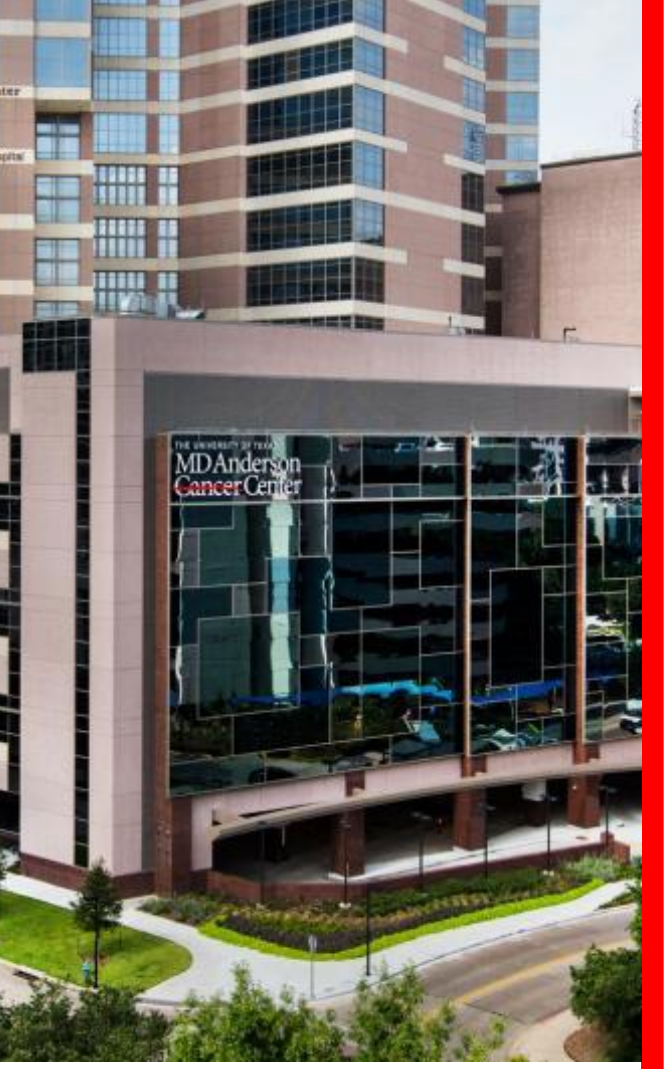


THE UNIVERSITY OF TEXAS
MD Anderson Cancer Center
Making Cancer History®

Characterization of Pre-Resistance Mechanisms Enabling Carbapenem Resistance in High-Risk *Escherichia coli* Lineages

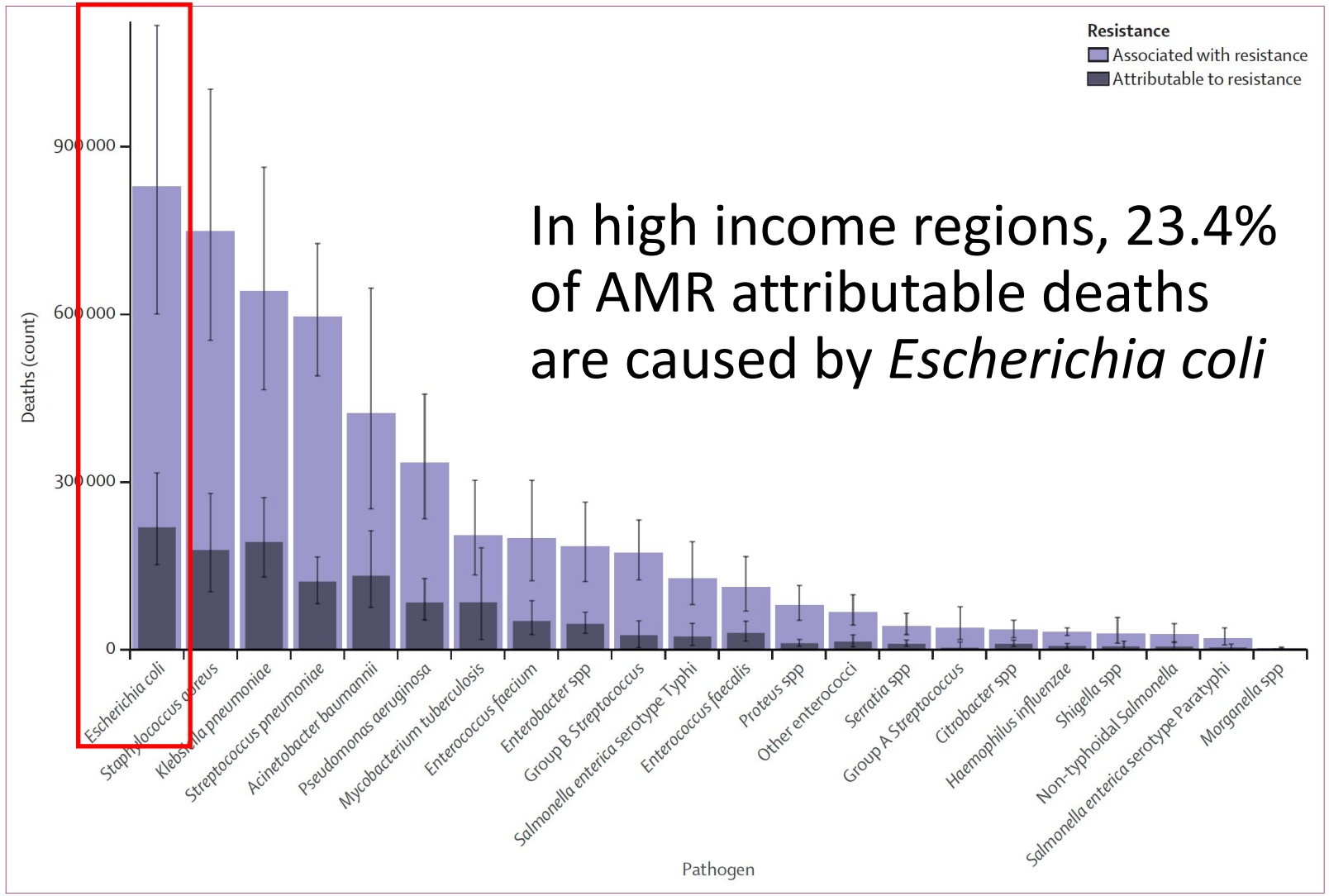
William Shropshire, PhD
wcshropshire@mdanderson.org
MD Anderson Cancer Center
Department of Infectious Diseases
2024-01-18

Mentors:
Samuel Shelburne, MD, PhD, MD Anderson
Yousif Shamoo, PhD, Rice University
Awdhesh Kalia, PhD, MD Anderson

