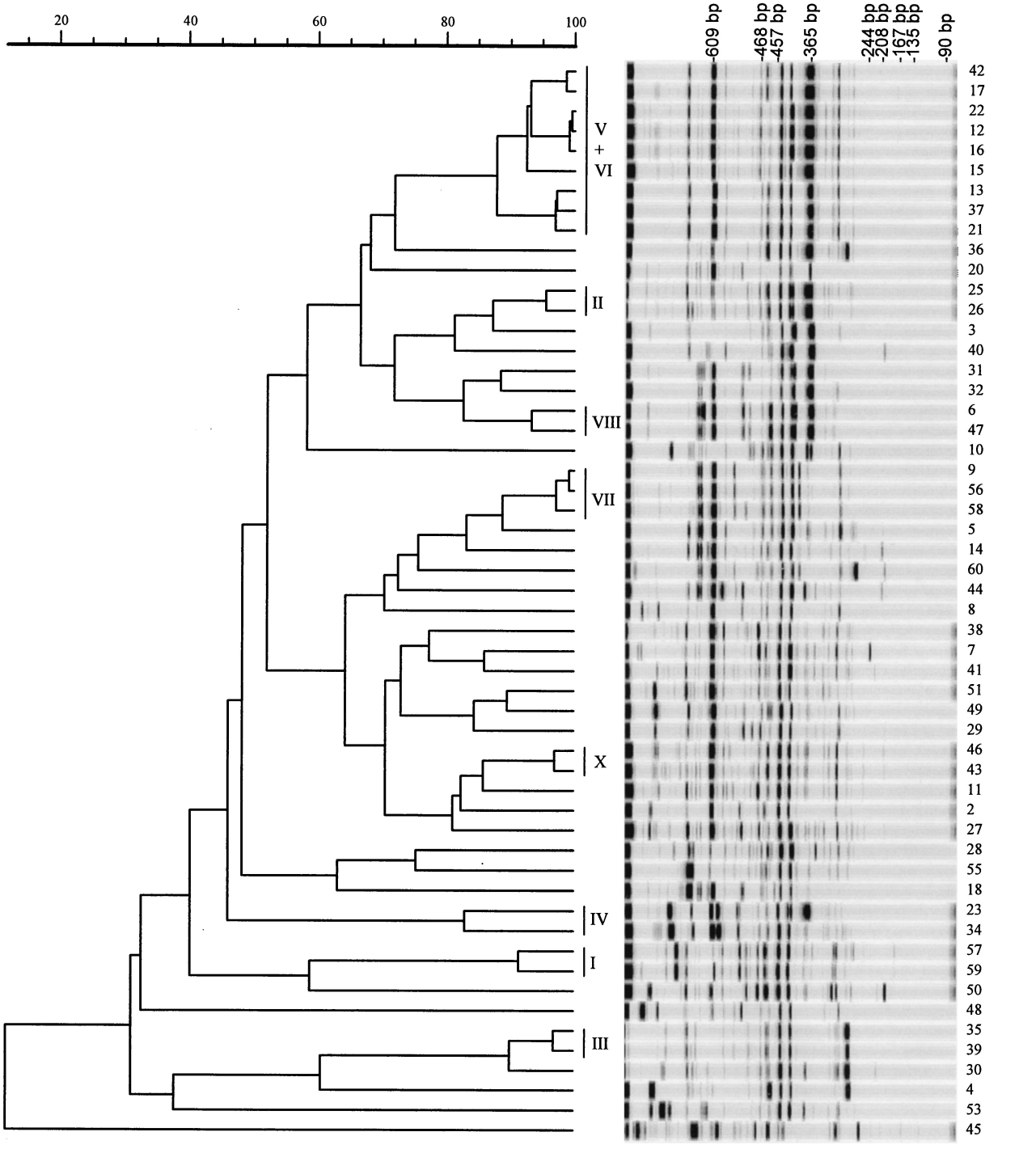

FIG. 3. Digitized RAPD (ERIC2 primer) patterns and dendrogram for 56 *P. aeruginosa* isolates obtained after selection of 1 isolate of each genotype from each of 44 patients colonized with *P. aeruginosa* during a 10-month period of study in an ICU. The dendrogram was constructed by cluster analysis by UPGMA with by GelCompar 3.1 software (Applied Maths). Percentages of similarity and molecular sizes are shown above the dendrogram. Roman numerals indicate clusters with ≥80% homology by PFGE and AFLP analysis; these almost correspond to ≥90% homology by RAPD analysis. Isolates from clusters V and VI obtained by PFGE and AFLP analysis are clustered in two other clusters by RAPD analysis, and cluster IV obtained by PFGE and AFLP analysis is not identified by RAPD analysis. Furthermore, an extra cluster (cluster X) is identified by RAPD analysis.

patients 4 weeks after the latter patient (i.e., the patient in whom isolates in clusters V and VI appeared after the September 1994 patients were dismissed) left the ICU. This is suggestive of a common source, but environmental samples for cultures were not taken except for the monthly retrieval of samples from water taps for cultures, but those cultures did not reveal *P. aeruginosa* isolates.

