Uncharted territories: applying "precision medicine" to understand the treacherous landscape of extensively and multidrug resistant (XDR and MDR) *Pseudomonas aeruginosa* in a patient with cystic fibrosis and lung transplantation

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ABSTRACT (modified)

Background. *Pseudomonas aeruginosa* is a persistent and difficult-to-treat pathogen in many patients, especially those with cystic fibrosis (CF). Herein, we describe our experience managing a young woman suffering from CF with XDR *P. aeruginosa* who underwent lung transplantation. We highlight the contemporary difficulties reconciling the clinical, microbiological, and genetic information.

Methods. Twenty-two sequential XDR and PDR *P. aeruginosa* isolates obtained from the patient within a 17-month period, before and after a double-lung transplant were analyzed by whole genome sequencing (WGS) and RNAseq in order to understand the genetic basis of the observed resistance phenotypes, establish the genomic population diversity, and define the nature of sequence changes over time

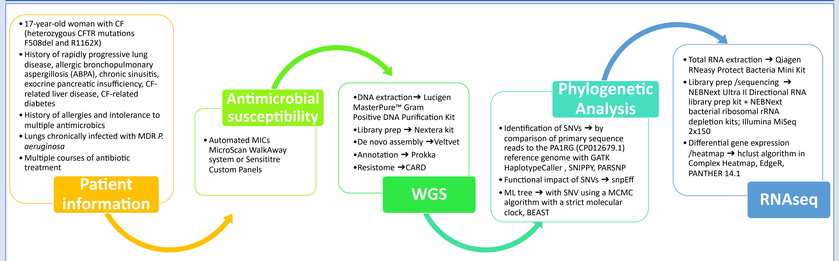
Results. Our phylogenetic reconstruction demonstrates that these isolates represent a genotypically and phenotypically heterogeneous population. The pattern of mutation accumulation and variation of gene expression suggests that a group of closely related strains was present in the patient prior to transplantation and continued to evolve throughout the course of treatment regardless of antibiotic usage. Our findings challenge antimicrobial stewardship programs that assist with the selection and duration of antibiotic regimens in critically ill and immunocompromised patients based on single-isolate laboratory-derived resistant profiles. We propose that an approach sampling the population of pathogens present in a clinical sample instead of single colonies be applied when dealing with XDR *P. aeruginosa*, especially in patients with CF.

Conclusion. In complex cases such as this, real-time combination testing and genomic/transcriptomic data could lead to the application of true "precision medicine" by helping clinicians choose the combination antimicrobial therapy most likely to be successful against a population of MDR pathogens present.

BACKGROUND

- The WHO and CDC have both designated *Pseudomonas aeruginosa* as one of the major pathogens for which antibiotics are desperately needed [1, 2].
- *P. aeruginosa* is a persistent and difficult-to-treat pathogen in many patients, especially those with Cystic Fibrosis (CF); where it is the most prevalent pathogen in the lungs and a major contributor to morbidity and mortality [3, 4].
- Our goal was to better understand the molecular basis of phenotypes of a set of *P. aeruginosa* isolates recovered during a 17-month observation period from a young woman suffering from CF who underwent double-lung transplantation to potentially inform the choice of therapies most likely to be successful in treating such complicated infections.

METHODS



RESULTS

Table 1. Antimicrobial susceptibility profiles and PDC variants found in *P.aeruginosa* isolates

