

Draft Genome Sequence of Colistin-Only-Susceptible *Pseudomonas aeruginosa* Strain ST235, a Hypervirulent High-Risk Clone in Spain

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We report the genome of colistin-only-susceptible *Pseudomonas aeruginosa* strain ST235 (PA_ST235). This isolate was obtained in the setting of an outbreak in a tertiary hospital in Spain. This clone was apparently associated with a significantly higher mortality rate. The ST235 clone also appears to be associated with greater virulence.

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Pseudomonas aeruginosa is an opportunistic pathogen in humans, frequently causing serious infections in immunocompromised and critically ill patients, due to its remarkable ability to combine mutation-driven and horizontally acquired resistance mechanisms (1, 2).

Molecular epidemiology studies, along with a deep genetic investigation of chromosomal and transferable resistance mechanisms, allowed us to describe a highly spread clone ST235 of colistin-only-susceptible (COS) *P. aeruginosa*, simultaneously producing the extended-spectrum β -lactamases (ESBLs) GES-1 and GES-5 in a class 1 integron in a Spanish hospital (3). The ST235 clone is considered a high-risk clone that has been reported in hospital outbreaks worldwide and associated with multidrug resistance patterns by acquisition of different ESBLs and carbapenemases (4–6). However, this clone totally disappeared in our institution and was replaced by another high-risk clone (ST175), which resulted in an endemic situation of multiresistant VIM-2-producing *P. aeruginosa* (7).

The *P. aeruginosa* ST235 genome (PA_ST235) was sequenced using a Roche 454 Junior sequencer. A total of 227,356,902 bp were obtained, providing approximately 26-fold coverage and 476,129 reads. Sequences obtained were used for *de novo* assembly using Newbler Assembler version 3.0 (Roche). The draft genome sequence consists of 130 contigs with an N_{50} contig size of 191,815 nucleotides and a total length of 6,930,611 bp. Sequences were annotated using the Rapid Annotation Using Subsystem Technology (RAST) server (8). A total of 6,539 coding DNA sequence (CDS) genes and 60 tRNAs were detected. This approach highlighted the presence of up to 136 genes related to antibiotic and antiseptic resistance. The verification of the class 1 integron harboring tandem duplication of GES1/GES5, as previously reported in this clone (3), was performed with BLAST (<http://www.ncbi.nlm.nih.gov/BLAST>). Several chromosomal mutations and proteins involved in antibiotic and antiseptic resistance, including many of those previously reported for other lineages, were also detected (9). Furthermore, a total of 62 related phage and

prophage elements were detected. The ST235 clone, besides its wide dissemination, appears to be associated with greater virulence (3, 10). PA_ST235 whole genome sequencing revealed the presence of type III secretion system cytotoxins ExoU and ExoY; in particular, ExoU has been associated with increased virulence (10). This strain was not a carrier of any plasmid as occurred with VIM-2-producing the *P. aeruginosa* ST175 clone (11). A genomic comparison of both clones performed with the RAST server revealed that no major differences in resistance mechanisms exist, as previously reported for each of the clones (3, 7). A few genes associated with resistance and virulence were detected in the PA_ST235 clone but not in the ST175 clone: ExoU protein, DedA protein, MacA protein (macrolide-specific efflux) or YdhE/NorM (multidrug and toxin extrusion [MATE] family efflux pump). A comprehensive genomic comparative analysis of the two clones, along with other sporadic and successful clones, might shed some hypotheses on the genetic factors determining clonal success and virulence, and thus establish new containment measures against the spread of this type of clone.

Nucleotide sequence accession number. This whole-genome shotgun project has been deposited at DDBJ/EMBL/GenBank under the accession number [JNHD00000000](https://www.ncbi.nlm.nih.gov/nuclink/JNHD00000000).

