

## Chronic Respiratory Infections by Mucoïd Carbapenemase-Producing *Pseudomonas aeruginosa* Strains, a New Potential Public Health Problem

*Pseudomonas aeruginosa* is a major cause of chronic respiratory infections (CRI) in patients with underlying diseases such as cystic fibrosis, bronchiectasis, or chronic obstructive pulmonary disease (COPD) (1, 6, 7). The establishment of *P. aeruginosa* CRI is driven by the acquisition of an important number of adaptive mutations required for long-term persistence (10, 12). Among them, the hallmark of *P. aeruginosa* CRI is the conversion to the mucoïd phenotype that drastically increases the organism's resistance to clearance by the immune system and antimicrobial treatments (2). Antimicrobial resistance development in this setting is frequent, although so far it is generally driven by mutations in chromosomal genes and not by the horizontal acquisition of resistance determinants (4, 8, 9).

In June 2007, a mucoïd multidrug-resistant (MDR) *P. aeruginosa* strain was isolated from the respiratory secretions of a 66-year-old male patient (patient 1) admitted to H. Son Dureta Intensive Care Unit due to a severe acute exacerbation of COPD. This isolate (P1jun07) was found to be resistant to ceftazidime, cefepime, piperacillin, piperacillin-tazobactam, imipenem, meropenem, ciprofloxacin, gentamicin, and tobramycin; intermediate to amikacin; and susceptible only to aztreonam and colistin by the Etest method. The screening test performed (Etest-MBL) was positive for class B carbapenemases (metallo- $\beta$ -lactamase [MBL]). The genetic element harboring the MBL-encoding gene was characterized by PCR and sequencing, following previously established protocols (3), revealing the presence of a class I integron in which the integrase-encoding *intI* gene was followed by *aacA4* [encoding an AAC(6')-Ib aminoglycoside-modifying enzyme that confers resistance to gentamicin and tobramycin] and *bla*<sub>VIM-2</sub>.

Patient 1 suffered a very severe obstruction to airflow (forced expiratory volume in 1 s [FEV<sub>1</sub>] of 15%) and had been admitted in the last few years to a chronic care hospital (H. Joan March) for long time periods. Pulsed-field gel electrophoresis (PFGE) analysis (Fig. 1), MDR pattern, and PCR revealed that the mucoïd MBL-producing clone had been present in this patient since at least April 2007 (isolate P1apr07). Isolate P1apr07 was obtained during one of the previous admissions to the chronic care hospital, which may suggest that it was acquired in that institution. The follow-up performed at the chronic care hospital revealed that a true CRI (defined as at least three sequential positive samples over a 6-month period) had been established: sequential cultures prospectively obtained up to December 2007 were always positive for the mucoïd MBL-producing strain (Fig. 1), despite several courses of antimicrobial treatments that included various combinations of amikacin, azithromycin, and inhaled colistin.

In August 2007, a second case of respiratory infection by mucoïd MBL-producing *P. aeruginosa* was prospectively detected in the chronic care hospital. This 80-year-old female patient (patient 2) suffered from chronic bronchiectasis and, like the first patient, had been admitted to the chronic care facility on several occasions over the last few years. The retrospective analysis of the *P. aeruginosa* isolates from this patient revealed the presence of the mucoïd MBL-producing strain since at least May 2007 (isolate P2may07), and the prospective

follow-up demonstrated the persistence of the strain in the respiratory tract at least up to December 2007, despite azithromycin and inhaled colistin treatment, again showing that a CRI had been established. PFGE analysis (Fig. 1) revealed that all of the sequential isolates belonged to the same clonal type of the MBL-producing strain of patient 1; the resistance pattern was also identical, and the presence of *bla*<sub>VIM-2</sub> was confirmed by PCR. These two patients were admitted to the chronic care facility in the same time frame before the detection of the first case in June 2007, and therefore before strict contact precautions could be adopted. Although they never shared rooms, cross transmission between them due to sporadic contact in common areas of the chronic care facility cannot be ruled out.