Gulf Coast Consortia
QUANTITATIVE BIOMEDICAL SCIENCES

Training Grant: T32 AI141349
NIAID: R21AI151536

Escherichia coli leading cause of AMR associated mortality

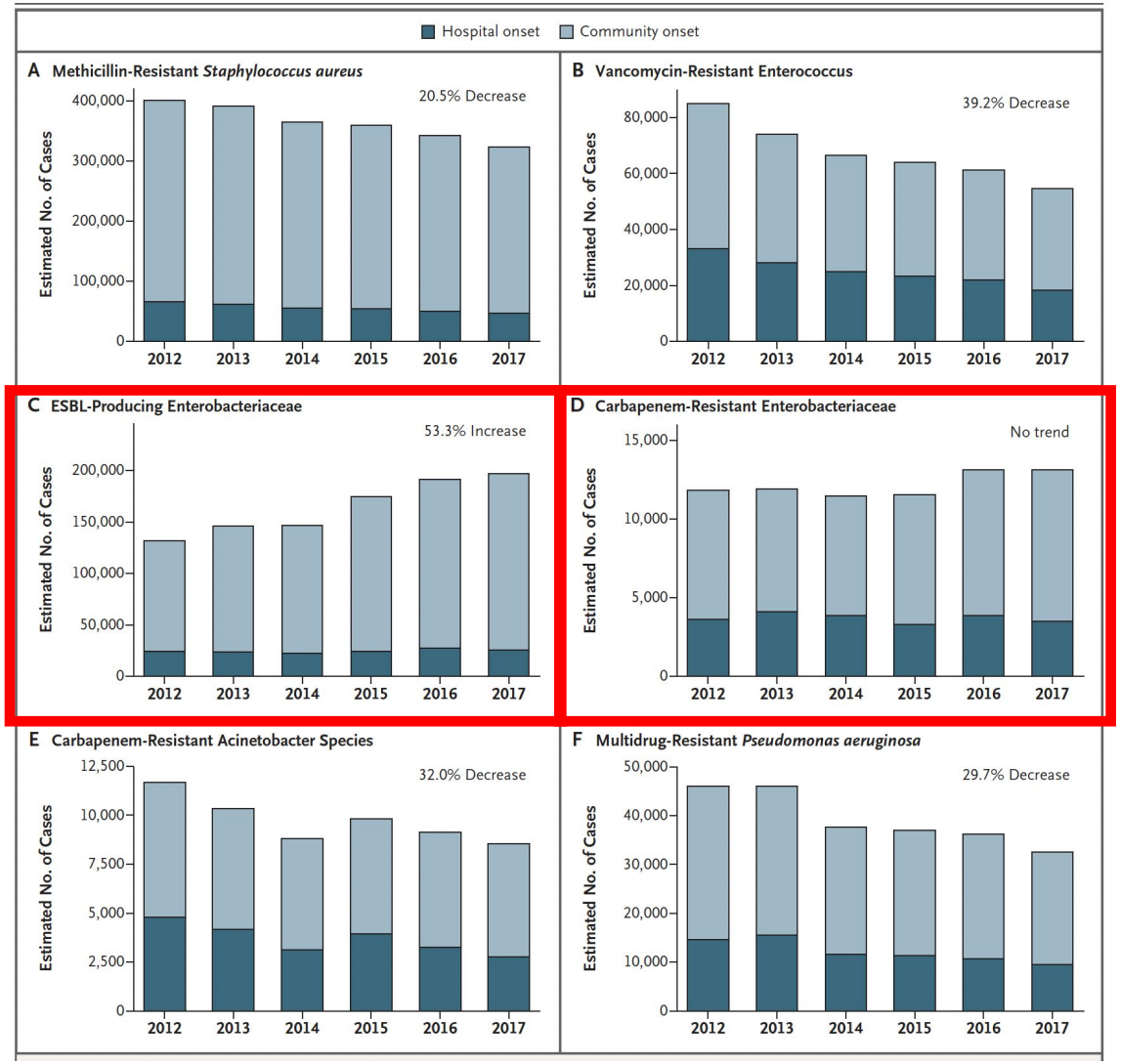


In high income regions, 23.4% of AMR attributable deaths are caused by *Escherichia coli*

THE UNIVERSITY OF TEXAS
MD Anderson Cancer Center
Making Cancer History®

Murray et al., 2022, *Lancet*

E. coli infections increasing in the US



E. coli infections accounted for 87% of the extended-spectrum β-lactamase (ESBL) *Enterobacterales* increase

Recurrent ESBL positive *E. coli* infections often lead to increased carbapenem MICs

Jernigan et al., 2020, *NEJM*

Carbapenems are recommended treatment for complicated ESBL *Escherichia coli* infections

Antibiotic Resistance

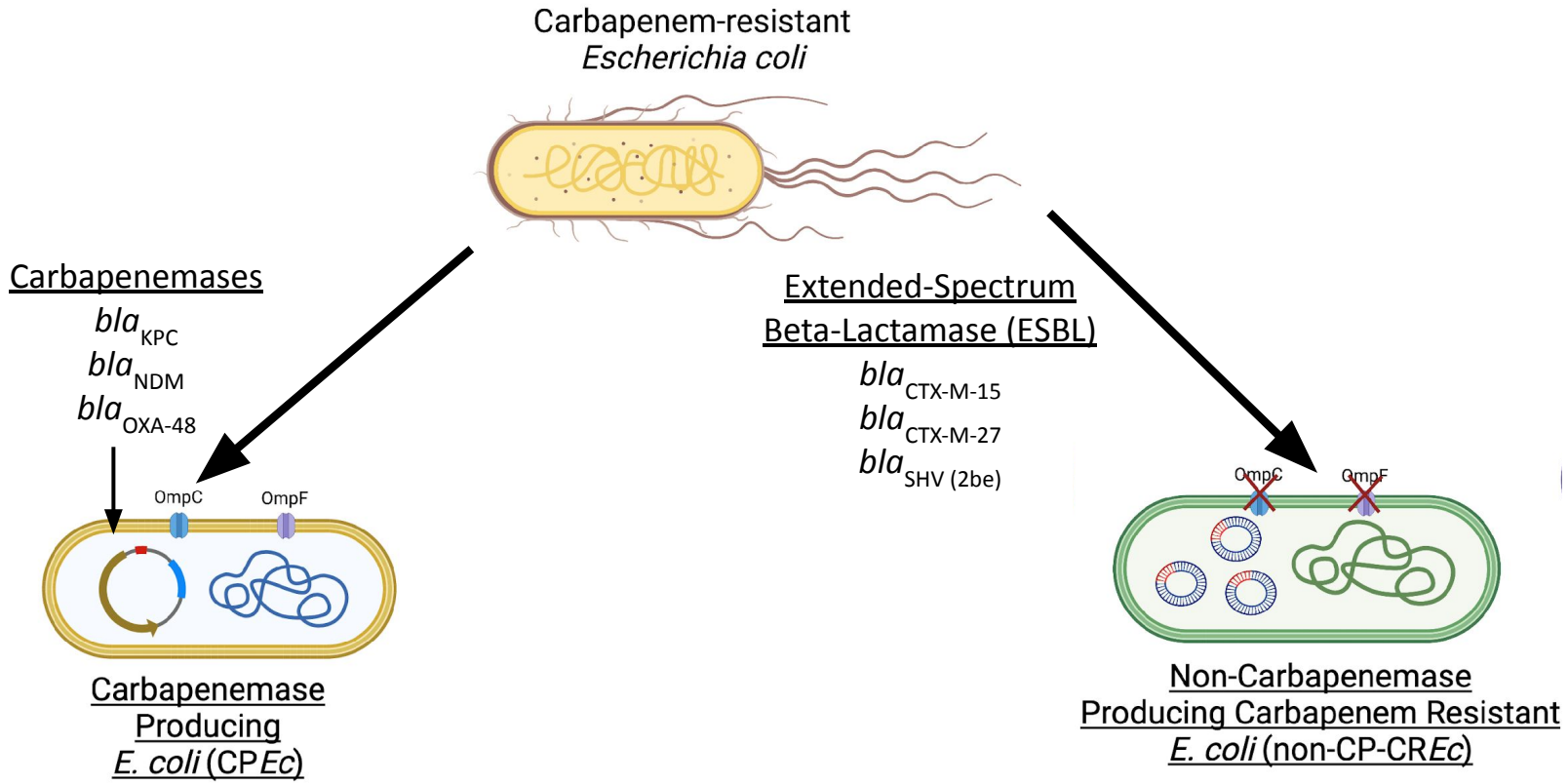
Aminopenicillin

Narrow-spectrum BL/BLIs

Cephalosporins/ Cephamycins

Carbapenems:
Ertapenem (ETP)
Meropenem (MEM)

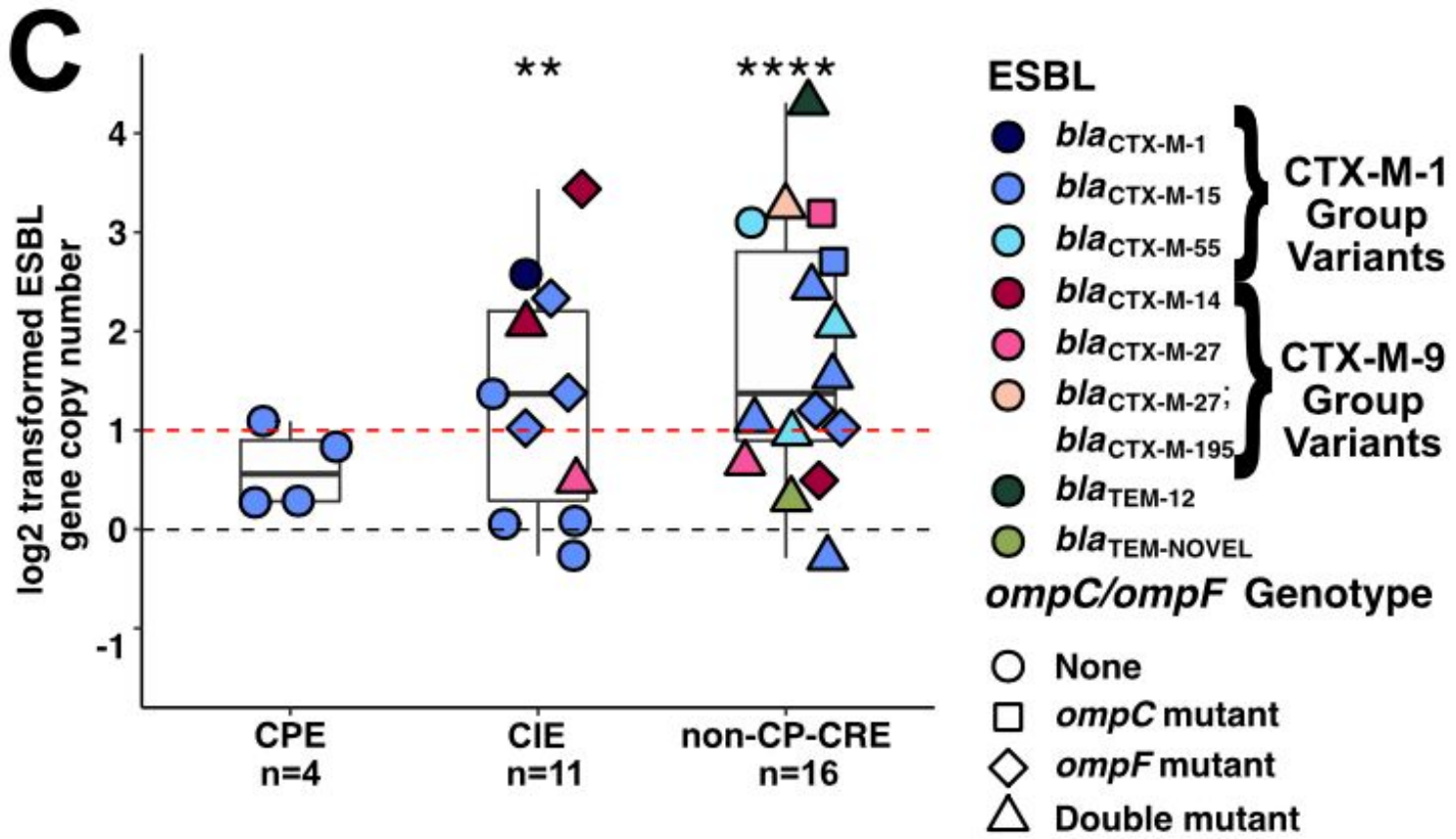
Cefiderocol
Novel BL/BLIs



THE UNIVERSITY OF TEXAS
MD Anderson Cancer Center
Making Cancer History®

ESBL Gene Copy Number Variation (CNV) + Outer Membrane Porin Disruptions Driving Non-CP-CRE

