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Ribotyping of *Pseudomonas aeruginosa*: Discriminatory Power and Usefulness as a Tool for Epidemiological Studies

DOMINIQUE S. BLANC,[1]* HANS H. SIEGRIST,[1] ROLAND SAHLI,[2] AND PATRICK FRANCIOLI[1]

Division Autonome de Médecine Préventive Hospitalière[1] and Institut de Microbiologie, Département de Virologie,[2] Centre Hospitalier Universitaire Vaudois, 1011 Lausanne, Switzerland

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Restriction fragment length polymorphism of ribosomal DNA regions (ribotyping) of *Pseudomonas aeruginosa* was evaluated as a tool for epidemiological purposes. Fifty-five epidemiologically unrelated isolates from three geographic areas of Switzerland and 11 isolates obtained during an outbreak of *P. aeruginosa* infections in a burn unit were typed by this method. Typeability and reproducibility of the method reached 100%. With four selected restriction enzymes (*Bam*HI, *Cla*I, *Eco*RI, and *Pst*I), the 55 unrelated isolates could be classified into 33 ribotypes. To assess the value of this method for the interpretation of epidemiological data, we calculated an index of discrimination (ID) which takes into consideration both the number of types defined by the typing method and their relative frequencies. Our ribotyping system obtained a high ID of 0.958 with only four restriction enzymes, comparing well with other different typing schemes for which ID values could be calculated from published data. All clinical isolates of the outbreak belonged to the same ribotype, whereas environmental isolates, initially thought to be the source of the epidemic, belonged to a different ribotype. Thus, the typeability, reproducibility, and discriminatory power of our method as well as its value established in an epidemiological investigation were found to be appropriate for further epidemiological studies of *P. aeruginosa*.

Pseudomonas aeruginosa is an important nosocomial pathogen. However, accurate investigations of the nosocomial epidemiology of *P. aeruginosa* have not been possible, mainly because the only typing methods available until recently (serotyping, phage typing, and bacteriocin typing) have poor discriminatory powers and use phenotypic markers, which are relatively unstable. With the advent of molecular typing methods, an increasing number of articles describing studies using genetic markers have been published. When applied to *P. aeruginosa*, these methods have shown that these markers were stable and that typeability and reproducibility were very good. In particular for *P. aeruginosa* infections in cystic fibrosis patients, molecular typing methods have contributed to a better understanding of the epidemiology of this infection. However, the discriminatory power of these methods could not be addressed, because only a small number of epidemiologically unrelated isolates were studied (3, 7, 12, 15, 16, 18). The discriminatory power of a typing method is of great importance for the precise interpretation of epidemiological data when this method is to be used for general epidemiological purposes. It is determined by the number of types defined by the typing method and the relative frequencies of these types. These two components of discriminatory power can be expressed by a single numerical value called an index of discrimination (ID) (10). This index is based on the probability that two unrelated strains will be placed into different typing groups.

In the present study, we decided to evaluate rRNA gene restriction fragment analysis (ribotyping) for the typing of *P. aeruginosa*. This technique can be used with many different bacterial species (1, 5), and therefore it is of great practical interest for a laboratory of epidemiology. We investigated the typeability, reproducibility, and discriminatory power of this method using a large number of epidemiologically unrelated isolates of *P. aeruginosa*. The value of the method in an epidemiological setting was assessed by using an outbreak of *P. aeruginosa* infections which had occurred earlier in the burn unit of our hospital.

MATERIALS AND METHODS

Bacterial isolates. Isolates 1 to 55 (51 clinical and 4 environmental) were presumed to be epidemiologically unrelated. They were isolated from three geographically distinct areas of Switzerland. Nineteen (no. 1 to 19) came from our hospital. Isolates 20 to 41 and 42 to 55 were kindly provided by the Institute of Microbiology of Lugano and the Institute of Medical Microbiology of Zürich, respectively. Isolates O1 to O11 were obtained during an outbreak of *P. aeruginosa* infections which had occurred in the burn unit of our hospital (eight clinical isolates from eight different patients and three environmental isolates) (4). All isolates from the burn unit belonged to serotype 2. The origins, sites of isolation, and serotypes (Habs scheme; Institut Pasteur, Paris, France) of the 66 isolates are summarized in Fig. 3.