ID		Isolation date	Source	Clade	PDC Variant	PDC Aminoacid substitutions	Minimum inhibitory concentrations (MICs, mg/L) Piperacillin-tazobactam	Ticarcillin/ Clavulanate	Ceftazidime/ Avibactam	Ceftolozane/ Tazobactam	Cefepime	Ceftazidime	Imipenem	Meropenem	Aztreonam	Ciprofloxacin	Levofloxacin	Gentamicin	Tobramycin	Amikacin	Colistin	Polymyxin	Fosfomycin**
Pae_AZ01		2/23/17	SPU	A	New 1	R79Q, T96I, T105A	32	>128	≤2	≤2	>16	>32	>8	16	>32	>2	>8	8	32	2	2	>64
Pae_AZ02		3/14/17	SPU		Not sequenced		16	64	≤2	≤2	8	4	>8	8	16	0.5	2	8	≤1	16	>4	>4	>64
Pae_AZ03	Pre-transplant	4/8/17	SPU	C	New 2	R79Q, T105A, F147L, E247K	16	**128**	4	≤2	16	16	>8	8	16	0.5	2	8	≤1	16	>4	>4	>64
Pae_AZ04		4/8/17	SPU	C	New 3	T105A, E247K	>64	>128	>16	>8	>16	>32	>8	>32	>32	>2	>8	>8	4	>32	2	1	>64
Pae_AZ05		5/27/17	SPU	C	New 2	R79Q, T105A, P180L, E247K	32	>128	>16	>8	>16	>32	4	8	>32	>2	>8	>8	2	>32	0.5	1	>64
Pae_AZ06		6/7/17	B-WA		Not sequenced		>64	>128	>16	>8	>16	>32	8	16	>32	>2	>8	>8	4	>32	2	1	>64
Pae_AZ07		7/7/17	BAL	C	New 2	R79Q, T105A, P180L, E247K	64	>128	>16	>8	>16	>32	≤1	16	>32	>2	>8	>8	2	>32	2	1	>64
Pae_AZ08		8/3/17	BAL	C	New 4	R79Q, T96I, T105A, P180L, E247K	>64	>128	>16	>8	>16	>32	8	8	>32	>2	>8	>8	4	>32	2	4	>64
Pae_AZ09		8/7/17	BAL	C	New 2	R79Q, T105A, P180L, E247K	>64	>128	>16	>8	>16	>32	>8	>32	>32	>2	>8	>8	4	>32	2	1	>64
Pae_AZ10		8/21/17	BAL	B	New 5	R79Q, T105A, D107N, ΔG240, E247K	>64	>128	>16	>8	>16	>32	8	>32	>32	>2	8	>8	>8	>32	1	1	>64
Pae_AZ11		8/24/17	BAL	C	New 2	R79Q, T105A, P180L, E247K	>64	>128	>16	>8	>16	>32	2	32	>32	>2	>8	>8	4	>32	2	1	>64
Pae_AZ12		9/25/17	B-Asp		Not sequenced		>64	>128	>16	>8	>16	>32	8	32	>32	2	>8	>8	>8	>32	0.5	1	>64
Pae_AZ13		10/15/17	Nasal	C	New 2	R79Q, T105A, P180L, E247K	>64	>128	>16	>8	>16	>32	8	32	>32	>2	>8	>8	4	>32	2	2	>64
Pae_AZ14		11/1/17	BAL	B	New 5	R79Q, T105A, D107N, ΔG240, E247K	>64	>128	>16	>8	>16	>32	8	>32	>32	2	4	>8	>8	>32	1	1	>64
Pae_AZ15		11/30/17	BAL	C	New 2	R79Q, T105A, P180L, E247K	>64	>128	>16	>8	>16	>32	4	32	>32	>2	>8	>8	>8	>32	2	1	>64
Pae_AZ16	Post-transplant	12/9/17	SPU	B	New 5	R79Q, T105A, D107N, ΔG240, E247K	>64	>128	>16	>8	>16	>32	8	>32	>32	>2	8	>8	>8	>32	1	1	>64
Pae_AZ17 *		12/20/17	BAL	C	New 2	R79Q, T105A, P180L, E247K	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Pae_AZ18		1/5/18	SPU	C	New 2	R79Q, T105A, P180L, E247K	>64	>128	>16	>8	>16	>32	>8	>32	>32	>2	>8	>8	>8	>32	2	1	>64
Pae_AZ19		1/15/18	SPU	C	New 2	R79Q, T105A, P180L, E247K	32	>128	>16	>8	>16	>32	>8	>32	>32	>2	>8	>8	>8	>32	1	1	>64
Pae_AZ20		1/25/18	B-WA	C	New 2	R79Q, T105A, P180L, E247K	>64	>128	>16	>8	>16	>32	>8	>32	>32	>2	>8	>8	>8	>32	2	2	>64
Pae_AZ21		2/21/18	SPU	C	New 2	R79Q, T105A, P180L, E247K	>64	>128	>16	>8	>16	>32	>8	32	>32	>2	>8	>8	>8	>32	1	1	>64
Pae_AZ22		3/4/18	B-WA	C	New 2	R79Q, T105A, P180L, E247K	>64	>128	>16	>8	>16	>32	>8	>32	>32	>2	>8	>8	>8	>32	2	2	>64

SPU: sputum; B-WA, bronchial wash; BAL, bronchoalveolar lavage; B-Asp, bronchial aspirate. * Isolate could not be regrown from frozen stock. NS, not sequenced. Numbers in bold indicate resistant according to CLSI breakpoints, except for fosmocyin (Ecoff value as per EUCAST). Colors indicate each one of the clades:** Clade A (gray), Clade B (orange), and Clade C (teal).