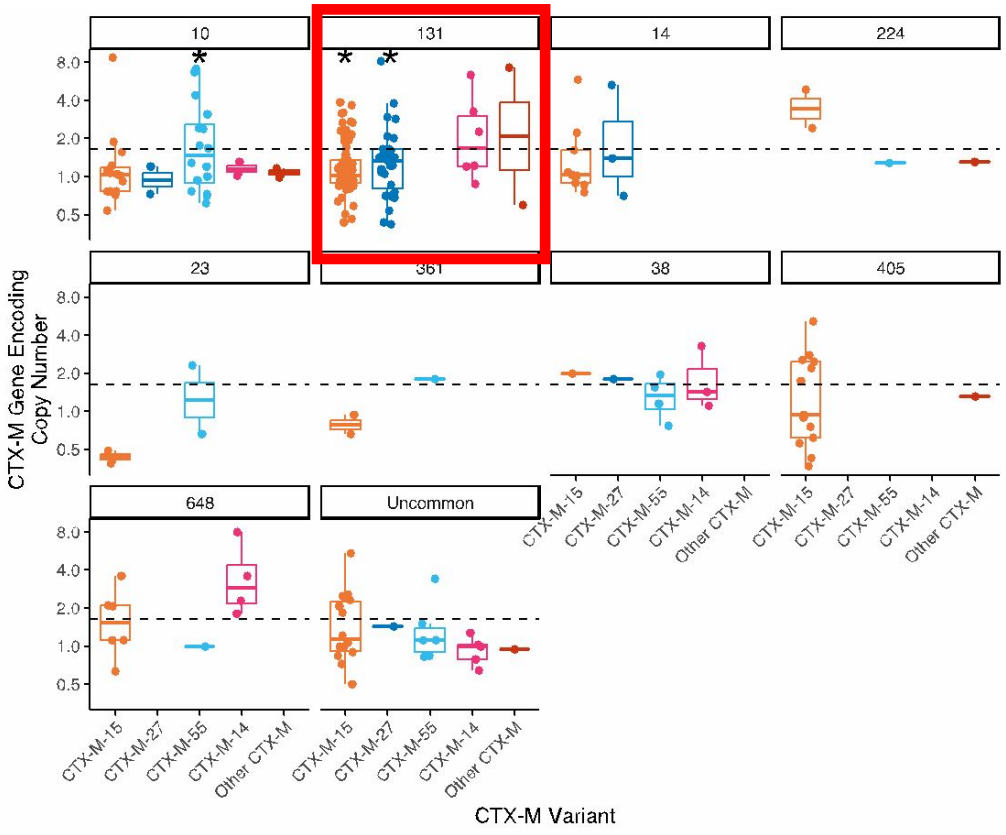
CPE = carbapenemase-producing *Enterobacterales*
CIE = carbapenem intermediate *Enterobacterales*
Non-CP-CRE = Non-carbapenemase-producing carbapenem resistant *Enterobacterales*