DNA isolation. Total DNA was extracted from a 5-ml overnight culture in nitrate broth medium. Cells from 1 ml of the culture were washed once in TE4 (40 mM Tris, 0.4 mM EDTA [pH 8.0]) and resuspended in 300 μl of the same buffer. Cells were lysed by the addition of 300 μl of a 4% sodium dodecyl sulfate solution in TE4 and 50 μl of RNase a (10 mg/ml) and incubation for 30 min at 37°C. Then, 5 μl of proteinase K (10 mg/ml) was added, and the reaction mixture was incubated for 60 min further at 50°C. DNA was extracted with phenol-chloroform-isoamyl alcohol (25:24:1) and chloroform-isoamyl alcohol (24:1) according to standard protocol (13). DNA in the aqueous phase was isopropanol

* Corresponding author.

precipitated after the addition of a 1/10 volume of 3 M sodium acetate, washed once with 70% ethanol, and resuspended in 50 to 100 μl of TE (10 mM Tris, 0.1 mM EDTA [pH 7.5]). The concentration and quality of DNA were assessed by agarose gel electrophoresis and ethidium bromide staining with DNA standards in adjacent wells.

Restriction digestion of DNA. Approximately 1 μg of DNA was incubated for 3 h with 10 to 20 U of restriction enzymes (RE) (Boehringer GmbH, Mannheim, Germany) according to the manufacturer's instructions. DNA fragments were separated by horizontal agarose gel electrophoresis (GNA200 apparatus; Pharmacia-LKB). Gels contained 0.8% agarose in TBE buffer (130 mM Tris, 80 mM boric acid, 2.5 mM EDTA). Electrophoresis was run overnight for a total of 60 $V \cdot h\ cm^{-1}$.

Southern blot and hybridization. Gels were soaked first in 0.25 N HCl and then in 0.5 M NaOH–0.5 M NaCl (denaturation solution). DNA fragments were transferred to nylon membranes (Sartorius GmbH, Göttingen, Germany) with a vacuum blotting apparatus (VacuGene, Pharmacia). Transfer was performed at 40 mb (ca. 40×10^5 mPa) for 90 min in the denaturation solution. Nylon membranes were washed twice in 2× SSC (20× SSC is 175.3 g of NaCl–88.2 g of sodium citrate [pH 7.4]–NaOH per liter). Hybridization was performed with plasmid pKK3535 containing an rDNA operon of *Escherichia coli*, as previously described (14). The DNA probe was labeled with biotin-7-dATP by using a nick translation kit (GIBCO-BRL, Life Technologies). Hybrids on the membrane were revealed by using the nonradioactive nucleic acid detection system BluGene (GIBCO-BRL, Life Technologies).

Data recording. In each gel, *Hin*dIII DNA fragments of λ and the restricted DNA of two to three characterized isolates were used as migration references. Very weak bands were not taken into consideration, as their presence fluctuated with the amount of DNA in the sample, making their differentiation from the background coloration difficult. Whenever a multitude of bands was recorded in one sample, partial digestion was suspected, especially when high-molecular-weight bands were present. In that case, the DNA of the sample was purified once again with chloroform-isoamyl alcohol prior to a new digestion. Restriction fragment sizes were estimated by using the standard set Raoul I (Appligene, Illkirch, France) as a reference.

The rDNA fingerprint of each isolate was visually scored for the presence or absence of each unique fragment (0, absence of a fragment; 1, presence of a fragment) for each RE. Thus, a rectangular matrix can be constructed with all isolates and a similarity index between isolates can be calculated. Since the absence of a particular band is as important as its presence, the similarity index of Sneath and Sokal (19), also known as the mismatch coefficient, was used. The construction of a dendrogram from the similarity matrix was done by using the unweighted pair group method of analysis (19).

ID. Hunter and Gaston (10) suggested the use of a single numerical ID to evaluate the discriminatory power of a typing method. It is based on the probability that two unrelated strains sampled from the test population will be placed into different typing groups. This probability is calculated by the following equation:

$$ID = 1 - [1/N(N-1)] \sum_{j=1}^{s} n_j (n_j - 1),$$

where N is the total number of strains, s is the total number of types, and n_j is the number of strains belonging to the jth type. Thus, this index can be calculated from the distribution of types, i.e., the number of isolates per each type.