An interesting sequence of isolation occurred in that four apparently genetically related isolates were obtained from two patients over a period of 4 months. It started with the isolation

TABLE 1. Comparison of number of band differences obtained by RAPD analysis, PFGE, and AFLP analysis (with *Mse*I+C and *Mse*I-Ad1 primers) of pairs of *P. aeruginosa* isolates with less than seven PFGE band differences

Isolate-patient	Isolate-patient	No. of band differences				Cluster
		RAPD analysis	PFGE	AFLP analysis (with *Mse*I+C)	AFLP analysis (with *Mse*I-Ad1)	
57-Mi[a]	59-Pa[a]	2	4	6	11	I
25-Ku[a]	26-We[a]	1	0	0	0	II
35-Me	39-Be[a]	0	3	0	3	III
23-Sm	34-Se	3	3	3	8	IV
13-So	21-Ge	0	0	0	4	V
13-So	37-Kl	0	0	0	0	V
13-So	42-Bee	0	0	0	0	V
13-So	17-Ke[a]	0	3	0	1	V
21-Ge	37-Kl	0	0	0	4	V
21-Ge	42-Bee	0	0	0	4	V
21-Ge	17-Ke	0	3	0	5	V
37-Kl	42-Bee[a]	0	0	0	0	V
37-Kl	17-Ke	0	3	0	1	V
42-Bee	17-Ke	0	3	0	1	V
22-Ge	12-So	0	1	1	3	VI
22-Ge	16-Ke	0	3	3	6	VI
22-Ge	19-Ke	1	6	3	7	VI
22-Ge	27-Ke	3	6	7	12	VI
16-Ke	19-Ke	1	3	1	3	VI
16-Ke	27-Ke	3	3	6	8	VI
19-Ke	27-Ke	2	2	4	5	VI
12-So	16-Ke	0	2	1	3	VI
12-So	19-Ke	1	5	1	4	VI
12-So	27-Ke	3	5	5	8	VI
Total differences		20	58	41	101	
(Sub)types		12	15	13	16	

[a] Patient pairs with overlaps in their admissions in the ICU.

of a *P. aeruginosa* strain from patient So (isolate 12), which was genetically related, as determined by all three methods, to an isolate from patient Ke (isolate 16). Thereafter, during follow-up, two isolates were obtained from patient Ke, and both of these isolates were genetically related to the isolates obtained earlier, but with an increase in the numbers of band differences. This may suggest the accumulation of new mutations.

DISCUSSION

In this report three different techniques for the molecular typing of *P. aeruginosa* were used to study the epidemiology of *P. aeruginosa* strains in a nonepidemic situation. *P. aeruginosa* colonization was observed for 49 patients, but only 19 of these patients were already colonized on admission. Therefore, most patients appeared to become colonized during their ICU stays. This suggests that cross-acquisition and/or a common exogenous source is an important route of *P. aeruginosa* acquisition. However, the majority of patients were colonized with *P. aeruginosa* isolates with unique genotypes. This implies that patients were probably colonized from an endogenous source and that isolates were not detectable on admission.

TABLE 2. Number of patterns and genotypes obtained with the 56 isolates of *P. aeruginosa* from 44 colonized ICU patients

Method	No. of patterns	No. of genotypes[a]
RAPD analysis	49	42
PFGE	52	44
AFLP analysis[b]	50	44

[a] The numbers of "ignored" band differences were as follows: RAPD analysis, 1; PFGE, 6; AFLP analysis, 4.
[b] With primer pair *Eco*RI+0–*Mse*I+C.

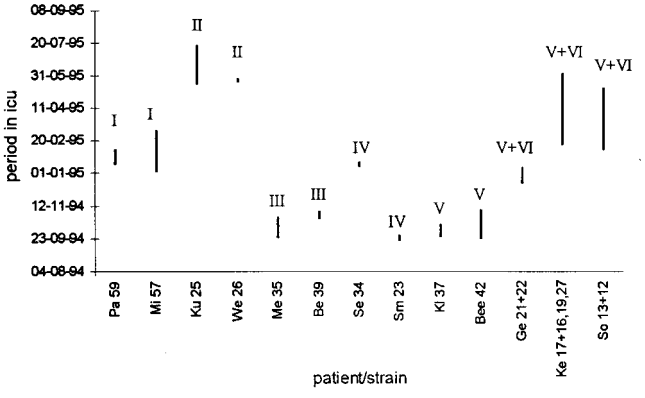

FIG. 4. Overlap in ICU stays of patients colonized with *P. aeruginosa* isolates clustered as similar types (cluster are given in roman numerals; see also Table 2). Period in the ICU is given as day-month-year.