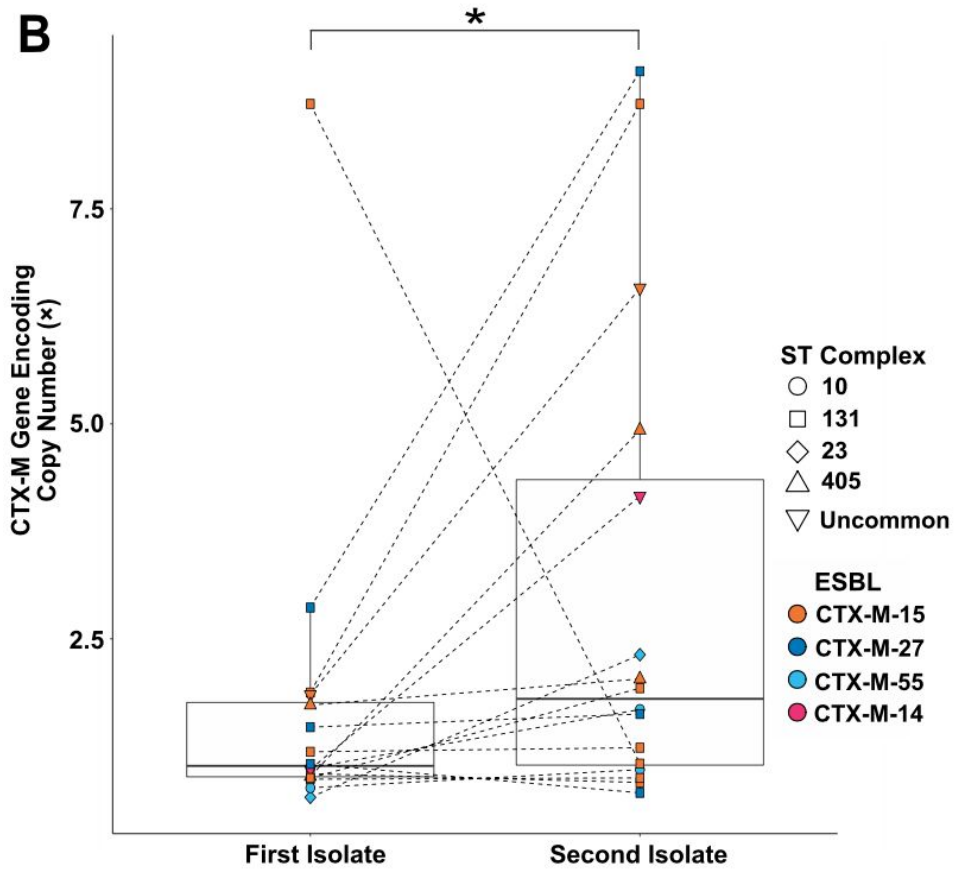


THE UNIVERSITY OF TEXAS MD Anderson Cancer Center
Making Cancer History®

Shropshire et al., 2022, *mSystems*

CTX-M gene amplification associated with bacteremia recurrence

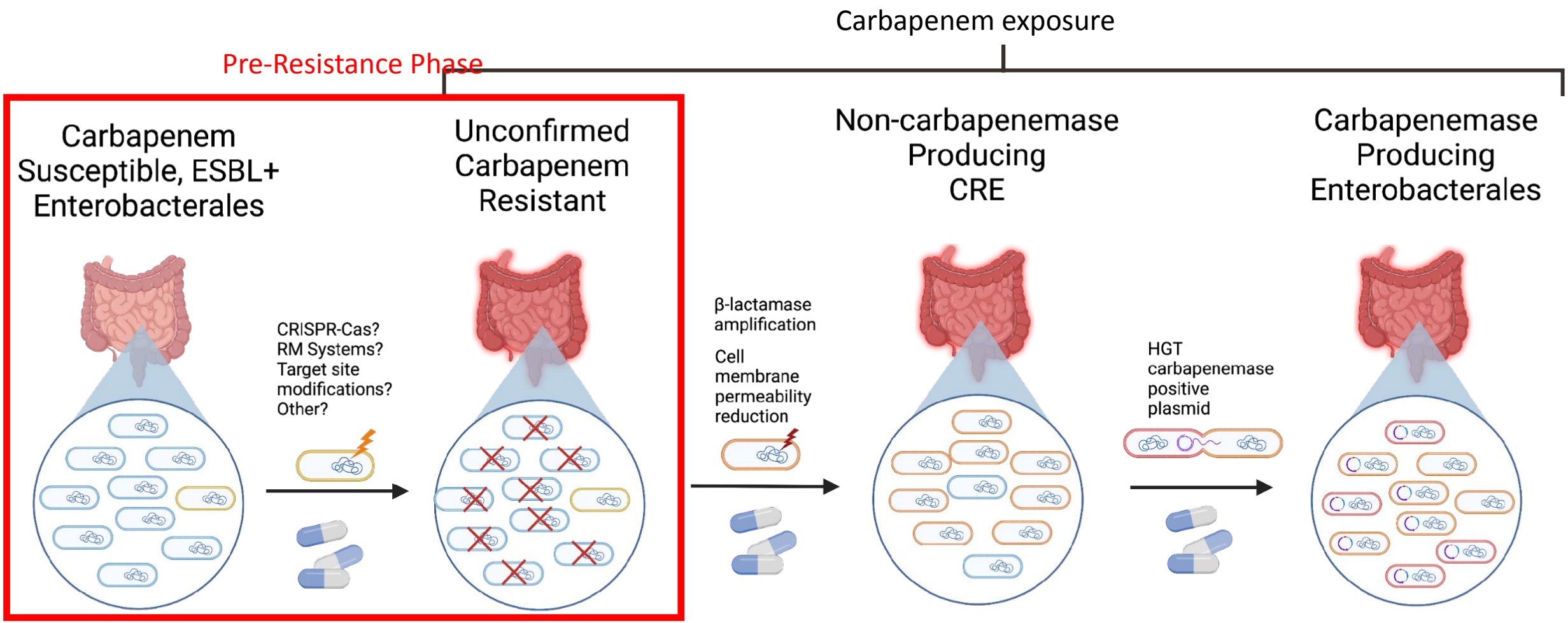




THE UNIVERSITY OF TEXAS MD Anderson Cancer Center
Making Cancer History®

Shropshire et al., 2023, *mSphere*

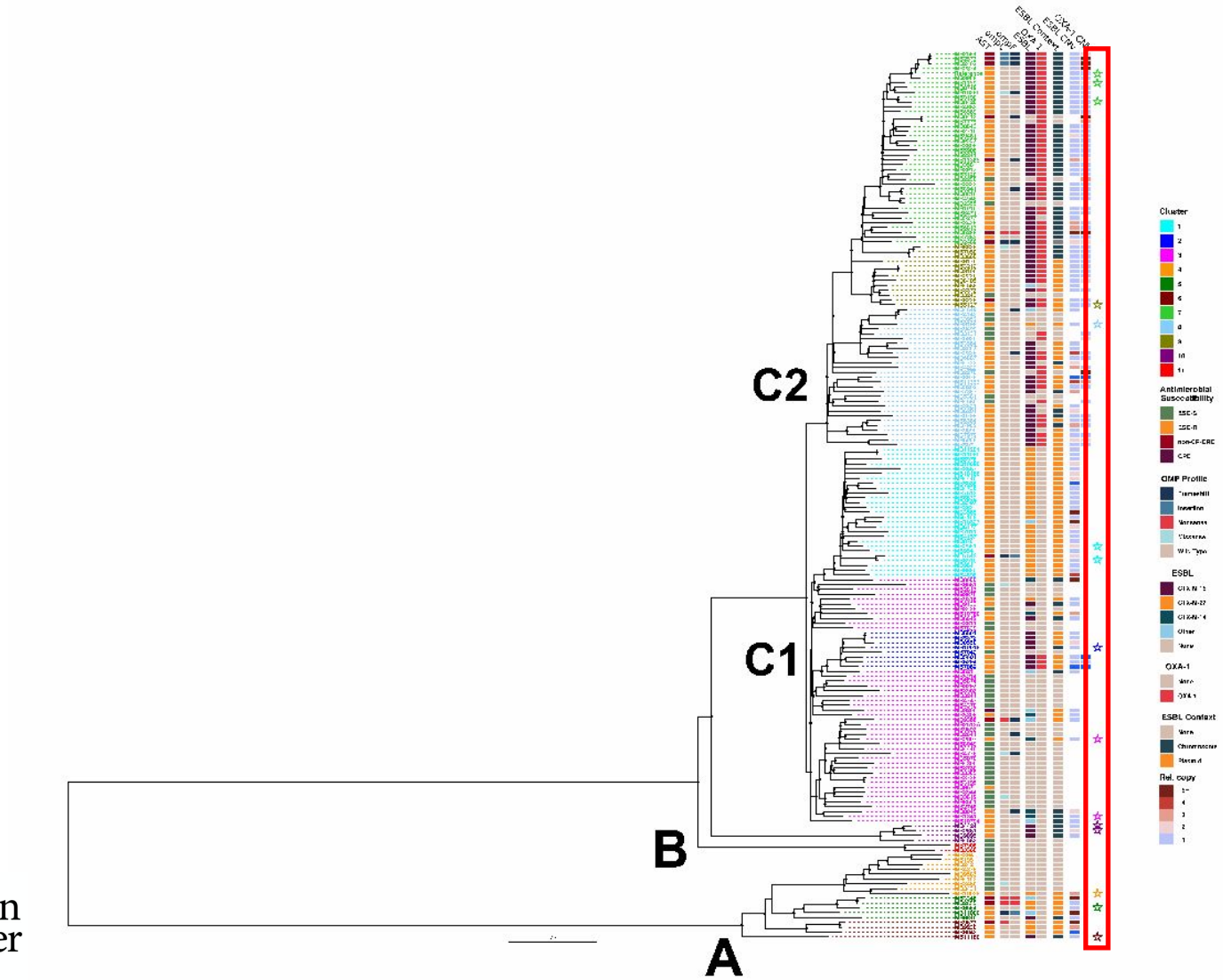
Working Model for Carbapenem Resistance Development

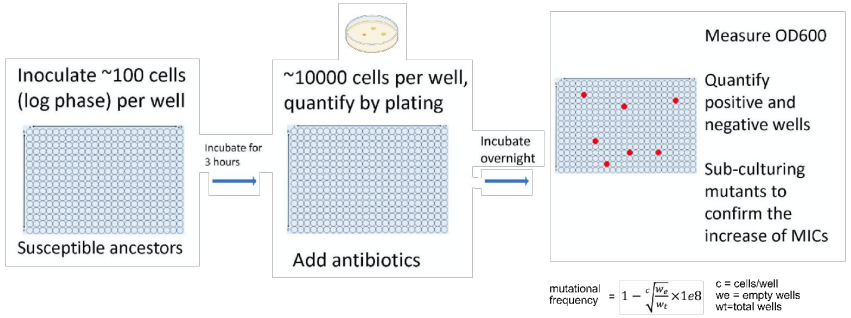


THE UNIVERSITY OF TEXAS MD Anderson Cancer Center
Making Cancer History®

Created with Biorender

Characterization of ST131 subclade capacity to develop carbapenem resistance





Adapted from Ma et al. 2022 *eLife*

Shropshire et al., 2022, *JAC*
Shropshire et al., 2022, *mSystems*
Shropshire et al., 2023, *mSphere*
Shropshire et al., 2023, *Micro*

THE UNIVERSITY OF TEXAS
MD Anderson Cancer Center
Making Cancer History®

Diverse array of ST131 subclones harboring ESBL genes can develop *in vitro* carbapenem resistance

Carbapenem Fluctuation Assay Results for Select ST131 ESBL Positive Strains

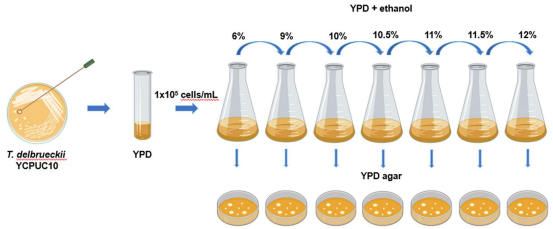
					Ertapenem (ETP) Fluctuation Assay Results				Meropenem (MEM) Fluctuation Assay Results			
Sample	Clade (sub-level)	ESBL	ESBL Context	P_0 ESBL CNV	P_0 ETP MIC (μg/mL)	P_0 Mut Freq Mut/10^8 cells	P_1 ETP MIC (μg/mL)	P_1 ETP Fold Change (×)	P_0 MEM MIC (μg/mL)	P_0 Mut Freq Mut/10^8 cells	P_1 MEM MIC (μg/mL)	P_1 MEM Fold Change (×)
MB2951	C1-M27 (1)	CTX-M-27	plasmid	1.67	0.088	6874	1.04	11.8	0.06	NA	NA	NA
MB6910	C1-M27 (1)	CTX-M-27	plasmid	1.47	0.063	749	0.32	5.2	0.03	88	NA	NA
MB10016	C1 (2)	CTX-M-15	chromosome	1.01	0.125	106	0.92	7.3	0.04	NA	NA	NA
MB1341	C1 (3)	CTX-M-14	chromosome	1.13	0.25	998	0.71	2.8	0.125	NA	NA	NA
MB2007	C1 (3)	CTX-M-14	plasmid	1.23	0.088	117	0.32	3.7	0.04	69	NA	NA
MB10029	A (4)	CTX-M-27	plasmid	2.97	0.088	12854	0.26	3	0.04	381	0.06	1.4
MB6054	A (5)	CTX-M-27	plasmid	1.42	0.063	564	0.46	7.3	0.06	NA	NA	NA
MB11180	A (6)	CTX-M-15	chromosome	0.93	0.5	967	1.54	3.1	0.06	NA	NA	NA
MB1159	C2 (7)	CTX-M-15	chromosome	1.06	0.13	1368	0.92	7.3	0.09	NA	NA	NA
MB1860	C2 (7)	CTX-M-15	chromosome	0.93	0.21	2242	1.3	6.2	0.04	97	0.1	2.5
MB8420	C2 (7)	CTX-M-15	chromosome	0.85	0.3	2031	1	3.4	0.125	NA	NA	NA
MB3196	C2 (8)	CTX-M-27	plasmid	0.76	0.063	106	0.52	8.4	0.03	130	NA	NA
MB5127	C2 (9)	CTX-M-15	plasmid	1.37	0.6	1149	0.77	1.3	0.06	185	NA	NA
MB2681	B0 (10)	CTX-M-15	chromosome	1.69	0.3	2879	0.52	1.8	0.06	261	NA	NA

