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# Epidemiological Analysis of Sequential *Pseudomonas aeruginosa* Isolates from Chronic Bronchiectasis Patients without Cystic Fibrosis

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**PCR fingerprinting was used for the epidemiological investigation of 64 *Pseudomonas aeruginosa* isolates collected from 16 chronic bronchiectasis patients without cystic fibrosis: 56% of the patients harbored one clone, 12.5% carried a single major type with minor variants, and 31.5% carried two clones. Only a minority of the acquisitions of antibiotic resistance was related to the acquisition of exogenous strains. Mucoid and nonmucoid sets of isolates did not display any consistent differences in their patterns. The genetic similarity among the clones ranged from 10 to 69%. Cross-infection or common-source exposure did not appear to have occurred.**

Most typing studies on *Pseudomonas aeruginosa* colonization and persistence in chronic lung disease have been centered on cystic fibrosis (CF) patients (1, 7, 16, 18, 19). However, this bacterium causes important infections in bronchiectasis patients without CF, and some reports have suggested that inflammation, injury, and damage, caused by harmful substances from *P. aeruginosa*, induce bronchiectasis (4, 12). This organism is very difficult to eradicate due to multiresistance to many antibiotics and to its ability to perform phenotypic alterations that enhance its capacity to survive in the lower respiratory tract (4, 11).

PCR fingerprinting has been successfully applied to the epidemiological study of *P. aeruginosa* (2, 8, 9, 17). In this work, *P. aeruginosa* strains isolated from bronchiectasis patients without CF were characterized by an antibiotic susceptibility test and two PCR fingerprinting techniques with the following objectives: (i) to assess the diversity of strains colonizing bronchiectasis patients over time, (ii) to determine how frequently antimicrobial resistance and mucoidy were related to the acquisition of new clones, and (iii) to estimate the variability degree of the genomic distance among the identified clones.

**Patients, isolates, and methods.** Sixty-four *P. aeruginosa* strains recovered from the sputa of 16 chronic bronchiectasis patients attending the Hospital of Santa Marina, Bilbao, Spain (a 200-bed respiratory illness-specialized institution) were prospectively studied for periods of time ranging from 2 to 38 months (median, 10.6 months). The patients, whose mean age was 66 years (range, 47 to 78 years), had been admitted to the hospital on 2 to 57 occasions (median, 22.5 occasions) during the study period. The diagnosis of bronchiectasis had been based on clinical (chronic or recurrent pulmonary infection and hemoptisis) and radiographic (e.g., thickening of the airways) features. The identifiable etiology factors were previous infections in 12 cases (tuberculosis in six cases, pneumonia in three cases, and measles in three cases), and the other four patients had a history of long-standing chronic bronchitis.

The MICs were determined by broth microdilution procedures as described by the National Committee for Clinical Laboratory Standards (13). The antibiotics tested were amikacin, aztreonam, cefotaxime, ceftazidime, cefuroxime, chloramphenicol, ciprofloxacin, imipenem, meropenem, and ofloxacin.

Total DNA was extracted, and two PCR typing assays were performed for each strain. The primers used were RD1 (5′-AGCGGGCCAA-3′) and ERIC2 (5′-AAGTAAGTGACTGGGGTGAGCG-3′), whose usefulness had been proved in previous assays. The patterns were analyzed by using MA/MAC Fingerprinting V1.0 (Bio-Rad Laboratories). The similarity was expressed as the Dice coefficient, and the matrix of similarity was clustered by the UPGMA algorithm. Each type was coded with a letter (similarity coefficients greater than 80%), and each subtype (similarity of 81% or more to the initial pattern) was coded with a number.

With the 64 isolates studied, the PCR with both primers generated 21 different patterns (Table 1). (i) In nine (56%) patients, with a median follow-up period of 10 months (range, 2 to 38 months), the initially acquired strain persisted over time (patients 1 to 9). (ii) Two (12.5%) patients, with a median follow-up period of 10 months (range, 4 to 18 months), carried a single major type, of which minor variants appeared (patients 10 and 11). (iii) Five (31.5%) patients, with a median follow-up period of 11 months (range, 2 to 22 months), lost their primary strain during the first year (patients 12 to 16). Patient number 16 was colonized by two different clones at the same time.

There were 38 distinct resistance phenotypes. Changes in susceptibility to one or more antibiotics were noticed in 59% of the sequential pairs of isolates and in 81% when the comparison was made with the initial isolate of each patient. In most cases, the latest isolate of each clone was more resistant than the initial one. Resistance changes arose in 60% of the sequential pairs and in 81% of the comparisons with the first isolate of each clone type.

An alginate-overexpressing morphology was observed in 26 of the 64 isolates (40%). Mucoid forms were present in the initial isolates of 10 patients, and in four patients such forms appeared 6 to 19 months later. Patients 6 and 10 were carrying nonmucoid pseudomonads exclusively. Mucoid and nonmucoid variants of the clones did not display any consistent differences in their genetic patterns.

