Towards a Genome-wide Fingerprint of Antibiotic Resistance Determinants in the Cystic Fibrosis Pathogen *Burkholderia cenocepacia* K56-2

University of Manitoba

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Andrew M Hogan[1], Lamprinos Frantzeskakis[1], Atif Ul Aftab[1], Silvia T Cardona[1,2]

1. Department of Microbiology, University of Manitoba, Canada;
2. Department of Medical Microbiology and Infectious Disease, University of Manitoba, Canada

@cardonalab

@cardona_lab
@MicrobeAndrew

silvia.cardona@umanitoba.ca
---
hogana34@myumanitoba.ca

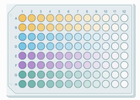
Introduction

Burkholderia cenocepacia is a Gram-negative opportunistic pathogen responsible for lethal pulmonary infection in individuals with cystic fibrosis. The lack of universal treatments for this infection stems from high intrinsic antibiotic resistance[1,2]. Next-generation sequencing coupled with transposon mutagenesis allows genome-wide explorations into the contributions of cryptic resistance mechanisms and routes to overcome them[3]. Here, we lay the foundation for such an exploration into the well-studied clinical isolate *B. cenocepacia* K56-2 with an in-depth survey into its resistance arsenal. We expect these studies to yield valuable insight into novel therapeutic avenues for treating infections caused by *B. cenocepacia* and related bacteria.

Objectives

1. Characterize the growth dose response of K56-2 to a panel of diverse antimicrobials
2. Identify genetic resistance and susceptibility determinants using a randomly-barcoded transposon mutant library in K56-2

Methods

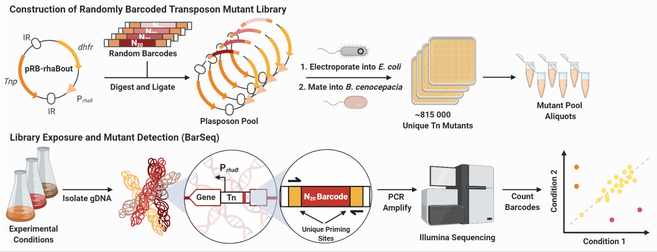


Dose Response Curves

- 87 antimicrobials from >30 diverse structural classes

K56-2 Genome: GCF_014357995.1

Unique hits >35% identity and 75-125% reference length[4]



Resistance Genes Cause High-level Antimicrobial Resistance

- The CARD predicts a broad-spectrum of putative resistance genes, mostly related to efflux
- As per CLSI guidelines, K56-2 is only sensitive to trimethoprim-sulfamethoxazole, ciprofloxacin, and minocycline

Figure 1. Overview of select resistance mechanisms encoded in strain K56-2 and their effect on antimicrobial dose-responses. Antimicrobials are grouped by structural class. The names of select putative resistance genes identified by CARD[4] are given in parentheses.

