A Hypermutator Strain of *Pseudomonas aeruginosa* in a COVID-19 Patient with Ventilator-Associated Pneumonia

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Abstract

Background: *P. aeruginosa* is a common cause of hospital-acquired and ventilator-associated pneumonia. Hypermutator *P. aeruginosa* strains have been described in patients with cystic fibrosis and other chronic respiratory infections but are rare in patients with acute *P. aeruginosa* infection. These isolates are associated with high genetic mutation rates leading to antimicrobial resistance. This case describes a hypermutator strain of *P. aeruginosa* found in a patient with COVID-19-associated acute respiratory distress syndrome (ARDS).

Methods: The patient was hospitalized at Northwestern Memorial Hospital in Chicago, IL, USA. Serial respiratory and blood cultures were collected throughout the patient's hospitalization and obtained from the clinical microbiology laboratory. Short-read sequencing libraries were prepared using the Illumina Nextera XT kit, and whole-genome sequencing was performed using the Illumina NextSeq platform. Long-read sequencing libraries were prepared from unsheared genomic DNA using ligation sequencing kit SQK-LSK109 and sequenced on the Oxford MinION platform. Single nucleotide variants were identified by aligning the reads from each isolate to the complete genome of the first available clinical isolate. Hypermutator assays were performed by measuring the mutation frequency rate for rifampin resistance, where hypermutator strains were defined as having a 20-fold higher mutation frequency than reference strain PAO1. Antibiotic minimal inhibitory concentrations were performed utilizing Sensititre plates (ThermoFisher Scientific). Growth curves were performed with a starting OD_{600} of 0.1 with measurements taken every 30 minutes for 24 hours.

Results: Seventeen respiratory and five blood isolates were obtained throughout 55 days of hospitalization. Fourteen of the 22 isolates exhibited hypermutator phenotypes by rifampin resistance assays and by whole-genome sequencing, which demonstrated rapid accumulation of mutations. All five bloodstream isolates were hypermutators. MIC testing noted increased resistance to aminoglycosides, fluoroquinolones, and aztreonam in the hypermutator isolates relative to the non-hypermutator isolates. Whole-genome sequencing noted that all bloodstream isolates descended from a single progenitor. Each hypermutator strain contained a mutation in the mismatch repair gene *mutL*, which has been previously associated with the hypermutator phenotype.

Conclusions: This case was notable for multiple isolates of hypermutator *P. aeruginosa* that persisted over weeks. The patient's COVID-19 infection and acute respiratory distress syndrome may have facilitated persistence of the *P. aeruginosa* lineage, allowing a hypermutator lineage to emerge.

Background

- *Pseudomonas aeruginosa* is a common cause of hospital-acquired and ventilator-associated pneumonia.
- Hypermutator *P. aeruginosa* isolates have been described in patients with cystic fibrosis and other chronic respiratory infections but are rare in patients with acute *P. aeruginosa* infection.
- Hypermutator isolates are associated with high genetic mutation rates as a result of a mutation in the mismatch repair genes (*mut* genes).
- Hypermutator isolates acquire resistance to antimicrobials quicker than non-hypermutator isolates.
- This case describes a hypermutator strain of *P. aeruginosa* found in a non-CF patient with COVID-19-associated acute respiratory distress syndrome (ARDS).

Methods

- The patient was hospitalized at Northwestern Memorial Hospital. Serial blood (n=5) and respiratory (n=17) cultures were collected throughout 55 days of hospitalization.
- MICs were performed using Sensitire GNX3F plates.
- Libraries for short-read sequencing were made using the Nextera XT Kit and was sequenced on the Illumina NextSeq.
- Long-read sequencing was performed on the Oxford Nanopore MinION.
- Growth curves were performed with a starting OD of 0.1 and measurements were taken every 30 minutes for 24 hours.
- The hypermutator assay measured the random mutation frequency against rifampin (300 ug/mL).
- Immunoblot assays were performed on bacterial supernatants (cultured in 20 mM $MgCl_2$ and 5mM EGTA in LB). The membrane was exposed to a 1:2000 concentration of polyclonal anti-ExoU antibodies and to a 1:5000 concentration of a goat anti-rabbit secondary antibody. Membranes were visualized using a LI-COR Odyssey Fc Imaging system.
- Cytotoxicity was performed on A549 cells. An MOI of 1 was inoculated into each well. An LDH Release Assay was performed after 7.5 hours.
- The murine pneumonia model of infection was performed on Balb/C mice. Fifty uL of the bacterial suspension was given intranasally. Mice were monitored daily for seven days.