RESULTS

In order to select REs giving higher levels of discrimination between isolates, restriction fragment length polymorphism analysis was initially performed on 15 isolates of *P. aeruginosa* with the following REs: *Bam*HI, *Cla*I, *Eco*RI, *Hae*III, *Hin*dIII, *Hpa*I, *Pst*I, and *Sma*I. The four enzymes which gave the greatest number of types with the 15 isolates, *Bam*HI, *Cla*I, *Eco*RI, and *Pst*I, were chosen for studying the remaining isolates. All of the 55 unrelated isolates and the 11 isolates of the outbreak could be typed by this method, so that the typeability reached 100%, whereas serotypeability did not exceed 78%. To evaluate the reproducibility of the method, 20 isolates were tested several times at different periods, and identical banding patterns (relative position and intensity of bands) were observed for each isolate with all four REs.

For all isolates, 8 different ribotypes were obtained with *Bam*HI (labeled B1 to B8), 12 were obtained with *Cla*I (C1 to C12), 12 were obtained with *Eco*RI (E1 to E12), and 14 were obtained with *Pst*I (P1 to P14). A schematic representation of the banding patterns of all these ribotypes is shown in Fig. 1, while Fig. 2 shows examples of the original blot. The 55 epidemiologically unrelated isolates were used to evaluate the discriminatory power by calculation of an ID. This index was calculated for both serotyping and ribotyping of each RE (Table 1). The variation of the ID with the use of two, three, or four REs was also evaluated. For this purpose, the data obtained by each RE were combined. Each combination of REs resulted in a different distribution of the 55 isolates into a certain number of combined ribotypes. These distributions and the corresponding ID are presented in Table 1. While this index was 0.859 for serotyping, it varied from 0.651 to 0.728 for ribotyping with one RE, from 0.807 to 0.902 with two REs, and from 0.900 to 0.945 with three REs. With all four REs, an ID of 0.958 was obtained.

With the four REs, an index of similarity between isolates was calculated, and a dendrogram was constructed from the similarity matrix (Fig. 3). Ribotypes showed no predilection for geographic area or type of specimens. However, some correlation between ribotypes and serotypes was observed. Indeed, four of five isolates of serotype 10 belonged to the same ribotype (Fig. 3). An epidemiological link between these isolates is not likely, since one came from a different geographic area and all three other isolates from the same hospital were sampled over a 3-year interval. Several isolates of the same ribotypes (B1 C1 E1 P1 and B1 C2 E1 P1, Fig. 3) were from all three geographic areas. This probably indicates that they are ubiquitous, since an epidemiological link between isolates from different geographic areas seems to be highly improbable. However, within a given geographic area, an epidemiological link cannot be excluded.

In order to evaluate ribotyping of *P. aeruginosa* for epidemiological purposes, we investigated an outbreak which had occurred in the burn unit of our hospital. From January to May 1987, 7 out of 15 patients admitted to the burn unit were colonized and/or infected by a *P. aeruginosa* strain which produced a brown pigment (4). Serotyping and pyocin typing suggested that a single strain was involved in this outbreak. A case-control study revealed that only the seven positive patients had been showered in the shower of