P_0 = ancestral clinical strain; P_1 = mutant progeny strain

- ESBL- isolates have mutation frequencies <LOD
- Positive correlation (r = 0.87, *P*<1e-5) ESBL gene amplification and ETP mutation frequency
- Median ETP Mutation Frequency Greater than MEM (1073 v 130 mutants/10^8 Cells; *P*=0.004)

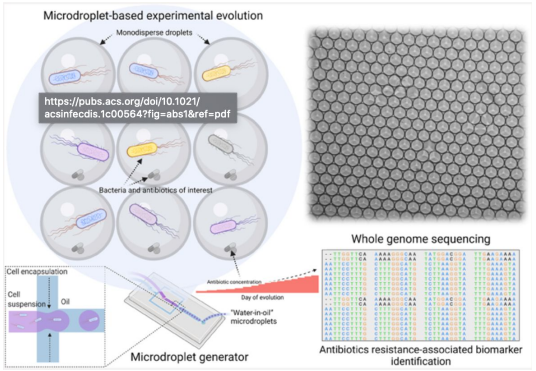
THE UNIVERSITY OF TEXAS
MD Anderson Cancer Center
Making Cancer History®

Distinct Experimental evolutionary platforms to elucidate evolutionary trajectories

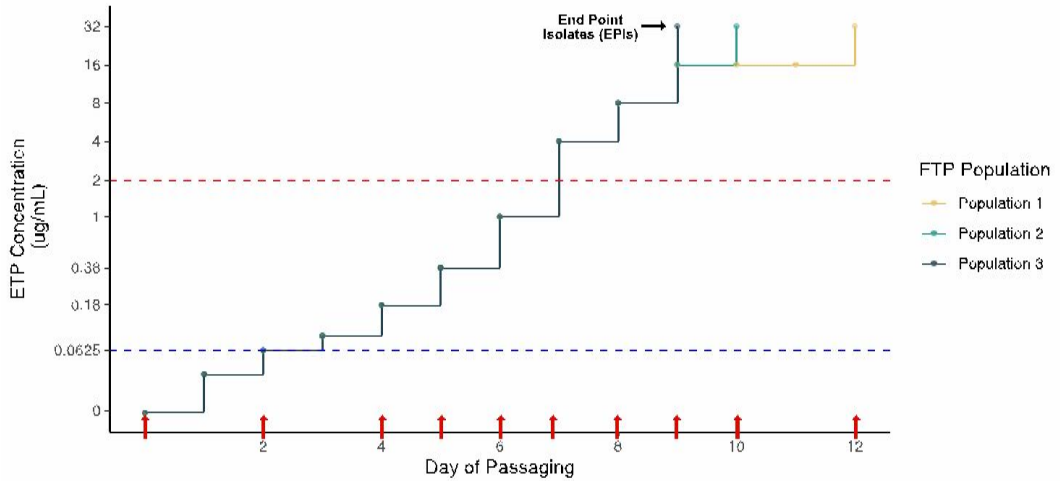


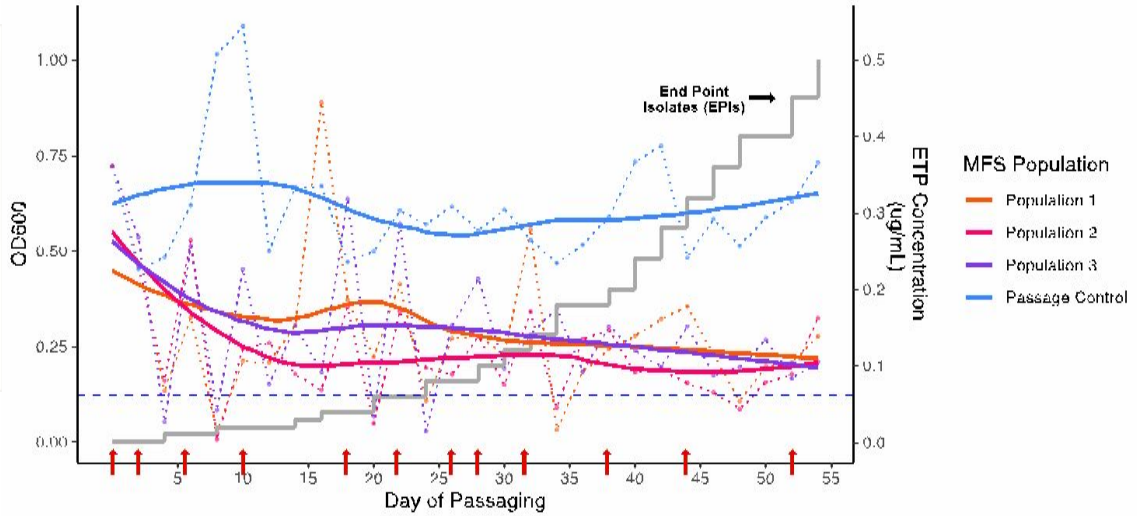
Catrileo et al., 2022, *Front Microbiol*