Case Presentation

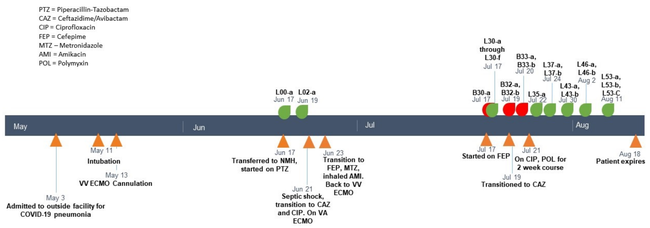

Figure 1: Clinical timeline of the patient.

Antimicrobial Resistance

		Amikacin	Gentamicin	Tobramycin	Ciprofloxacin	Levofloxacin	Aztreonam	Imipenem	Cefepime	Meropenem	Colistin	Polymixin B	Ceftazidime	Doripenem	Piperacillin/ Tazobactam constant 4	Non-synonymous Mutations
Non-Hypermutators	L00-a	≤4	2	≤1	0.12	≤1	≤2	≤1	≤2	≤1	2	1	≤1	≤0.5	≤8/4	
	L02-a	≤4	2	≤1	0.12	≤1	4	≥16	≤2	4	1*	1	2	≥8	≤8/4	
	L30-a	≤4	2	≤1	0.25	2	16	≥16	≥32	≥16	2	2	16	≥8	32/4	*mexR, mexB, oprD*
	L30-d	≤4	2	≤1	0.25	≤1	16	≥16	≥32	≥16	2	2	≥32	≥8	64/4	*mexR, mexB, oprD*
	L35-a	≤4	≤1	≤1	0.5	2	16	≥16	16	≥16	2	1	16	≥8	16/4	*mexR, mexB, oprD*
	L43-b	≤4	2	≤1	0.5	2	16	≥16	≥32	≥16	2	2	≥32	≥8	16/4	*mexR, mexB, oprD*
	L55-a	≤4	2	≤1	0.5	2	16	≥16	≥32	≥16	4*	2	16	≥8	32/4	*mexR, mexB, oprD*
	L55-c	≤4	2	≤1	0.5	2	≥32*	≥16	≥32	≥16	2	2	≥32	≥8	32/4	*mexR, mexB, oprD*
Hypermutators	L30-b	16	≥16	2	0.25	≤1	16	≥16	≥32	≥16	2	1	≥32	≥8	16/4	*mexR, mexB, oprD*
	L30-e	8	4	≤1	0.12	≤1	16	8	≥32	8	2	1	≥32	≥8	16/4	*mexR, mexB, oprD*
	L30-f	16	≥16	4	0.25	≤1	16	8	≥32	≥16	2	1	≥32	≥8	32/4	*mexR, mexB, oprD*
	B30-a	32	≥16	4	0.25	≤1	≥32	≥16	≥32	≥16	2	1	≥32	≥8	32/4	*mexR, mexB, oprD*
	B32-a	32	≥16	4	0.25	≤1	≥32	≥16	≥32	≥16	1	1	≥32	≥8	64/4	*mexR, mexB, oprD*
	B32-b	32	≥16	4	0.25	≤1	≥32	≥16	≥32	≥16	1	2	≥32	≥8	64/4	*mexR, mexB, oprD*
	B33-a	32	≥16	4	0.25	≤1	≥32	8	≥32	≥16	1*	1	≥32	≥8	64/4	*mexR, mexB, oprD*
	B33-b	32	≥16	2	0.25*	≤1	≥32	≥16	≥32	≥16	2	2	≥32	≥8	64/4	*mexR, mexB, oprD*
	L37-a	16	8	2	≥4	8	≥32	≥16	≥32	≥16	2	2	≥32	≥8	64/4	*gyrA, mexR, mexB, oprD*
	L37-b	16	8	2	≥4	≥16	≥32	≥16	≥32	≥16	2	2	≥32	≥8	32/4	*gyrA, mexR, mexB, oprD*
	L43-a	32	≥16	4	≥4	8	≥32	8	≥32	≥16	2	1	≥32	≥8	32/4	*gyrA, mexR, mexB, oprD*
	L46-a	32	≥16	2	≥4	8	≥32	≥16	≥32	≥16	2	2	≥32	≥8	64/4	*gyrA, mexR, mexB, oprD*
	L46-b	16	≥16	2	≥4	≥16	≥32	≥16	≥32	≥16	2	2	≥32	≥8	32/4	*gyrA, mexR, mexB, oprD*
	L55-b	≤4	≤1	≤1	≥4	≥16	≥32	8	≥32	≥16	1	1	≥32	≥8	64/4	*gyrA, mexR, mexB, oprD*

n = two biological replicates; all replicates were within two-fold;
* indicates MICs ≥ 4 fold

Figure 2: Antibiotic minimal inhibitory concentrations were performed using Sensititre plates (Susceptible, Intermediate, Resistant).