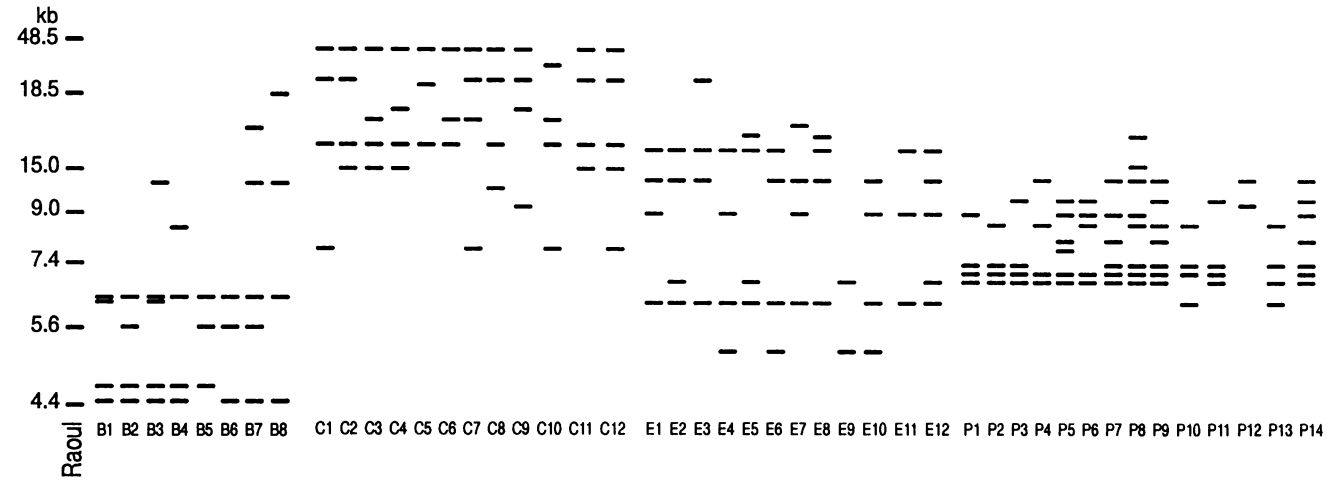


FIG. 1. Schematic representation of the banding patterns of ribotypes among all isolates of *P. aeruginosa*. Represented are ribotypes obtained from DNA digestion with *Bam*HI (B1 to B8), with *Cla*I (C1 to C12), with *Eco*RI (E1 to E12), and with *Pst*I (P1 to P14). Lane Raoul, DNA molecular weight marker.

the burn unit, unlike the eight other patients who remained negative. Moreover, one of the patients colonized with *P. aeruginosa* was brought to the burn unit only to be showered. Of the numerous environmental samplings, *P. aeruginosa* strains belonging to the outbreak serotype and pyocin type was repeatedly recovered only from the waste pipe. On the basis of the case-control study and microbiological data, it was assumed that the shower and, in particular, the waste pipe were the source of the epidemic. Indeed, the waste pipe of the shower had been wrongly conceived, since backward projections of wastewater occurred during the utilization of the shower. The system was consequently modified, but two additional cases appeared some months later. Ribotyping was performed on isolates from these two latter patients (O7 and O8, Fig. 2), on those from six of the seven patients (O1 to O6), and on three isolates from the waste pipe (O9 to O11). The analysis strongly suggested that all clinical isolates were identical. On the other hand, the three environmental isolates, though identical to one another, were clearly different from the clinical isolates. Thus, it was highly unlikely that the shower was the source of the outbreak.

DISCUSSION

All typing systems for epidemiological investigation should have established typeability and reproductibility, be sensitive enough to distinguish organisms that are similar but not identical, and be of proven value on the basis of previous testing in an epidemiological investigation (17). The typeability and the reproducibility of the ribotyping method used in the present study appear to be very good, since both reached 100%. The typeability achieved with some other restriction fragment length polymorphism typing methods might be lower if the probe does not hybridize with all strains. The exotoxin A probe, for example, is able to hybridize with only 95% of *P. aeruginosa* strains (21). The stability of a marker is another important feature. For this purpose, we have chosen rDNA genes, since they are highly conserved in evolution.

Usually, the evaluation of typing methods for epidemiological purposes is based on strains that are linked to well-investigated outbreaks. However, this may not be sufficient, since related strains cannot be used to establish the

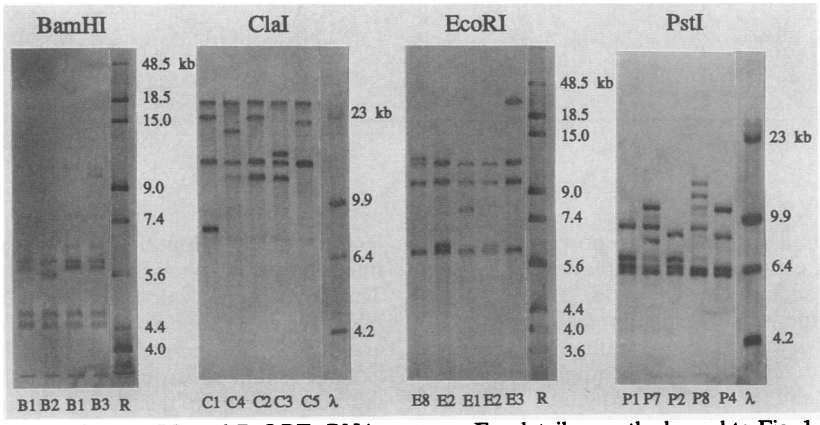


FIG. 2. Examples of *Bam*HI, *Cla*I, *Eco*RI, and *Pst*I RE rRNA patterns. For details, see the legend to Fig. 1. The lanes containing DNA molecular weight markers are R (Raoul) and λ (λ-*Hin*dIII DNA).