Blue line = minimum inhibitory concentration (MIC)
Red line = ETP-R breakpoint



Seo et al., 2021, *ACS*

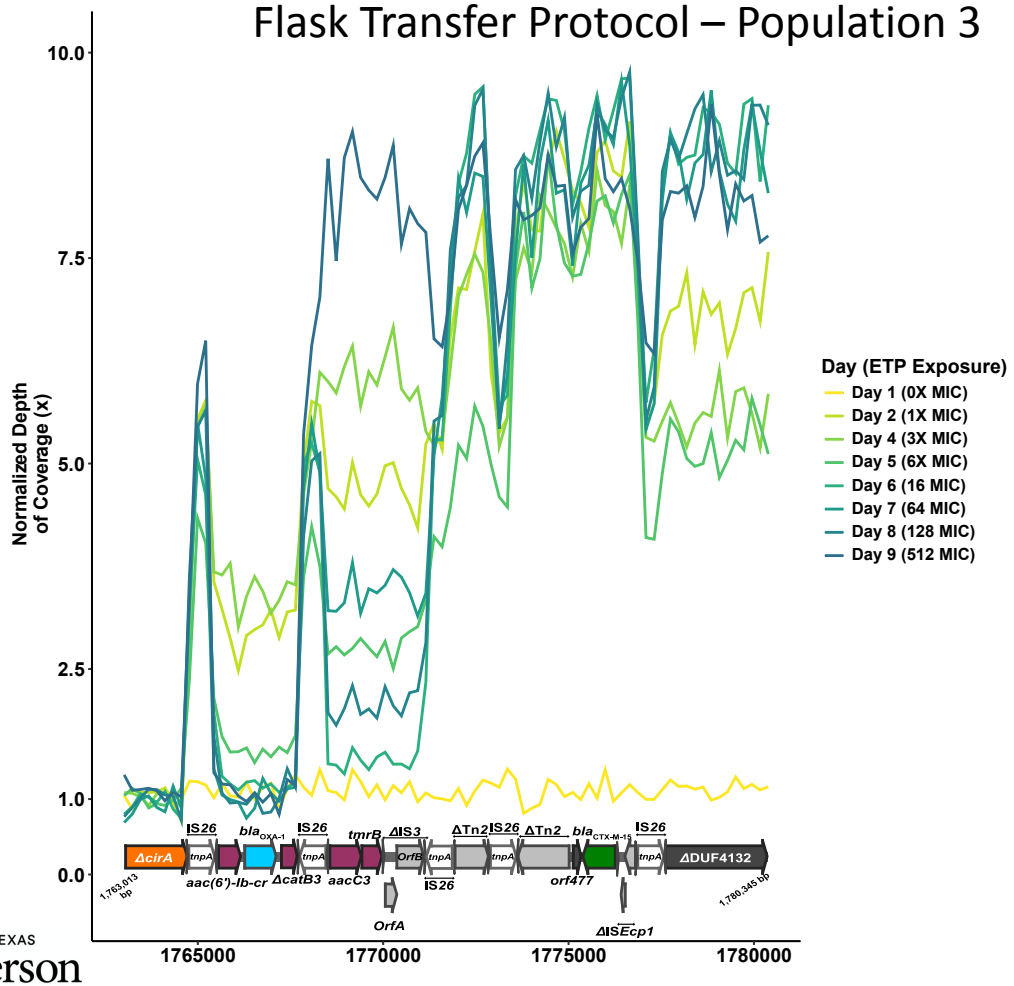


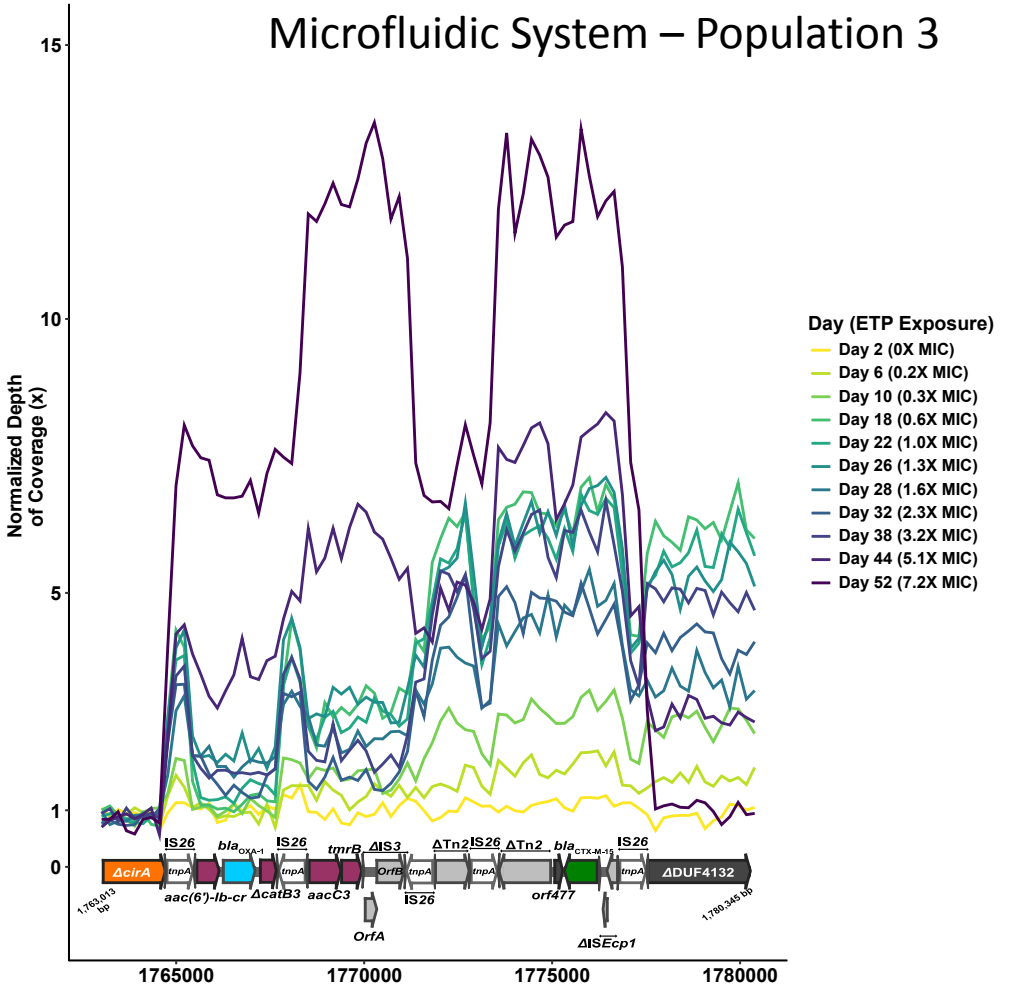


1. Evolution is ‘slowed down’ in MFS
2. Stepwise selection of ETP mutants is stronger in FTP vs MFS

THE UNIVERSITY OF TEXAS
MD Anderson Cancer Center
Making Cancer History®

Beta-lactamase amplification occurs during early ETP exposures

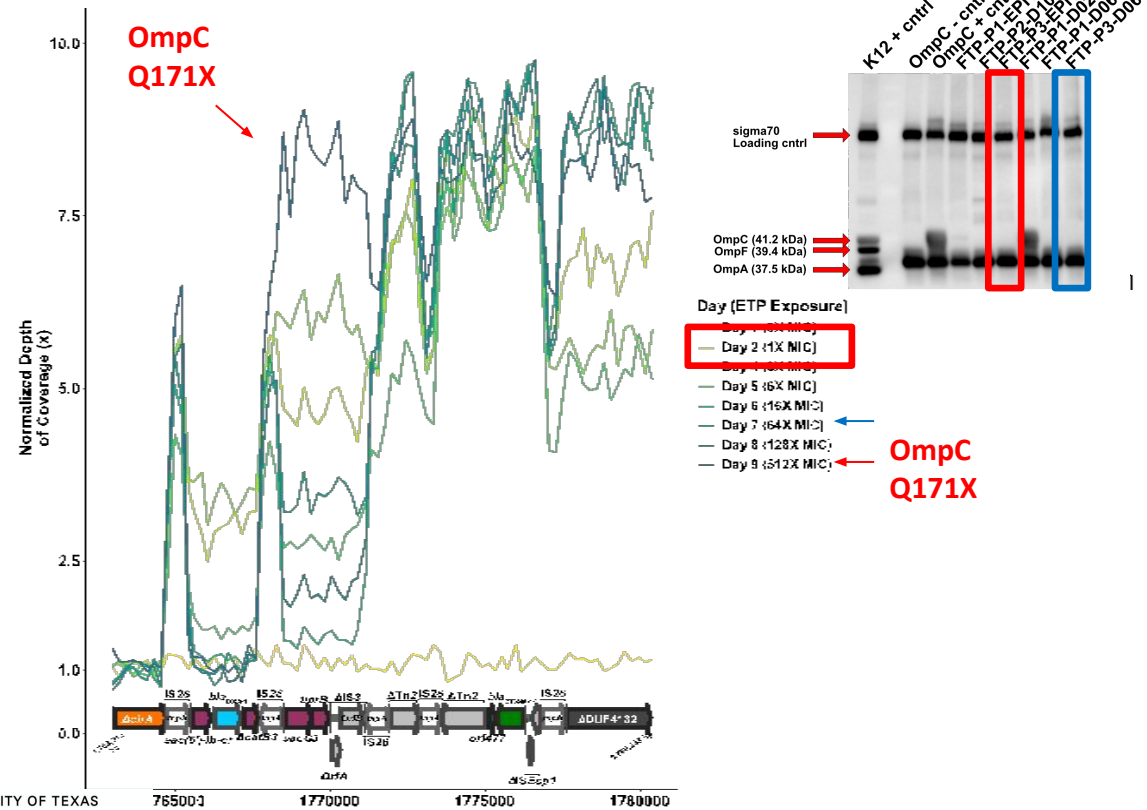




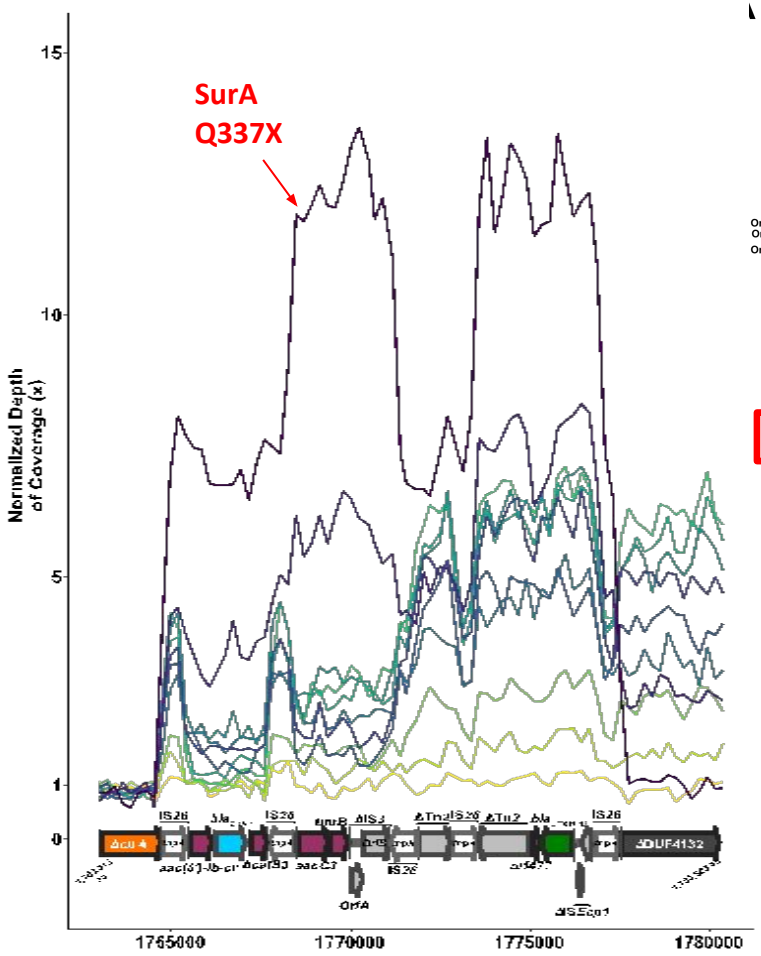
THE UNIVERSITY OF TEXAS
MD Anderson Cancer Center
Making Cancer History®

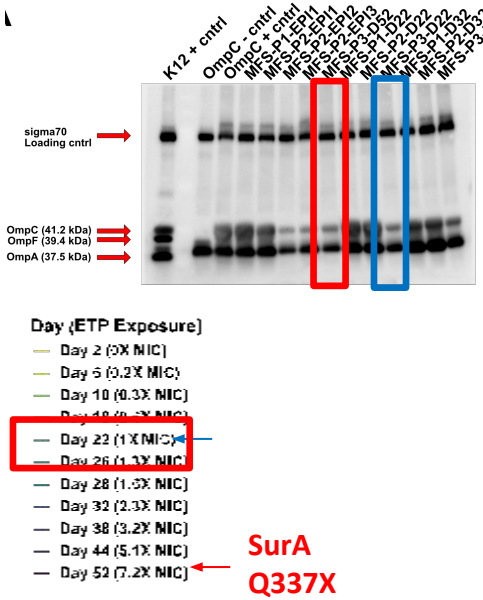
Longitudinal comparison of copy number variation and outer membrane porin profiling between FTP and MFS