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REFERENCES

1. Aloush V, Navon-Venezia S, Seigman-Igra Y, Cabili S, Carmeli Y. 2006. Multidrug-resistant *Pseudomonas aeruginosa*: risk factors and clinical impact. *Antimicrob. Agents Chemother.* 50:43–48. <http://dx.doi.org/10.1128/AAC.50.1.43-48.2006>.
2. Obritsch MD, Fish DN, MacLaren R, Jung R. 2004. National surveil-

- lance of antimicrobial resistance in *Pseudomonas aeruginosa* isolates obtained from intensive care unit patients from 1993 to 2002. *Antimicrob. Agents Chemother.* 48:4606–4610. <http://dx.doi.org/10.1128/AAC.48.12.4606-4610.2004>.
3. Viedma E, Juan C, Acosta J, Zamorano L, Otero JR, Sanz F, Chaves F, Oliver A. 2009. Nosocomial spread of colistin-only-sensitive sequence type 235 *Pseudomonas aeruginosa* isolates producing the extended-spectrum beta-lactamases GES-1 and GES-5 in Spain. *Antimicrob. Agents Chemother.* 53:4930–4933. <http://dx.doi.org/10.1128/AAC.00900-09>.
 4. Woodford N, Turton JF, Livermore DM. 2011. Multiresistant Gram-negative bacteria: the role of high-risk clones in the dissemination of antibiotic resistance. *FEMS Microbiol. Rev.* 35:736–755. <http://dx.doi.org/10.1111/j.1574-6976.2011.00268.x>.
 5. Riera E, Cabot G, Mulet X, García-Castillo M, del Campo R, Juan C, Cantón R, Oliver A. 2011. *Pseudomonas aeruginosa* carbapenem resistance mechanisms in Spain: impact on the activity of imipenem, meropenem and doripenem. *J. Antimicrob. Chemother.* 66:2022–2027. <http://dx.doi.org/10.1093/jac/dkr232>.
 6. Edelstein MV, Skleenova EN, Shevchenko OV, D'Souza JW, Tapalski DV, Azizov IS, Sukhorukova MV, Pavlukov RA, Kozlov RS, Toleman MA, Walsh TR. 2013. Spread of extensively resistant VIM-2-positive ST235 *Pseudomonas aeruginosa* in Belarus, Kazakhstan, and Russia: a longitudinal epidemiological and clinical study. *Lancet Infect. Dis.* 13: 867–876. [http://dx.doi.org/10.1016/S1473-3099\(13\)70168-3](http://dx.doi.org/10.1016/S1473-3099(13)70168-3).
 7. Viedma E, Juan C, Villa J, Barrado L, Orellana MA, Sanz F, Otero JR, Oliver A, Chaves F. 2012. VIM-2-producing multidrug-resistant *Pseudomonas aeruginosa* ST175 clone, Spain. *Emerg. Infect. Dis.* 18: 1235–1241. <http://dx.doi.org/10.3201/eid1808.111234>.
 8. Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, Formsma K, Gerdes S, Glass EM, Kubal M, Meyer F, Olsen GJ, Olson R, Osterman AL, Overbeek RA, McNeil LK, Paarmann D, Paczian T, Parrello B, Pusch GD, Reich C, Stevens R, Vassieva O, Vonstein V, Wilke A, Zagnitko O. 2008. The RAST server: rapid annotations using subsystems technology. *BMC Genomics* 9:75. <http://dx.doi.org/10.1186/1471-2164-9-75>.
 9. Cabot G, Ocampo-Sosa AA, Domínguez MA, Gago JF, Juan C, Tubau F, Rodríguez C, Moyà B, Peña C, Martínez-Martínez L, Oliver A, Spanish Network for Research in Infectious Diseases (REIPI). 2012. Genetic markers of widespread extensively drug-resistant *Pseudomonas aeruginosa* high-risk clones. *Antimicrob. Agents Chemother.* 56: 6349–6357. <http://dx.doi.org/10.1128/AAC.01388-12>.
 10. Roy-Burman A, Savel RH, Racine S, Swanson BL, Revadigar NS, Fujimoto J, Sawa T, Frank DW, Wiener-Kronish JP. 2001. Type III protein secretion is associated with death in lower respiratory and systemic *Pseudomonas aeruginosa* infections. *J. Infect. Dis.* 183:1767–1774. <http://dx.doi.org/10.1086/320737>.
 11. Viedma E, Juan C, Otero JR, Oliver A, Chaves F. 2013. Draft genome sequence of VIM-2-producing multidrug-resistant *Pseudomonas aeruginosa* ST175, an epidemic high-risk clone. *Genome Announc.* 1(2):e00112-13. <http://dx.doi.org/10.1128/genomeA.00112-13>.