

## Draft Genome Sequences of Two *Pseudomonas aeruginosa* Clinical Isolates with Different Antibiotic Susceptibilities<sup>∇</sup>

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***Pseudomonas aeruginosa* is a primary cause of opportunistic infections. We have sequenced and annotated the genomes of two *P. aeruginosa* clinical isolates evidencing different antibiotic susceptibilities. Registered differences in the composition of their accessory genomes may provide clues on *P. aeruginosa* strategies to thrive in different environments like infection loci.**

*Pseudomonas aeruginosa* is a versatile Gram-negative bacterium that is ubiquitous in a wide variety of environments, including health care centers (5). Therefore, it is not surprising that *P. aeruginosa* has emerged as a leading cause of nosocomial acute infections, including pneumonia, bacteremia, urosepsis, and wound infections (3, 4, 6–8). During a seasonal epidemiological survey carried out in a collaboration with Hospital de Braga, located in northern Portugal, that handles roughly 500 *P. aeruginosa* isolates per year, we have selected two clinical isolates obtained from sputum samples from two patients with pneumonia, named 138244 and 152504, representatives of allelic sequence types ST175 (widely disseminated and associated with multidrug resistance) and ST560 (rare allele), respectively, for further analysis. Besides allelic variation, we have observed that, under standardized experimental procedures, isolate 138244 did not produce pigments and evidenced an antibiotic-panresistant phenotype, whereas 152504 produced a large amount of pyocyanin pigment and was susceptible to all antibiotics tested. To unveil the global genetic variations that may justify, at least in part, the registered phenotypic differences, a comparative genomics strategy was set. In this context, we have determined the full genome sequences of the two clinical isolates using the high-throughput system Illumina Genome Analyzer IIx. Sequencing of the susceptible strain genome yielded 4,135,435 paired-end sequence reads, whereas the panresistant isolate yielded 3,866,276 paired-end sequence reads. Raw pair-ended reads were *de novo* assembled using the Ray assembler (version 1.30) (2). The minimum contig size was set to 500 nucleotides, which generated 381 contigs for isolate 138244 (N50, 32,770 bp) and 389 contigs for isolate 152504 (N50, 32,834 bp), respectively. For both isolates, a percent GC content of 66.2% was estimated. Based on assembly data, the estimated genome sizes of isolates 138244 and 152504 are 6,357,409 bp and 6,813,259 bp, respectively, evidencing an almost 0.5-Mbp difference between the two isolates. Both genome drafts were annotated using the NCBI Prokaryotic Genomes Automatic Annotation Pipeline, revealing that isolate 138244 apparently possesses 6,153 open read-

ing frames (ORFs), whereas isolate 152504 has 6,454 ORFs. A comparative genomics analysis of the genomes of the two isolates and the fully sequenced genomes of *P. aeruginosa* (GenBank accession numbers NC\_011770.1, NC\_008463.1, NC\_009656.1, and NC\_002516.2), using the RAST server (1), provided the first clues on the accessory genomes of both isolates. Most of the unique genes identified in each isolate are annotated as “hypothetical proteins.” However, it was possible to verify that for the panresistant isolate, some of the unique genes are related to antibiotic resistance (e.g., puromycin *N*-acetyltransferase-like gene), phages, and transposase elements. In the case of the susceptible isolate, unique genes include functions like resistance to arsenic and chromate and cell wall biogenesis (e.g., glycosyltransferases). Further exploitation of the genomic information of these two clinical isolates will provide valuable information regarding the importance of the accessory genome component in the strategies used by *P. aeruginosa* clinical strains to infect hospitalized patients.

**Nucleotide sequence accession numbers.** This genome shotgun project has been deposited in DDBJ/EMBL/GenBank under accession numbers AEVV00000000 (*P. aeruginosa* 138244) and AEVV00000000 (*P. aeruginosa* 152504). The versions described in this paper are the first versions, DDBJ/EMBL/GenBank accession numbers AEVV01000000 and AEVV01000000.

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### REFERENCES

1. Aziz, R. K., et al. 2008. The RAST server: rapid annotations using subsystems technology. *BMC Genomics* 9:75.
2. Boisvert, S., F. Laviolette, and J. Corbeil. 2010. Ray: simultaneous assembly of reads from a mix of high-throughput sequencing technologies. *J. Comput. Biol.* 17:1519–1533.
3. Cholley, P., et al. 2010. Molecular epidemiology of multidrug-resistant *Pseudomonas aeruginosa* in a French university hospital. *J. Hosp. Infect.* 76:316–319.
4. Jarvis, W. R., and W. J. Martone. 1992. Predominant pathogens in hospital infections. *J. Antimicrob. Chemother.* 29(Suppl. A):19–24.
5. Kerr, K. G., and A. M. Snelling. 2009. *Pseudomonas aeruginosa*: a formidable and ever-present adversary. *J. Hosp. Infect.* 73:338–344.
6. Lister, P. D., D. J. Wolter, and N. D. Hanson. 2009. Antibacterial-resistant *Pseudomonas aeruginosa*: clinical impact and complex regulation of chromosomally encoded resistance mechanisms. *Clin. Microbiol. Rev.* 22:582–610.
7. Oliver, A., R. Canton, P. Campo, F. Baquero, and J. Blazquez. 2000. High frequency of hypermutable *Pseudomonas aeruginosa* in cystic fibrosis lung infection. *Science* 288:1251–1254.
8. Richards, M. J., J. R. Edwards, D. H. Culver, and R. P. Gaynes. 2000. Nosocomial infections in combined medical-surgical intensive care units in the United States. *Infect. Control Hosp. Epidemiol.* 21:510–515.

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