TABLE 1. Distribution of serotypes and ribotypes among 55 epidemiologically unrelated strains of *P. aeruginosa*

RE and characteristic	No. of isolates	ID
Serotypes[a]		0.859
1	1	
3	5	
4	2	
5	4	
6	14	
7	3	
9	1	
10	5	
11	4	
13	1	
14	1	
15	1	
16	1	
PA	9	
NT	3	
Individual ribotypes		
*Bam*HI		0.651
B1	28	
B2	17	
B3	3	
B4	2	
B5	2	
B6	1	
B7	1	
B8	1	
*Eco*RI		0.693
E1	25	
E2	18	
E3	2	
E4	2	
E5	2	
E6	1	
E7	1	
E8	1	
E9	1	
E10	1	
E11	1	
*Cla*I		0.723
C1	20	
C2	21	
C3	5	
C4	2	
C5	1	
C6	1	
C7	1	
C8	1	
C9	1	
C10	1	
C11	1	
*Pst*I		0.728
P1	27	
P2	10	
P3	3	
P4	2	
P5	3	
P6	2	
P7	1	
P8	1	
P9	1	
P10	1	
P11	1	
P12	1	
P13	1	
P14	1	
Combined ribotypes		0.807
*Bam*HI + *Eco*RI		
1	20	
2	14	
3	3	
4	2	
5	2	
6	2	
7–18	1[b]	
*Bam*HI + *Pst*I		0.808
1	22	
2	10	
3	3	
4	3	
5	2	
6	2	
7	2	
8–18	1[b]	
*Cla*I + *Pst*I		0.874
1	16	
2	10	
3	4	
4	4	
5	2	
6	2	
7–23	1[b]	
*Eco*RI + *Pst*I		0.879
1	17	
2	9	
3	3	
4	2	
5	2	
6	2	
7	2	
8	2	
9–24	1[b]	

Continued on following page

discriminatory power of a method. This power is rarely reported, and it is often estimated very subjectively by the authors. Terms like "acceptable," "good," and "very good" are used without mention of the appreciation criteria.

Discriminatory power is the ability to distinguish between unrelated isolates. It is determined by the number of types identified by the method and the relative frequencies of these types. Therefore, its evaluation should be done with a large sample of epidemiologically unrelated and randomly selected strains. Hunter and Gaston (10) proposed a very interesting way of assessing this power by calculating an ID. This index can be calculated for all typing methods, allowing objective comparison of their individual discriminatory powers (8, 9). The index is the probability that two unrelated isolates sampled from a population will belong to two different typing groups. Thus, if two typing methods give the same number of groups, but one method clusters most of the isolates in one or two groups while the second method