Whole Genome Sequencing

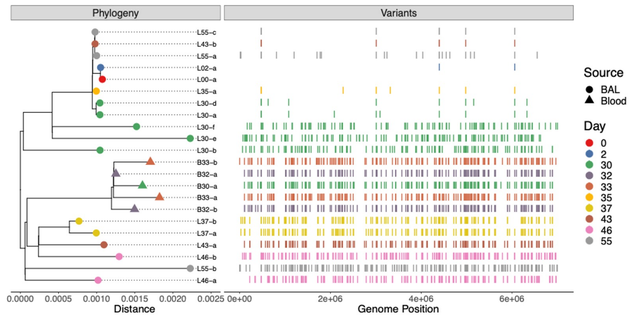

Figure 3: A complete genome of the first isolate (L00-a) was obtained using hybrid assembly of Nanopore and Illumina reads. All other isolates were sequenced on the Illumina platform and were aligned to the L00-a genome to identify single nucleotide variants. Phylogenetic analyses revealed all isolates were of the same lineage.

Growth Curve

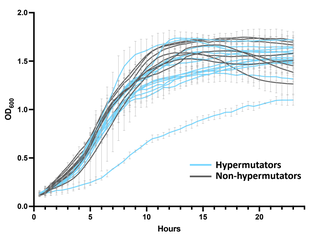

Figure 4: Growth rates differed among the isolates. The slowest growing isolate was a hypermutator strain, L55-b.

Hypermutator Assay

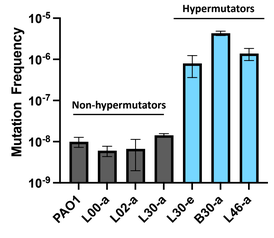

Figure 5: All hypermutator isolates contained a SNV in the *mutL* gene. The hypermutator spontaneous emergence of rifampin resistance was about 100-fold greater.

Non-ExoU Secretor

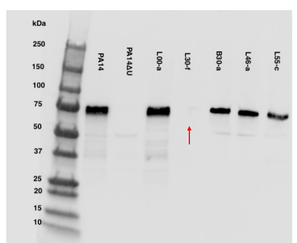

Figure 6: A representative immunoblot showing type III secretion of ExoU. All isolates except for hypermutator L30-f secreted ExoU.

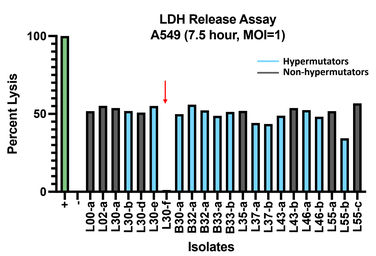

Figure 7: Cytotoxicity assay showing that L30-f (non-ExoU secretor) does not have cytotoxic effects on A549 cells.

Pneumonia Model of Infection

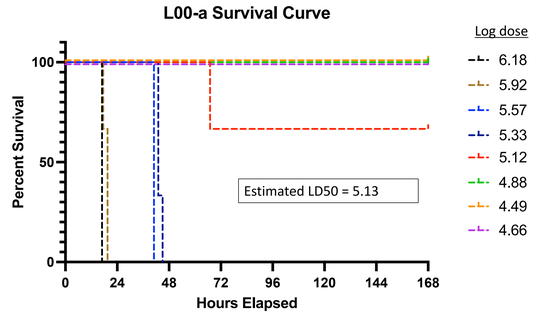

Figure 8: Balb/C mice were infected with L00-a to determine the LD50. The estimated LD50 is 5.13.

Results

- Seventeen respiratory and five blood isolates were collected throughout the patient's hospitalization.
- Fourteen of the 22 isolates exhibited hypermutator phenotypes, and all bloodstream isolates were hypermutators.
- Each hypermutator strain contained a mutation in the *mutL* gene.
- MIC testing revealed increased resistance to aminoglycosides, fluoroquinolones, and aztreonam in hypermutator isolates
- One hypermutator lung isolate did not secrete ExoU and did not have cytotoxic effects on A549 cells.

Conclusions

- This case demonstrates multiple isolates of hypermutator *P. aeruginosa* persisting over weeks in a patient with an acute and subacute respiratory infection.
- Hypermutator lineages acquired higher levels of antibiotic resistance than non-hypermutator lineages.
- Hypermutator and non-hypermutator lineages co-existed throughout much of the infection.
- COVID-19 infection and ARDS may have facilitated persistence of the hypermutator *P. aeruginosa* lineage.
- More investigations are necessary to determine whether the prolonged episodes of mechanical ventilation associated with COVID-19 pneumonia are a risk factor for development of hypermutator *P. aeruginosa* infections.

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