Flask Transfer Protocol – Fast Evolution



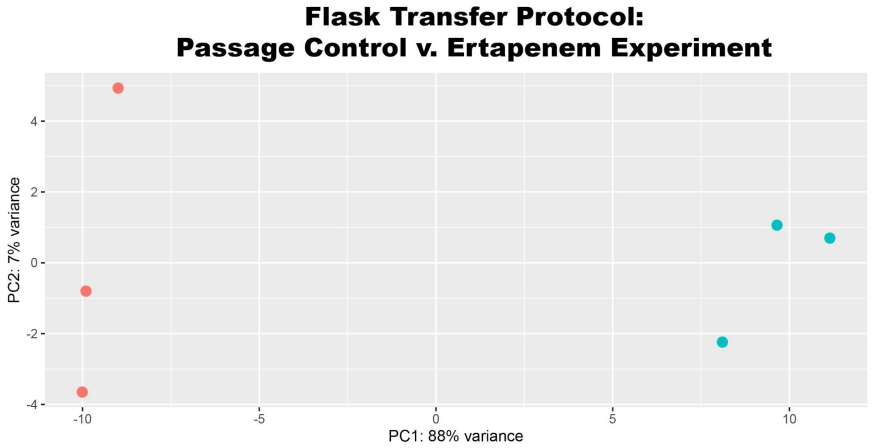
Microfluidic System – Slow evolution

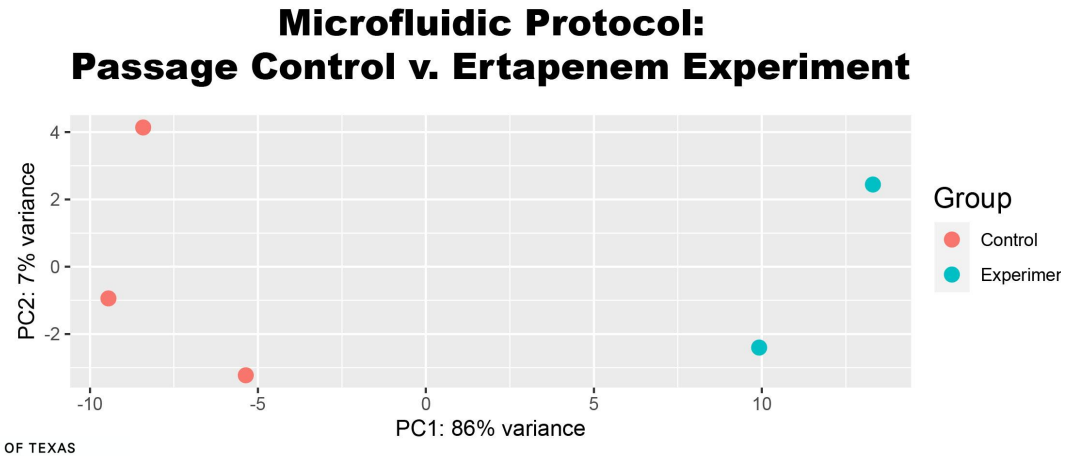


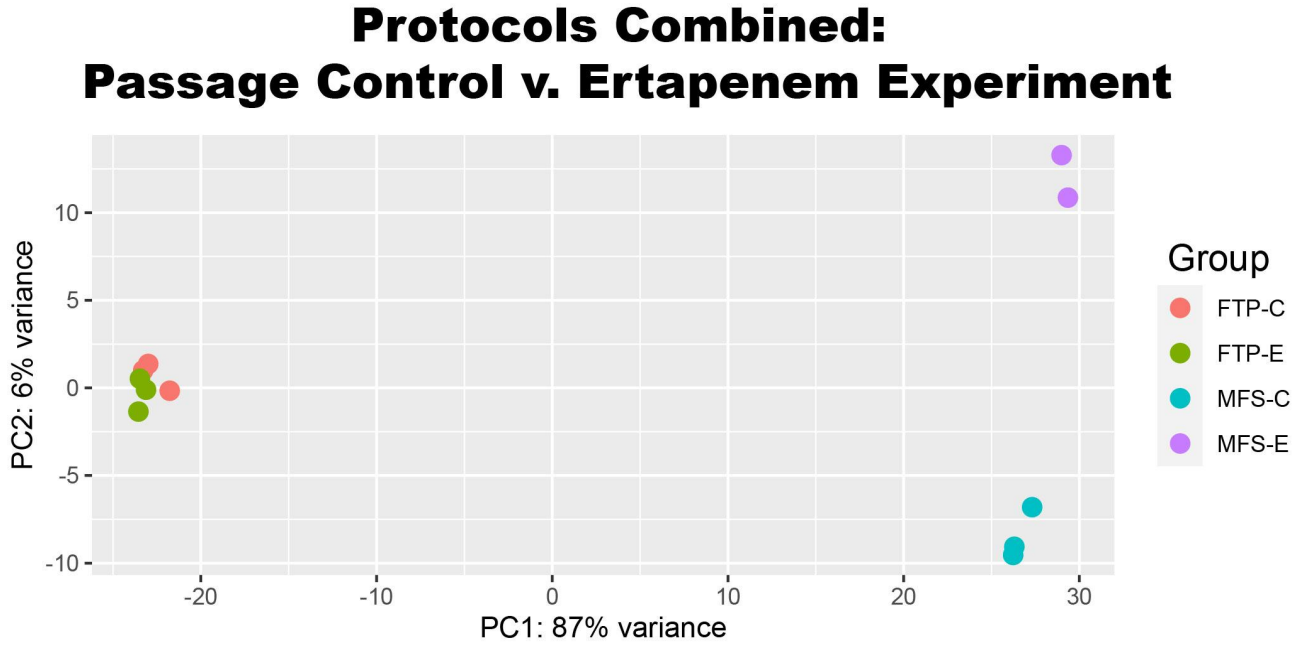


THE UNIVERSITY OF TEXAS
MD Anderson Cancer Center
Making Cancer History®

Transcriptomic Data Reveals Differential Expression Patterns Across Experimental Evolutionary Platforms