Cross-acquisition was established for only 10 patients (five pairs). Isolates in three clusters (clusters IV, V, and VI) reappeared in the ICU, even though the stays did not overlap for any of the patients. These results are suggestive of a common exogenous source. This was not found, as no environmental samples for culture were obtained. By definition, the strains in these clusters can be called endemic (11). However, isolates (in cluster IV) that belonged to one genotype were found to colonize two patients whose stays in the ICU were separated by several months. This makes epidemiological but also genetic relatedness (defined as strains from one clone) rather unlikely. This is probably an example of the limits of these genotyping methods if studies are done over a long period of time and with large populations of organisms (11).

Computer-assisted analysis of the banding patterns revealed several groups of strains with more or less related genotypes. Grouping was virtually independent of the typing method applied, although the discriminatory power of RAPD analysis appeared to be less than those of PFGE and AFLP analysis. This is apparent, for instance, from the increase in the cutoff value applied for strain differentiation, which increased in the order PFGE = AFLP analysis < RAPD analysis (80, 80, and 90%, respectively). The same order was observed by comparing the sum of band differences obtained by each method between isolates with related genotypes (Table 1). In addition, RAPD analysis clustered strains in clusters V and VI differently than AFLP analysis and PFGE did and did not identify cluster IV, even though AFLP analysis and PFGE identified them as three clearly separate clusters.

On the basis of these data and published criteria for RAPD analysis (8) and PFGE (11) during outbreaks, interpretive criteria for *P. aeruginosa* typing by AFLP analysis were derived. Strains that differ by no more than three bands by PFGE or one band by RFLP analysis are probably epidemiologically related. Thus, strains that differ by up to two bands by AFLP analysis can be classified as probably related. Strains with three or four band differences by AFLP analysis can be classified as possibly related, in analogy with four to six band differences by PFGE typing.

Computer-assisted analysis is a very useful tool and is even indispensable for the analysis of large numbers of strains. It also allows a database of patterns to be built for comparison of the patterns of present and future isolates. However, a few discrepancies were obtained when isolates within computationally obtained clusters were visually inspected and compared with epidemiological data. Of the 10 clusters identified by any method with the software, 4 could not be confirmed by visual inspection, as too many band differences were obtained and epidemiological data did not point to an overlap in the ICU stays of most patients. Furthermore, AFLP patterns within cluster V that were found to be identical by visual examination were found to have a range of 85 to 98% similarity with software, and two isolates in cluster III with three band differences by PFGE were clustered with slightly less than 80% homology. This makes visual inspection of clusters obtained with GelCompar 3.1 software always necessary, and as already mentioned by others (10), epidemiological data should always be taken into account when deciding whether genetically related strains are also epidemiologically related.

The three typing techniques have their advantages and disadvantages. RAPD analysis had the lowest discriminatory power but gives the fastest typing results with the least hands-on time. Reproducibility is affected by many factors (13). This is a major drawback if typing results are used to generate a large database of typing patterns. PFGE has a highly reproducible discriminatory power, but it has the most hands-on time. In addition, the agarose gel used for PFGE has a lower resolving power compared to those of polyacrylamide-urea gels, which can be used for RAPD and AFLP analyses. This may be a disadvantage for reliable comparison of patterns and computer analysis. The hands-on time of AFLP analysis is between those of RAPD analysis and PFGE. This is partly explained by the need for purified DNA, which should be easier to obtain with the commercial DNA purification kits now available. Preliminary data indicate that restriction and ligation can be combined in one reaction of 2 to 4 h by decreasing the amount of digested DNA to 10 ng. Patterns were virtually indistinguishable when some of the strains were typed a second time by starting with fresh *P. aeruginosa* colonies. However, single band differences did occur. AFLP analysis with *Mse*I-Ad1 as the primer was the most discriminatory typing method in this study. However, we recommend for general application AFLP analysis with primers with one selective base (*Mse*I+C). The large number of band differences obtained by use of primers without a selective base may be confusing and will usually not give additional information about whether strains are epidemiologically related.

In conclusion, cross-acquisition does occur in a situation of endemicity, but most isolates are probably derived from the host itself. In this study all three methods correlated equally well with the epidemiology in a situation of endemicity. RAPD analysis is useful as a first screening genotyping method; this can be followed by either PFGE or AFLP analysis. However, with faster DNA isolation methods and shorter incubation times, AFLP analysis can be performed with the ease of direct computational analysis only if an automatic sequencer analyzes the gels. The criteria for AFLP analysis with primers with one selective base are that isolates with up to two band differences by AFLP analysis are probably related. Strains with three or four band differences by AFLP analysis can be classified as possibly related, in analogy with four to six band differences by PFGE typing. However, epidemiological data should always be taken in account when deciding whether genetically related strains are also epidemiologically related.

ACKNOWLEDGMENT

We thank J. Stoof for expert technical assistance and computer analysis.

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