

1 **Supplementary Information**

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3 **In-patient evolution of a high-persister *Escherichia coli* strain with reduced in-vivo**
4 **antibiotic susceptibility**

5 Joshua B. Parsons^{1,2*}, Ashelyn E. Sidders², Amanda Z. Velez², Blake M. Hanson³, Michelle
6 Angeles-Solano², Felicia Ruffin¹, Sarah E. Rowe², Cesar A. Arias^{4,5}, Vance G. Fowler Jr¹, Joshua
7 T. Thaden¹, Brian P. Conlon^{2*}

8 * Co-corresponding author

9 Corresponding Authors: Joshua B. Parsons and Brian P. Conlon

10 **Email:** joshua.parsons@duke.edu (JBP), brian_conlon@med.unc.edu (BPC)

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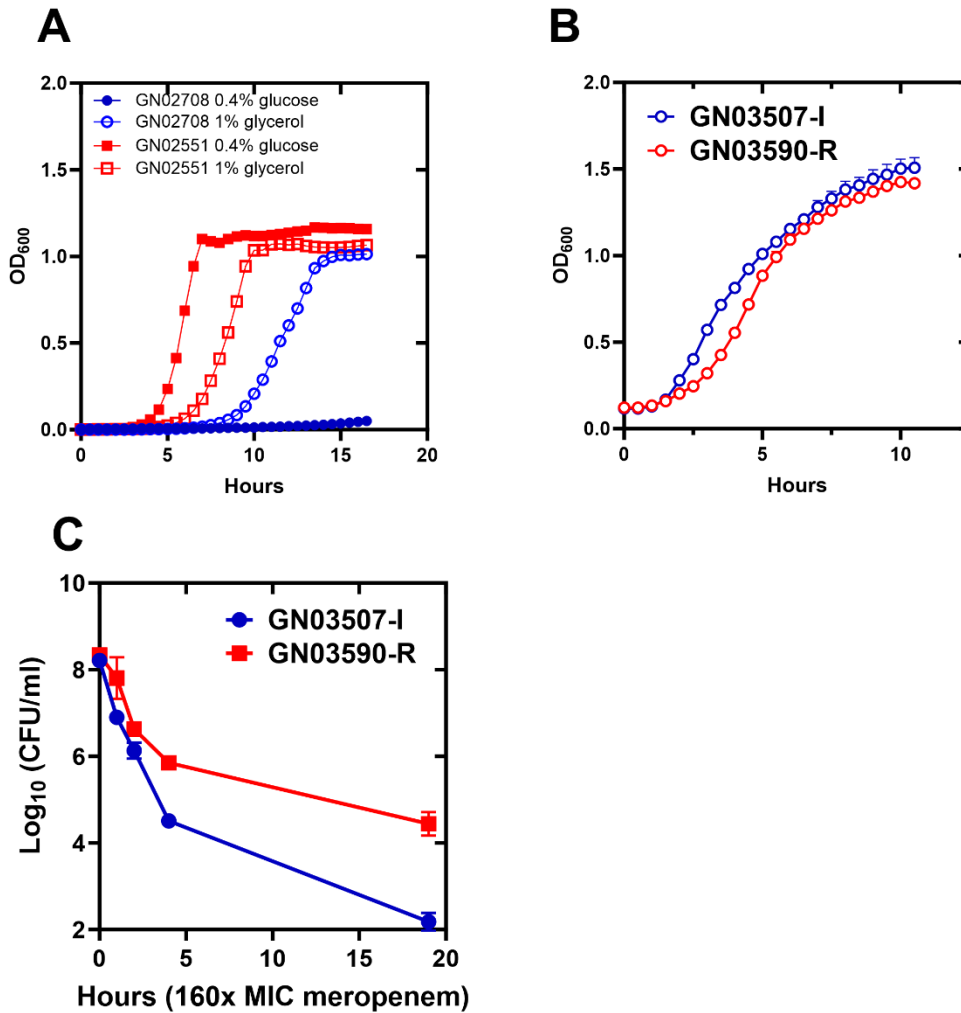
- 13 • Figures S1-S4
- 14 • Tables S1-S7
- 15 • SI References