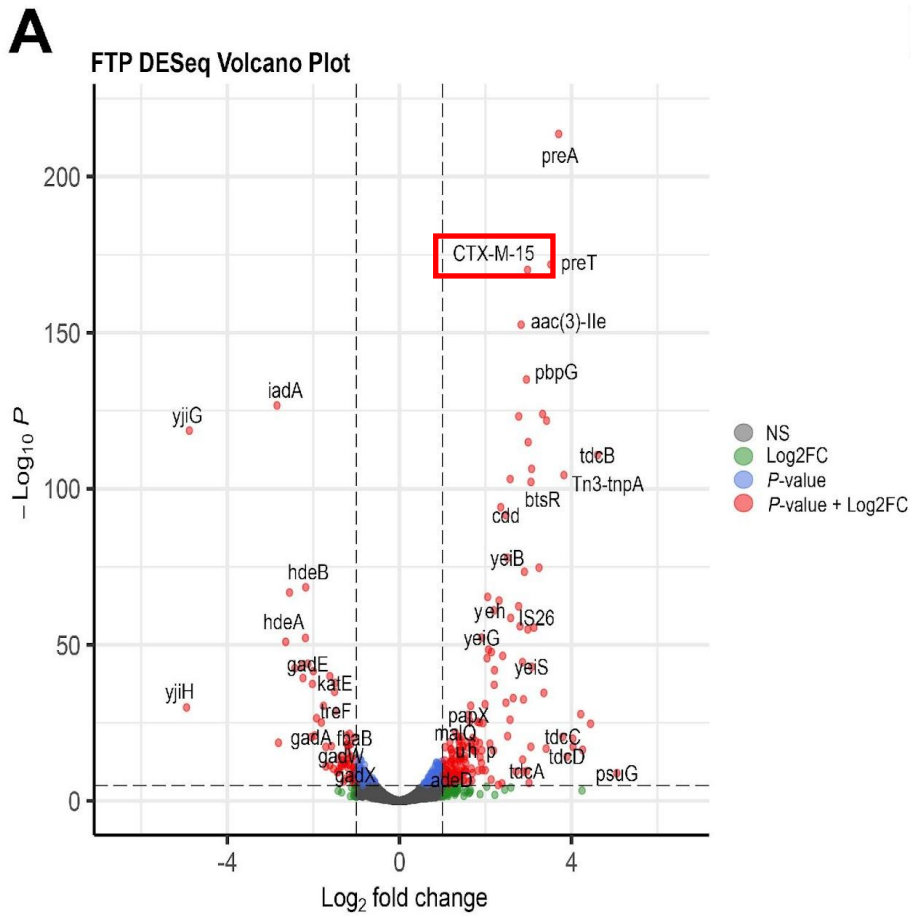


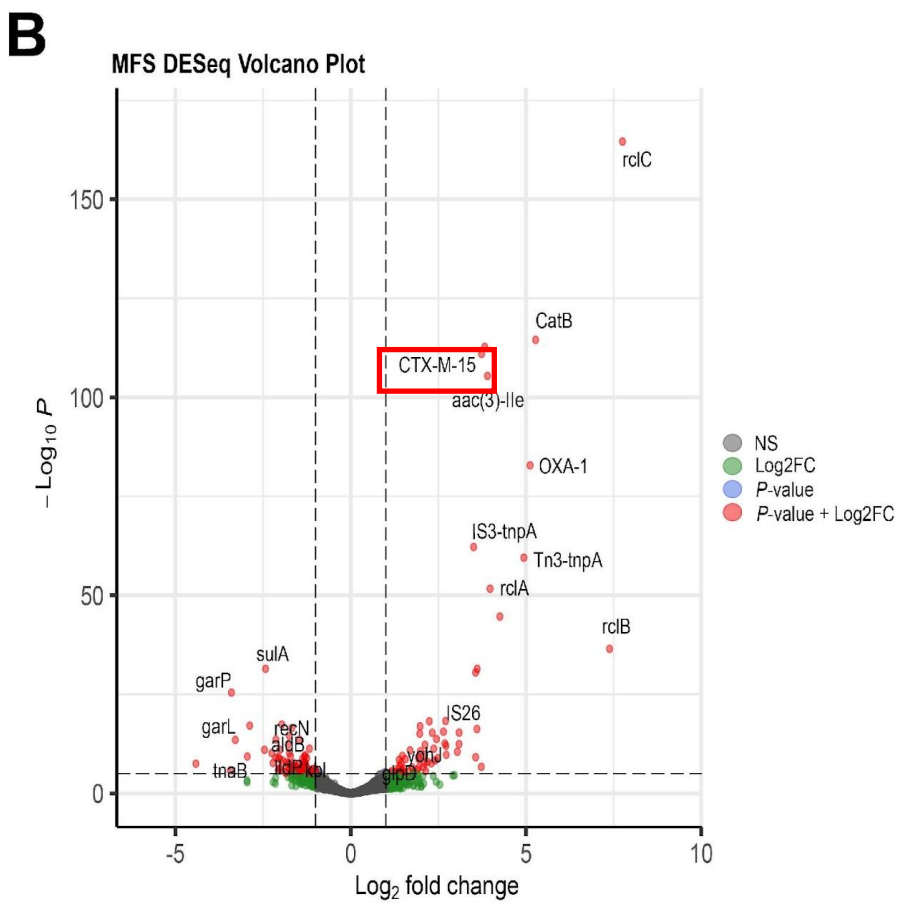




THE UNIVERSITY OF TEXAS
MD Anderson Cancer Center
Making Cancer History®

Both platforms have increased expression of AMR genes and transposases

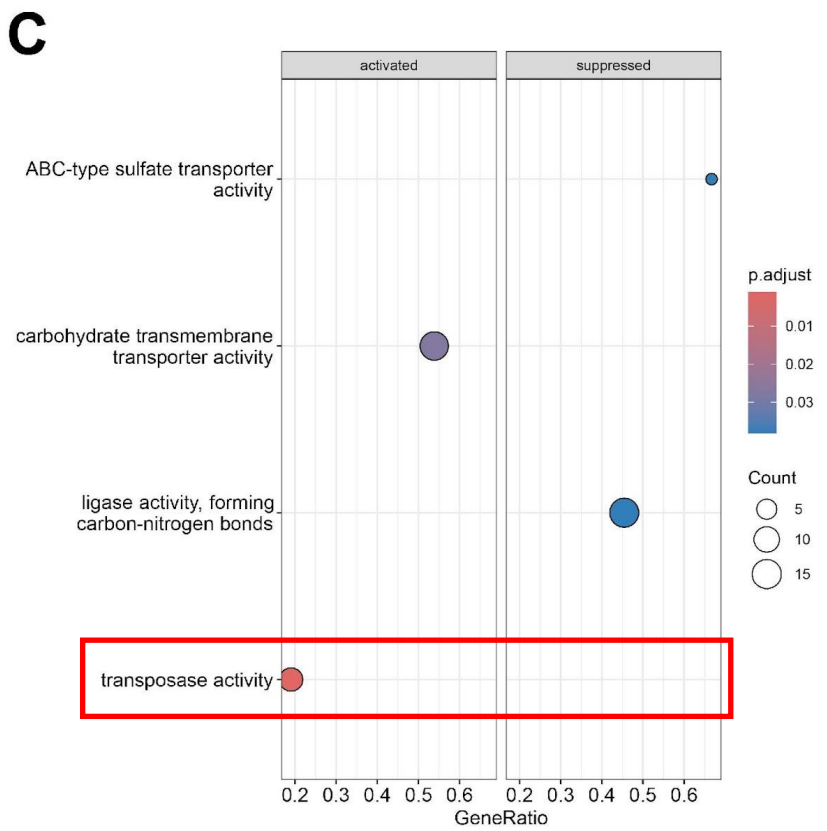




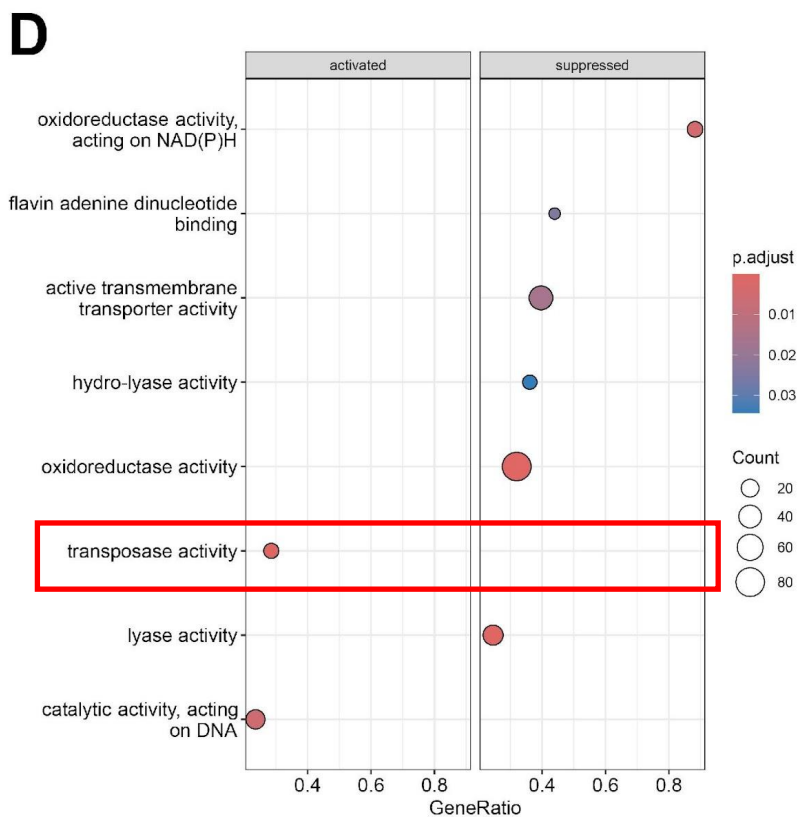
THE UNIVERSITY OF TEXAS
MD Anderson Cancer Center
Making Cancer History®

Both platforms have increased expression of AMR genes and transposases

FTP – Fast Evolution



MFS – Slow Evolution



THE UNIVERSITY OF TEXAS
MD Anderson Cancer Center
Making Cancer History®

Conclusions/Future Directions

- ESBL positive ST131 *E. coli* can develop positive carbapenem MIC shifts across multiple cladal backgrounds.
- Amplification of beta-lactamase genes via increased transposase activity is initial adaptation to carbapenem selective pressure.
- Characterize tolerance and heteroresistance across these ESBL positive ST131 populations.
- The goal is to extend these studies to patients colonized with high-risk *E. coli* undergoing carbapenem therapy to better understand recurrence risk.

Acknowledgements

Shelburne Lab
Sam Shelburne, MD, PhD
Sruti DebRoy, PhD
Chioma Odo
Nicola Horstmann, PhD
Past Members
Jordan Bremer
Chau Tran, PhD
Pranoti Saharsbhojane

Shamoo Lab
Yousif Shamoo, PhD
Xinghao Song, PhD
Seokju Seo, PhD

MDACC SHP
Awdhesh Kalia, PhD
Chin-Ting Wu

Konovalova Lab
Anna Konovalova, PhD
Susana Rodriguez, PhD

Arias Lab
Cesar Arias, MD, PhD
An Dinh, MS
Haley Greenia
Alex Deyanov

Hanson Lab
Blake Hanson, PhD

Others
Micah Bhatti, MD
Samuel Aitken, PharmD
Patrick McDaneld, PharmD
Yohei Doi, MD, PhD
William Miller, MD
Selva Selvaraj Anand, MS

Gulf Coast Consortia
QUANTITATIVE BIOMEDICAL SCIENCES

NIH National Institute of Allergy and Infectious Diseases

Funding:

(1) Training Program in Antimicrobial Resistance (TPAMR); T32 AI141349

(2) National Institute of Allergy and Infectious Diseases (NIAID); R21AI151536

Poster #:
71