TABLE 1—*Continued*

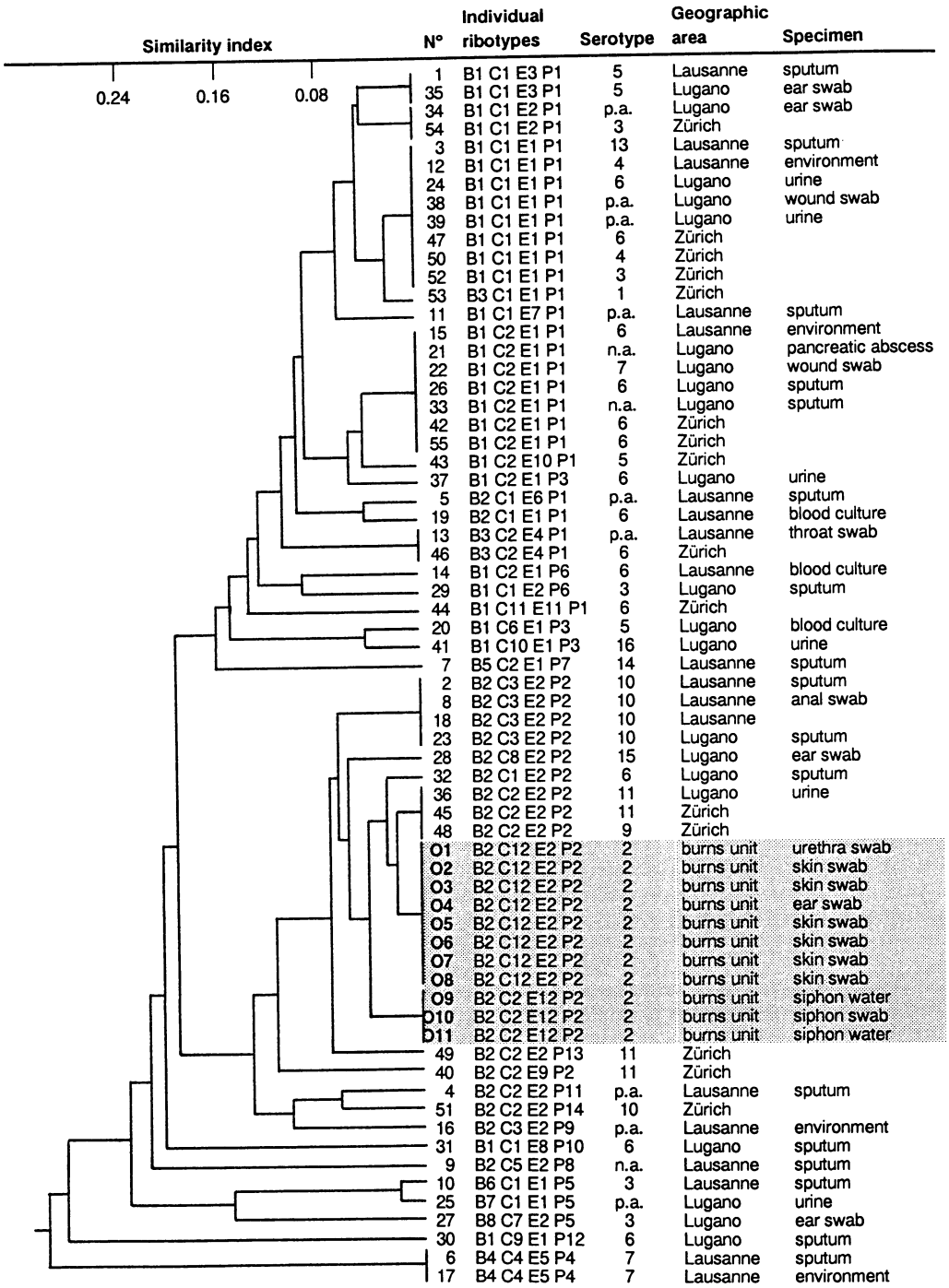
RE and characteristic	No. of isolates	ID
*Bam*HI + *Cla*I		
1	14	0.884
2	10	
3	7	
4	5	
5	3	
6	2	
7	2	
8	2	
9–18	1[b]	
*Cla*I + *Eco*RI		0.902
1	12	
2	10	
3	6	
4	5	
5	4	
6	2	
7	2	
8	2	
9–20	1[b]	
*Bam*HI + *Eco*RI + *Pst*I		0.900
1	15	
2	9	
3	3	
4	2	
5	2	
6	2	
7	2	
8–27	1[b]	
*Bam*HI + *Cla*I + *Pst*I		0.919
1	13	
2	8	
3	4	
4	4	
5	2	
6	2	
7	2	
8–27	1[b]	
*Bam*HI + *Cla*I + *Eco*RI		0.936
1	9	
2	8	
3	6	
4	5	
5	3	
6	2	
7	2	
8	2	
9–26	1[b]	
*Cla*I + *Eco*RI + *Pst*I		0.945
1	10	
2	7	
3	4	
4	3	
5	2	
6	2	
7	2	
8	2	
9–29	1[b]	
*Bam*HI + *Cla*I + *Eco*RI + *Pst*I		0.958
1	8	
2	7	
3	4	
4	3	
5	2	
6	2	
7	2	
8	2	
9–33	1[b]	

[a] Habs scheme, Institut Pasteur. PA, polyagglutinable; NT, nontypeable.
[b] One isolate for each ribotype.

distributes them more homogeneously over all groups, the ID of the second method will be greater than that of the first one. It has also been demonstrated that this index is an unbiased measure of the population from which the sampling is done.