The majority of isolates were resistant to almost all antibiotics tested, with only colistin and Polyxmyxin B remaining active. Only 3/21 isolates were susceptible to caz/avi and tol/tazo

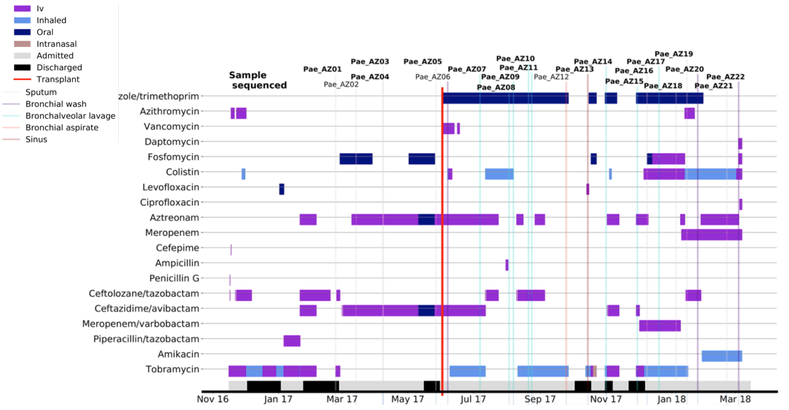


Figure 1. Timeline of antibiotic treatment and isolates recovered from the CF patient within the 17-month period until the withdrawal of care. Indicated in bold are isolates that underwent WGS.

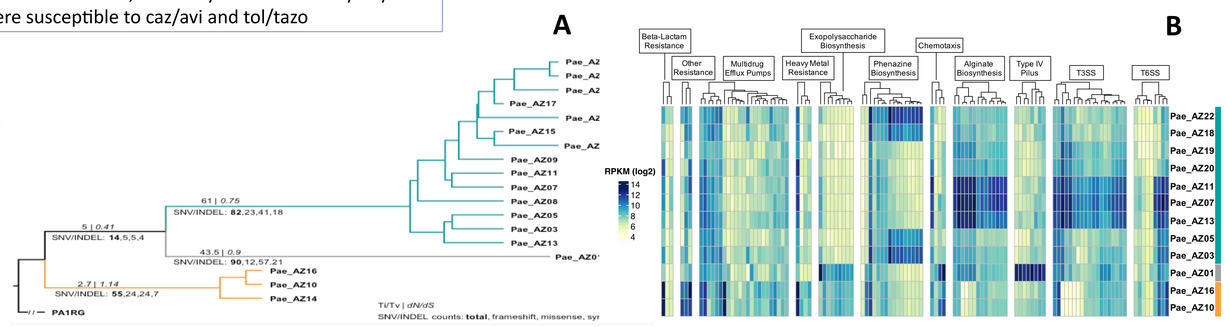


Figure 2A. Maximum Likelihood tree indicating relatedness among sequenced isolates. Tree was built in BEAST using a concordant set of 459 SNVs from GATK, Snippy, and Parsnp. Three main clades shown are Clade A (gray), Clade B (orange), and Clade C (teal). On each branch, the transition to transversion ratio (Ti/Tv) and the *dN/dS* ratio are shown above the line, with total numbers of variants of each type below the line. **B. Expression patterns of antibiotic resistance/pathogenicity genes amongst *P. aeruginosa* isolates**. RNA-seq data from 12 isolates representing all 3 clades showed variable gene expression in multiple resistance and pathogenicity functional categories (indicated at top). Hierarchical clustering of the gene expression data was used to group isolates for differential expression (DE) analysis. Differentially expressed genes were tested for overrepresentation of Gene Ontology Biological Process categories compared to the PAO1 genome in PANTHER; DE and overrepresented categories are shown.

- A phylogenetic tree constructed using 455 SNVs and 129 short indels revealed 3 clades (A, B, and C; with 1, 3, and 14 members respectively) that share a recent common ancestor.
- Clades A and C contained different mutations in the DNA mismatch repair gene *mutL* and exhibited an elevated ratio of transition to transversion mutation
- RNAseq performed on representative isolates from each clade, revealed substantial differences in the expression of genes associated with antibiotic resistance and virulence traits
- Interestingly, bla_{PDC} expression varied 50-fold across isolates, with the highest expression in Clade B, but with several other isolates exhibiting 2-10x higher expression than the oldest isolate, Pae_AZ01.

CONCLUSIONS

- The genomic/transcriptomic data provided a much richer view of the extent of heterogeneity among isolates, not evident by antibiotic susceptibility profiles.
- As our phylogenetic reconstruction demonstrates, these isolates represent a genotypically and phenotypically heterogeneous population, therefore profiling of a single isolate to inform antibiotic choice may yield a treatment that is potentially ineffective against the rest of the population; highlight the need for a more comprehensive sampling when gathering microbiological data.
- In complex infections such as the one presented, real-time combination testing and genomic/transcriptomic data could help to the application of true "precision medicine" by helping clinicians choose the combination antimicrobial therapy most likely to be successful against a population of MDR pathogens.

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