Molecular Epidemiological Analysis of Relapsed Gram-negative Bloodstream Infection Isolates

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Introduction

- Gram negative bacterial bloodstream infections (GNB-BSI) are common and frequently lethal
- Many patients experience multiple GNB-BSI for unclear reasons
- Repeat infections can be classified as relapsed (infection with same isolate) or reinfection (infection with genetically distinct isolate)
- Bacteria can undergo microevolution in the host, which can lead to genetic changes with alterations in antimicrobial resistance and antimicrobial tolerance.
- The mechanism of alterations in antibiotic tolerance *in-vivo* remains incompletely understood

Objectives

1. To identify the molecular changes occurring in Gram-negative bloodstream isolates from patients with relapsed GNB-BSI.
2. Determine how these molecular changed affect antibiotic resistance and antibiotic tolerance.

Methods

Patients

- The Bloodstream Infection Biorepository (BSIB) prospectively enrolled adult, hospitalized, non-neutropenic patients with monomicrobial GNB-BSI at Duke from 2002-2015.

Bacterial Isolates

- Clinical isolates were obtained from the BSIB. This collection of recurrent GNB-BSI patients was previously studied by Bock et al (Clin Infect Dis, Aug 2022, In press, PMID 35929656)

Definitions

- Recurrent GNB-BSI: ≥2 episodes of GNB-BSI with the same bacterial species
- Relapsed GNB-BSI: Recurrent GNB-BSI due to same bacterial strain
- Reinfection GNB-BSI: Recurrent GNB-BSI due to a different bacterial strain

Whole Genome Sequencing

- Bacterial genomic DNA was extracted with the Qiagen Bacterial DNA Isolation Kits. Genomic DNA was prepared for Illumina short-read sequencing and Oxford Nanopore long read sequencing. Libraries prepared for sequencing on Illumina NextSeq 2000 using Illumina DNA Prep kit with unique dual indexes. Libraries were prepared for sequencing using the Rapid Barcoding Kit and the FLO-MIN106D chemistry.

Determination of relapsed vs reinfection GNB-BSI

- All isolates underwent pulsed-field gel electrophoresis (PFGE) as an initial screen to determine relapse vs. reinfection
- PGFE results consistent with relapse underwent WGS
- Bioinformatic analysis able to distinguish true relapse from reinfection and identified single-nucleotide polymorphisms between initial and relapsed isolates

Time-Kill study to assay antibiotic tolerance

- *E. coli* cultures grown in LB until OD_{600} = 0.4. Meropenem added at 160x the minimum inhibitory concentration. 100 ml removed to enumerate colony forming units (CFU) at the specified time point

Results

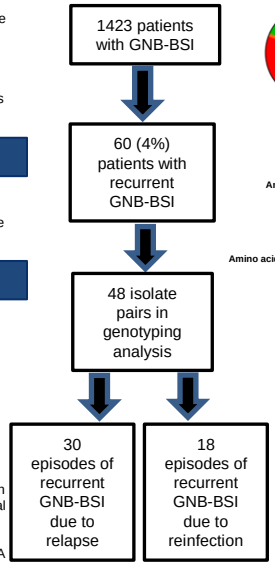


Figure 1. Most episodes of recurrent GNB-BSI are due to relapsed infection with the same bacterial strain.

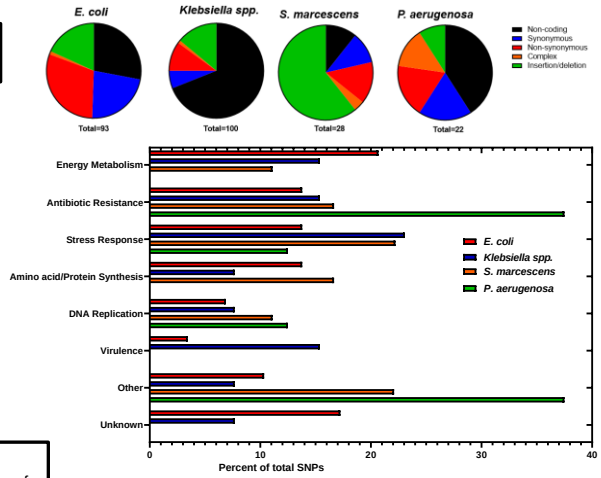


Figure 2: Distribution of single-nucleotide polymorphisms (SNP) varies by species

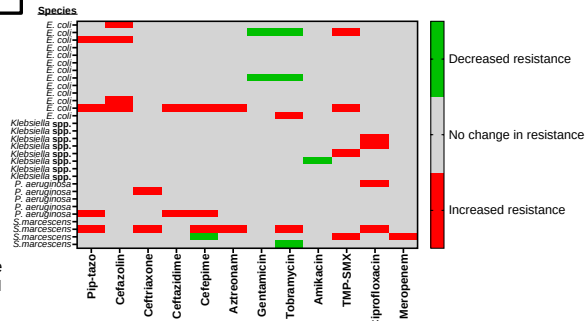
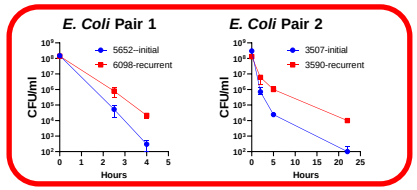


Figure 3: Antibiotic resistance changes in relapsed GNB-BSI isolate relative to initial isolate



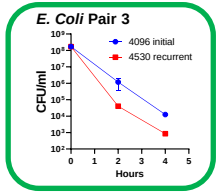


Figure 4: Alterations in antibiotic tolerance occurs in 3/10 relapsed *E. coli* isolates. Increased antibiotic tolerance shown in red, decreased tolerance in green.

E. Coli Pair	Species	Mutation type	Gene	Product	Mutation	Function
Pair 1	E. coli	Missense	*gpmI*	2,3-bisphosphoglycerate-independent phosphoglycerate mutase	G410V	CHO metabolism
		Missense	*mtlA*	PTS system mannitol-specific EIICB component	A122V	CHO metabolism
		Frameshift	*mnmC*	tRNA 5-methylaminomethyl-2-thiouridine biosynthesis bifunctional protein MnmC	S30fs	Amino acid/Protein synthesis
		Missense	*yedI*	Inner membrane protein YedI	A122V	Unknown
		Stop	*riml*	[Ribosomal protein S18]-alanine N-acetyltransferase	Y130 stop	Amino acid/Protein synthesis
		Missense	*oxyR*	Hydrogen peroxide-inducible genes activator	A100T	Stress response
		Conservative deletion	*traD*	Coupling protein TraD	Q638-P640	Conjugation
Pair 2	E. coli	Missense	*argD*	Acetylornithine/succinyldiaminopimelate aminotransferase	P85L	Amino acid/Protein synthesis
		Missense	*ptsI*	Phosphoenolpyruvate-protein phosphotransferase	V488F	CHO Metabolism
Pair 3	E. coli	Inframe Insertion	*gatC*	PTS system galactitol-specific EIIC component	Insertion	CHO Metabolism
		Missense	*lapB*	LPS assembly protein B	R375W	Stress Response

Table 1: SNPs associated with alterations in antibiotic tolerance in *E. coli*

Conclusions

1. In recurrent GNB-BSI, relapse is more common than reinfection
2. Alterations in antibiotic resistance are common in relapsed GNB-BSI
3. Antibiotic resistance changes in relapsed GNB-BSI isolates are driven by both acquisition of resistance genes and SNPs that modulate antibiotic tolerance