In some studies, molecular typing of *P. aeruginosa* was done with a number of unrelated strains too small for the evaluation of the discriminatory power of the method (7, 12, 18). In studies using more unrelated strains (about 15 to 20), the discriminatory power appeared to be very promising (3, 15, 16). In three studies, the number of unrelated strains was large enough so that the ID could be calculated retrospectively. An ID of 0.832 can be derived from the data of Speert et al. (20), who used a pilin gene probe to study *P. aeruginosa*. In that study, however, 210 isolates were obtained from 75 patients. The discriminatory power of the method would certainly be higher if only unrelated isolates could be considered (i.e., one isolate per patient). As in the present study, Denamur et al. (2) used ribotyping but with different REs and with a slightly different probe. With 29 presumably unrelated strains, an ID of 0.889 could be calculated. However, by the addition of a second typing method (esterase electrophoresis), an ID of 0.995 was obtained. High discriminatory power was also obtained by the field inversion gel electrophoresis method. Grothues et al. (6) stated that all 72 unrelated patients were colonized with different strains, which implies an ID of 1.00. Ojeniyi et al. (16), using a smaller number of strains, found that field inversion gel electrophoresis analysis has better discriminatory power than restriction fragment length polymorphism analysis with three REs. In the present study, an ID of 0.958 was obtained with four REs. Izard et al. (11) suggested that the discriminatory power could be enhanced by using more REs. Our data show that the choice of the REs is more important than the indefinite addition of REs. Depending on which REs are selected, the ID varies from 0.81 to 0.90 with two REs and from 0.90 to 0.95 with three REs.

We assumed that the discriminatory power of a typing method required for epidemiological purposes is appropriate when the probability that two unrelated isolates belong to



N°	Individual ribotypes	Serotype	Geographic area	Specimen
1	B1 C1 E3 P1	5	Lausanne	sputum
35	B1 C1 E3 P1	5	Lugano	ear swab
34	B1 C1 E2 P1	p.a.	Lugano	ear swab
54	B1 C1 E2 P1	3	Zürich	
3	B1 C1 E1 P1	13	Lausanne	sputum
12	B1 C1 E1 P1	4	Lausanne	environment
24	B1 C1 E1 P1	6	Lugano	urine
38	B1 C1 E1 P1	p.a.	Lugano	wound swab
39	B1 C1 E1 P1	p.a.	Lugano	urine
47	B1 C1 E1 P1	6	Zürich	
50	B1 C1 E1 P1	4	Zürich	
52	B1 C1 E1 P1	3	Zürich	
53	B3 C1 E1 P1	1	Zürich	
11	B1 C1 E7 P1	p.a.	Lausanne	sputum
15	B1 C2 E1 P1	6	Lausanne	environment
21	B1 C2 E1 P1	n.a.	Lugano	pancreatic abscess
22	B1 C2 E1 P1	7	Lugano	wound swab
26	B1 C2 E1 P1	6	Lugano	sputum
33	B1 C2 E1 P1	n.a.	Lugano	sputum
42	B1 C2 E1 P1	6	Zürich	
55	B1 C2 E1 P1	6	Zürich	
43	B1 C2 E10 P1	5	Zürich	
37	B1 C2 E1 P3	6	Lugano	urine
5	B2 C1 E6 P1	p.a.	Lausanne	sputum
19	B2 C1 E1 P1	6	Lausanne	blood culture
13	B3 C2 E4 P1	p.a.	Lausanne	throat swab
46	B3 C2 E4 P1	6	Zürich	
14	B1 C2 E1 P6	6	Lausanne	blood culture
29	B1 C1 E2 P6	3	Lugano	sputum
44	B1 C11 E11 P1	6	Zürich	
20	B1 C6 E1 P3	5	Lugano	blood culture
41	B1 C10 E1 P3	16	Lugano	urine
7	B5 C2 E1 P7	14	Lausanne	sputum
2	B2 C3 E2 P2	10	Lausanne	sputum
8	B2 C3 E2 P2	10	Lausanne	anal swab
18	B2 C3 E2 P2	10	Lausanne	
23	B2 C3 E2 P2	10	Lugano	sputum
28	B2 C8 E2 P2	15	Lugano	ear swab
32	B2 C1 E2 P2	6	Lugano	sputum
36	B2 C2 E2 P2	11	Lugano	urine
45	B2 C2 E2 P2	11	Zürich	
48	B2 C2 E2 P2	9	Zürich	
O1	B2 C12 E2 P2	2	burns unit	urethra swab
O2	B2 C12 E2 P2	2	burns unit	skin swab
O3	B2 C12 E2 P2	2	burns unit	skin swab
O4	B2 C12 E2 P2	2	burns unit	ear swab
O5	B2 C12 E2 P2	2	burns unit	skin swab
O6	B2 C12 E2 P2	2	burns unit	skin swab
O7	B2 C12 E2 P2	2	burns unit	skin swab
O8	B2 C12 E2 P2	2	burns unit	skin swab
O9	B2 C2 E12 P2	2	burns unit	siphon water
O10	B2 C2 E12 P2	2	burns unit	siphon swab
O11	B2 C2 E12 P2	2	burns unit	siphon water
49	B2 C2 E2 P13	11	Zürich	
40	B2 C2 E9 P2	11	Zürich	
4	B2 C2 E2 P11	p.a.	Lausanne	sputum
51	B2 C2 E2 P14	10	Zürich	
16	B2 C3 E2 P9	p.a.	Lausanne	environment
31	B1 C1 E8 P10	6	Lugano	sputum
9	B2 C5 E2 P8	n.a.	Lausanne	sputum
10	B6 C1 E1 P5	3	Lausanne	sputum
25	B7 C1 E1 P5	p.a.	Lugano	urine
27	B8 C7 E2 P5	3	Lugano	ear swab
30	B1 C9 E1 P12	6	Lugano	sputum
6	B4 C4 E5 P4	7	Lausanne	sputum
17	B4 C4 E5 P4	7	Lausanne	environment