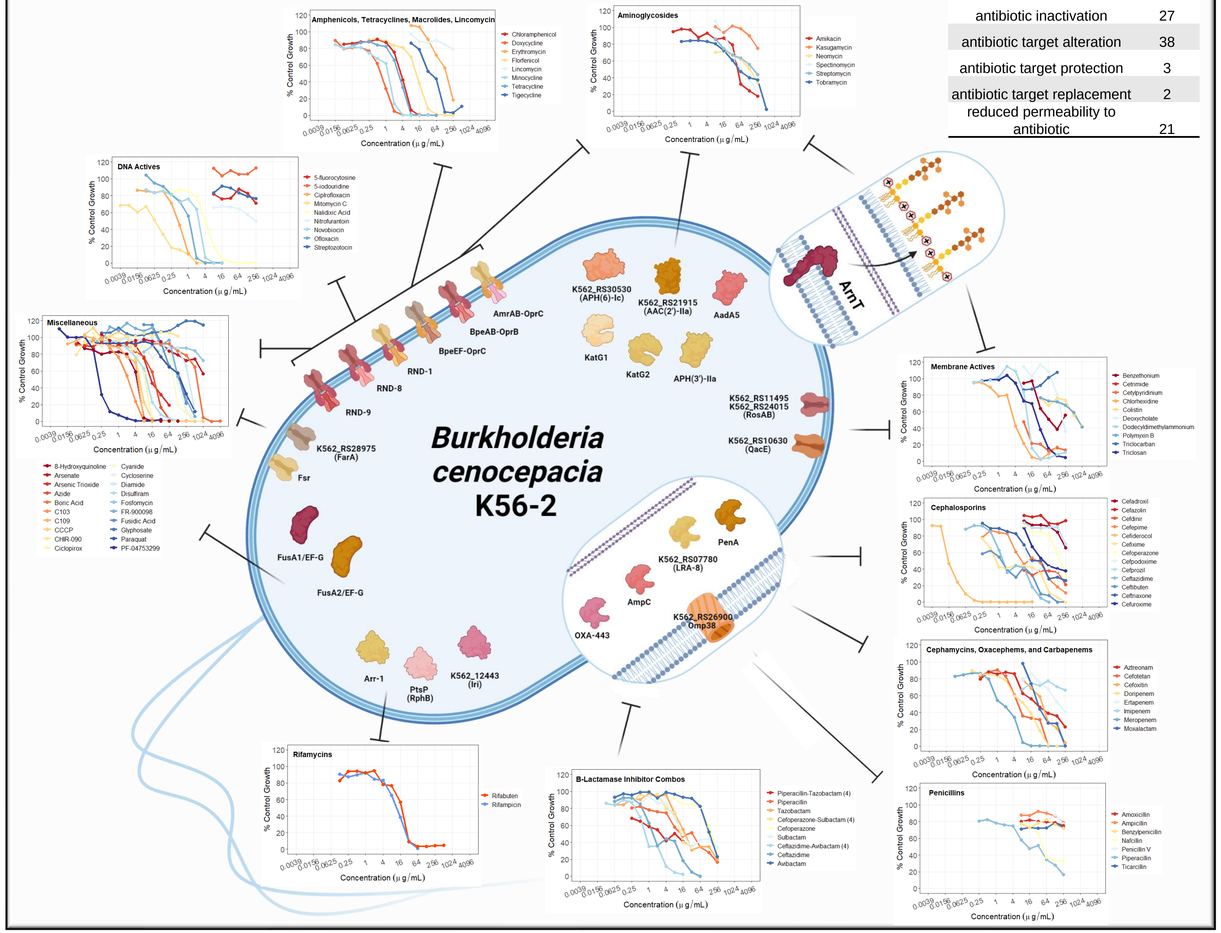


Table 1. Summary of putative resistance genes from CARD in K56-2

Mechanism	Count
antibiotic efflux	160
antibiotic inactivation	27
antibiotic target alteration	38
antibiotic target protection	3
antibiotic target replacement	2
reduced permeability to antibiotic	21

Tn Library Exposed to β-Lactams

- BarSeq reveals mechanisms of resistance and susceptibility
- Shared (e.g. *lolA, wbxA*) and unique (*e.g. hldD, dacB, K562_RS31510*) factors are linked to mechanisms of action

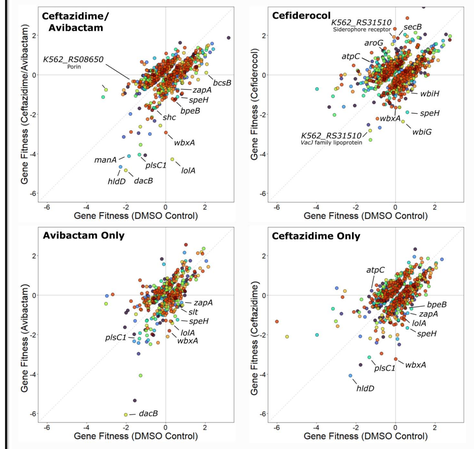


Figure 2. A Tn library with 340 000 mutants was exposed to the IC_{25} of the stated β-lactams. gDNA was harvested after 6hrs. Barcodes amplified by PCR and sequenced by Illumina NextSeq. Analysis was done as per refs 5 and 6. Shown are genes with significantly different fitness from DMSO control ($P < 0.05$).

Disruption impairs fitness ← -6 Gene Fitness +2 → Disruption enhances fitness

COG Category

- Not assigned
- Chromatin structure and dynamics
- Energy production and conversion
- Cell cycle control, cell division, chromosome partitioning
- Amino acid transport and metabolism
- Nucleotide transport and metabolism
- Carbohydrate transport and metabolism
- Coenzyme transport and metabolism
- Lipid transport and metabolism
- Translation, ribosomal structure and biogenesis
- Transcription
- Replication, recombination and repair
- Cell wall/membrane/envelope biogenesis
- Cell motility
- Post-translational modification, protein turnover, chaperones
- Inorganic ion transport and metabolism
- Secondary metabolite biosynthesis, transport and catabolism
- Function Unknown
- Signal transduction mechanisms
- Intracellular trafficking, secretion, and vesicular transport
- Defense mechanisms

Summary and Future Directions

- K56-2 encodes diverse mechanisms resulting in high levels of antimicrobial resistance
- Tn methods offer genome-wide view; first study on cefiderocol
- Profile more compounds to predict gene function and antimicrobial mechanisms of action

References and Acknowledgements

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