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Complete Genome Sequences of Three Pseudomonas aeruginosa Isolates with Phenotypes of Polymyxin B Adaptation and Inducible Resistance

Brian Boyle, Lucia Fernandez, Jerome Laroche, Irena Kukavica-Ibrulj, Caio M. F. Mendes, Robert W. Hancock and Roger C. Levesque

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GENOME ANNOUNCEMENT

Complete Genome Sequences of Three *Pseudomonas aeruginosa* Isolates with Phenotypes of Polymyxin B Adaptation and Inducible Resistance

Brian Boyle,[a] Lucia Fernandez,[b] Jerome Laroche,[a] Irena Kukavica-Ibrulj,[a] Caio M. F. Mendes,[c] Robert W. Hancock,[b] and Roger C. Levesque[a]

Institut de biologie intégrative et des systèmes, Université Laval, Québec, Canada[a]; Centre for Microbial Diseases & Immunity Research, University of British Columbia, Vancouver, British Columbia, Canada[b]; and Fleury Medicina e Saude, Setor de Microbiologia, Sao Paulo, Brazil[c]

Clinical "superbug" isolates of *Pseudomonas aeruginosa* were previously observed to be resistant to several antibiotics, including polymyxin B, and/or to have a distinct, reproducible adaptive polymyxin resistance phenotype, identified by observing "skipped" wells (appearance of extra turbid wells) during broth microdilution testing. Here we report the complete assembled draft genome sequences of three such polymyxin resistant *P. aeruginosa* strains (9BR, 19BR, and 213BR).

The emergence of multidrug-resistant Gram-negative organisms, including *Pseudomonas aeruginosa*, and the simultaneous lack of new clinically available antimicrobial agents have led to a resurgence of the polymyxins as a drug of last choice in the clinic (2, 4). The mode of action of polymyxin B is poorly understood (10), but resistance is usually associated with decreased uptake into the bacterial cell (1). Polymyxin B and other polycationic compounds normally enter the cell by self-promoted uptake (3) whereby these polycations bind to polyanionic outer membrane lipopolysaccharide (LPS), displacing Mg^{2+} and disrupting Mg^{2+} cross bridges between anionic LPS molecules in the outer membrane, thus leading to membrane destabilization.

It is well established that in the lab and the clinic, two separate two-component regulators, PhoP-PhoQ and PmrA-PmrB, when dysregulated, result in polymyxin B resistance in *P. aeruginosa* due to increased expression of the *arnB* operon, which mediates arabinosaminylation of bacterial LPS (1, 6, 7), while other antibiotics show an enormous diversity of mutational types leading to resistance (2).

Previously, strains collected as part of a Brazilian surveillance study between 2002 and 2004 were defined as multidrug resistant to meropenem, ciprofloxacin, polymyxin B, and at least one β-lactam from the set of cefepime, ceftazidime, or piperacillin-tazobactam (7). Intriguingly, two types of polymyxin resistance were observed: stable mutational resistance in the case of strain 9BR and, for 24 isolates typified by strains 19BR and 213BR, the occurrence of "skipped" wells (appearance of extra turbid wells) during polymyxin B MIC testing (7). Real-time PCR revealed that the skipped-well isolates 19BR and 213BR had similar basal levels of expression compared to that of wild-type PAO1 for three polymyxin resistance genes, *phoQ*, *arnB*, and PA4773 (from the *pmrAB* operon), but that these three genes were all overexpressed only at the concentration in the skipped well. In contrast, 9BR had constitutive 52- and 280-fold-higher expression of the resistance genes *arnB* and PA4773, respectively, when grown in the absence or presence of polymyxin B. Previous sequencing of the *pmrAB* and *phoPQ* operons for all three isolates revealed a number of unique mutations compared to PAO1 (7), while 1-*N*-phenylnaphthylamine (NPN) indicated a correlation between resistance and the efficiency of disruption of the outer membrane by polymyxin B.

To more fully understand multidrug and polymyxin resistance in *P. aeruginosa*, the genomes of 9BR, 19BR, and 213BR were sequenced using 454 FLX Titanium (Roche) with 27-fold, 33-fold, and 31-fold coverages, respectively. Each draft genome was assembled using Newbler version 2.5.3 and went through two rounds of manual finishing. The draft genome sequences of *P. aeruginosa* strain 9BR consisted of two scaffolds of 6,795,619 bp and 48,358 bp, while the sequences of 19BR and 213BR were assembled into singletons of 6,742,964 bp and 6,719,211 bp, respectively. Preliminary analysis revealed 12% genomic differences from the known PAO1 (8), PA14 (5), and LESB58 (9), available at http://www.pseudomonas.com/.

Nucleotide sequence accession numbers. This whole-genome shotgun project has been deposited at DDBJ/EMBL/GenBank under the accession numbers AFXI00000000, AFXJ00000000, and AFXK00000000 for strains 9BR, 19BR, and 213BR, respectively.

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Address correspondence to Roger Levesque, rclevesq@ibis.ulaval.ca.

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