FIG. 3. Dendrogram of similarities among the 55 epidemiologically unrelated isolates (no. 1 to 55) and the 11 isolates of an outbreak in a burn unit (no. O1 to O11 [shaded]). The ribotypes, serotypes, geographic areas of isolation, and types of specimen are listed.

the same typing group is <5%, which means that the ID is >0.95. With the enhancement of the discriminatory power, one could ask whether the method might not become too sensitive, so that even natural mutations of a single strain would be detected. By typing strains at the beginning of culture and after several passages in culture, some authors have found that their isolates were stable (7, 11). One could also argue that these in vitro conditions might be far different from those encountered by bacteria in a natural setting, e.g., during an outbreak. The calculation of similarities between isolates may be of great help, since the genetic similarities of the strains can be visualized on a dendrogram and natural mutations of a strain during an outbreak would give rise to isolates of a degree of similarity still higher than that of unrelated strains.

The dendrogram obtained from our similarity data (Fig. 3)

also shows that the correlation between the results of ribotyping and those of serotyping was low. This low correlation between molecular and traditional (serotype, pyocin type, morphology, and antibiogram) typing methods of *P. aeruginosa* was already reported by others (3, 15, 16, 20).

The present ribotyping method with four REs proved to be valuable for the epidemiological investigation of *P. aeruginosa* infections. It confirmed that the clinical isolates of the outbreak in the burn unit all belong to the same strain. The water system of the bath was initially suspected on the basis of epidemiological data, serotype, and pyocin type. However, ribotyping suggested that this was probably not the source of the epidemic, since environmental and clinical isolates belonged to different ribotypes. This illustrates the value of ribotyping in providing precise data in *P. aeruginosa* outbreaks.

In conclusion, we found that the typeability and reproducibility of *P. aeruginosa* ribotyping are very high. The discriminatory power of this method was assessed by an ID and with a large number of isolates which were epidemiologically unrelated. The calculation of this index was found to be very useful for comparing different typing methods and assessing their value for epidemiological purposes. In our case, a high ID was obtained with four REs (*Bam*HI, *Cla*I, *Eco*RI, and *Pst*I), which should be adequate for this method to be used in epidemiological investigations.

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