Finally, a third case of MBL-producing *P. aeruginosa* was detected in the same institution in October 2007. It was isolated from the respiratory tract of an 83-year-old female patient (patient 3) diagnosed with moderate COPD (FEV<sub>1</sub> of 69%) and bronchiectasis. PFGE analysis showed that this isolate also belonged to the clone described above (Fig. 1), although, interestingly, this isolate did not show the mucoïd phenotype. Furthermore, follow-up cultures obtained in November and December 2007 after treatment with inhaled colistin yielded negative results; the fact that a CRI was apparently

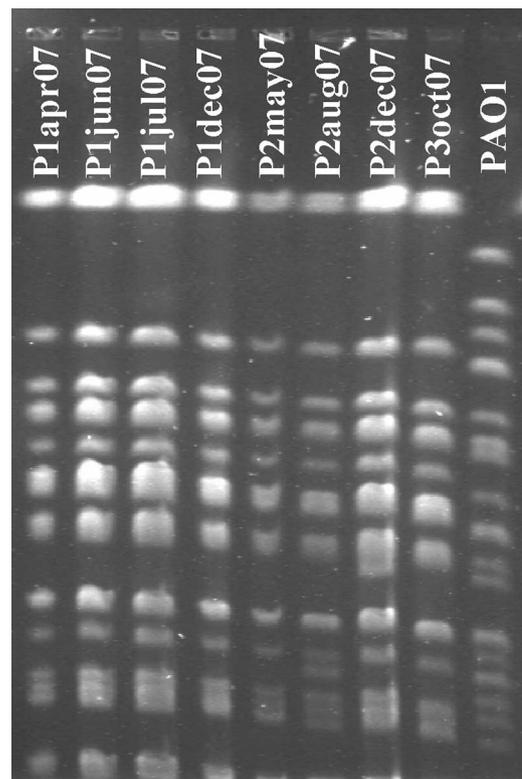


FIG. 1. SpeI DNA restriction patterns obtained through PFGE of the sequential MDR *P. aeruginosa* isolates from patients 1, 2, and 3.

not established in this patient could be related to the absence of the mucoid phenotype in this isolate.

In summary, we describe the first cases of CRI by mucoid *P. aeruginosa* strains harboring horizontally acquired MBL-producing integrons in patients with COPD and bronchiectasis. Furthermore, a single *P. aeruginosa* clone infected the three documented patients, highlighting the spreading capacity of this strain. Horizontally acquired MDR, driven by MBL-producing integrons, in *P. aeruginosa* is emerging as a major clinical problem in the hospital setting worldwide (5, 11, 13). On the other hand, CRI by mucoid *P. aeruginosa* in patients with chronic underlying respiratory diseases are extremely difficult to manage with antimicrobial agents; once they are fully established, eradication is generally no longer possible (10). Certainly, the confluence of these two major threatening features of *P. aeruginosa* infections, chronicity and horizontally acquired MDR, represents a potential major public health problem, since despite our therapeutic efforts, these patients will likely become chronic reservoirs of these highly concerning MDR determinants. Since patients with CRI are periodically admitted to both chronic and acute care hospitals, they may represent a high risk for the spread of MDR *P. aeruginosa*. Therefore, active surveillance of MBL-producing *P. aeruginosa* strains in patients with chronic underlying respiratory diseases should be a priority in the epidemiological control of MDR *P. aeruginosa*.

This work was supported by the Ministerio de Sanidad y Consumo, Instituto de Salud Carlos III through the Spanish Network for the Research in Infectious Diseases (REIPI C03/14 and RD06/0008) and by the Govern de les Illes Balears (PROGECIC-4C). The contribution of the RESC Program of the chronic care public hospital Hospital Joan March to this work is acknowledged.

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<sup>†</sup> Published ahead of print on 7 April 2008.