Cluster analysis of PCR fingerprinting patterns revealed that

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TABLE 1. Colonization period and typing results of *P. aeruginosa* isolates from chronically infected bronchiectasis patients without cystic fibrosis

| Patient (sex)[a] | No. of isolates | Follow-up period (mo) | Genotype[b] | | No. of isolates with genotype | Persistence period of genotype (mo)[c] | Antibiotype(s) associated with genotype[d] |
|---|---|---|---|---|---|---|---|
| | | | RAD1 | ERIC2 | | | |
| 1 (F) | 8 | 38 | A | a | 8 | 38 | 1, 2, 3 |
| 2 (F) | 4 | 13 | B | b | 4 | 13 | 12, 13 |
| 3 (M) | 3 | 2 | C | c | 3 | 2 | 2, 20, 21 |
| 4 (M) | 4 | 6 | D | d | 4 | 6 | 25 |
| 5 (F) | 2 | 5 | E | e | 2 | 5 | 24 |
| 6 (M) | 3 | 7 | F | f | 3 | 7 | 25 |
| 7 (F) | 4 | 2 | G | g | 4 | 2 | 29, 6, 30, 31 |
| 8 (M) | 3 | 12 | H | h | 3 | 12 | 32, 33, 34 |
| 9 (F) | 4 | 9 | I | i | 4 | 9 | 35, 36, 37, 38 |
| 10 (F) | 4 | 3 | J1 | j1 | 1 | 2 | 4 |
| | | | J2 | j2 | 3 | 2 | 5, 6, 7 |
| 11 (F) | 3 | 18 | K1 | k1 | 1 | 7 | 17 |
| | | | K2 | k2 | 2 | 11 | 18, 19 |
| 12 (M) | 4 | 7 | L | l | 2 | 2 | 8, 9 |
| | | | M | m | 2 | 5 | 10, 11 |
| 13 (M) | 4 | 22 | N | n | 1 | 12 | 14 |
| | | | O | o | 3 | 10 | 15, 16 |
| 14 (M) | 7 | 21 | P | p | 6 | 15 | 22, 1, 18 |
| | | | Q | q | 1 | 6 | 23 |
| 15 (M) | 2 | 2 | R | r | 1 | 1 | 24 |
| | | | S | s | 1 | 1 | 24 |
| 16 (M) | 5 | 4 | T1 | t1 | 2 | 4 | 25 |
| | | | T2 | t2 | 1 | 1 | 27 |
| | | | U1 | u1 | 1 | 1 | 26 |
| | | | U2 | u2 | 1 | 1 | 28 |

[a] F, female; M, male.
[b] Major types are designated by letters, and their subclonal variants are designated by number suffixes.
[c] Time period between first and last date of isolation.
[d] Resistance profile towards 10 antimicrobial agents.

the similarity among the clones from the same patient (if more than one was detected) ranged from 23 to 67% (average of 50%), and from different patients it ranged from 10 to 54% (average of 40%).

The phenotypic variation observed among isolates of *P. aeruginosa* collected from sputum supports the assumption that different clonal lineages can be involved in chronic lung infections. This work reveals that two-thirds of the studied patients harbored the same clone and that the other third carried a maximum of two genotypes. These results suggest that a limited number of strains colonize chronic bronchiectasis patients, which agrees with other studies carried out with CF patients (18). However, some reports suggest that if *P. aeruginosa* is monitored for periods longer than 2 years, more complex epidemiologic phenomena, such as coinfection or strain replacement, may be observed (9). In the present work the appearance of new clones was detected after 1 to 15 months. However, several patients were colonized by a unique strain for longer periods and without strain substitution. Along with what other authors have reported, our data suggest that there is not a significant relation between the period of colonization and strain replacement in chronic bronchiectasis patients (19).

Each clone appeared to have a mean of two different antibiotic resistance patterns, and although seven clone types had only one antibiotype, in many cases it was not exclusive. Thus, a common resistance pattern does not result from the spread of a single clone. Given this remarkable phenotypic variability, the strain identification of *P. aeruginosa* for epidemiological purposes should be based on genetic rather than phenotypic traits.

When susceptibility patterns of isolates collected from the same patient were compared with PCR fingerprinting, 30% of the modifications in the susceptibility profile seemed to be related to mutational determinants of resistance. A total of 17% of these modifications were associated with a clonal substitution, and 13% were associated with subclonal variations. These results agree with previous studies which establish that only a minority of the acquisitions of resistance is related to the acquisition of exogenous strains (19). However, 70% of the shifts in antibiotic resistance occurred in the absence of a detectable change in PCR fingerprinting. These resistant variants could be mutants which had altered their outer membrane permeability, had modified gyrase activity or depression of beta-lactams, or had activated the multidrug efflux pump systems (10, 14, 15).

In addition to those from CF patients, mucoid variants have also been isolated from chronic bronchiectasis patients (6), and this conversion may have taken place in response to environmental factors encountered during chronic infections (CF or bronchiectasis) or in vitro under adverse growth conditions (5). Differences in the PCR fingerprinting of serial mucoid and nonmucoid sets of isolates were not observed, and this may be due to the inability of the primers we used to detect the genetic changes associated with the conversion to mucoidy (3).

Hla et al. discovered that the range of similarity among bronchiectasis patients was 55 to 65% (6). The level of interpatient similarity in the present work is higher (10 to 69%), which may be due to a higher genomic variability in the strain population or to the higher discriminative power of the randomly amplified polymorphic DNA technique. According to the present results, the genetic variability among clones from the same patient (when more than one were detected) or from different patients was not significantly important. Likewise, no

evidence of strain transmission among the studied patients was detected.

In conclusion, although there was phenotypic heterogeneity among the isolates from individual patients, the fact that the studied bronchiectasis patients remained colonized with a single *P. aeruginosa* strain or with a limited number of strains (in this report a maximum of two) for long periods of time was shown by PCR fingerprinting. This work also demonstrates that most of the antibiotic susceptibility changes and the overproduction of alginate are due not to strain replacement but to strain adaptation to the lung environment.

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