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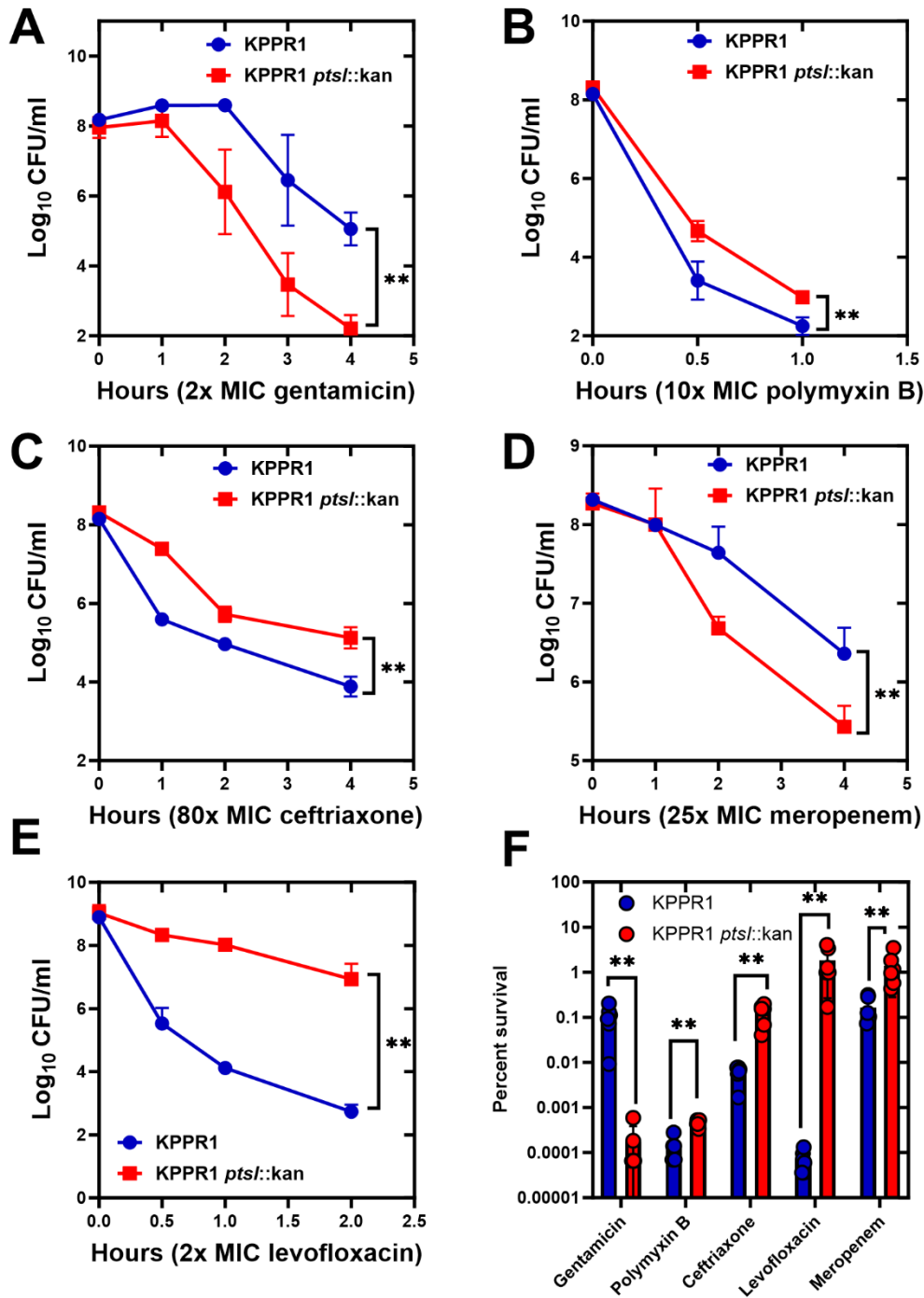
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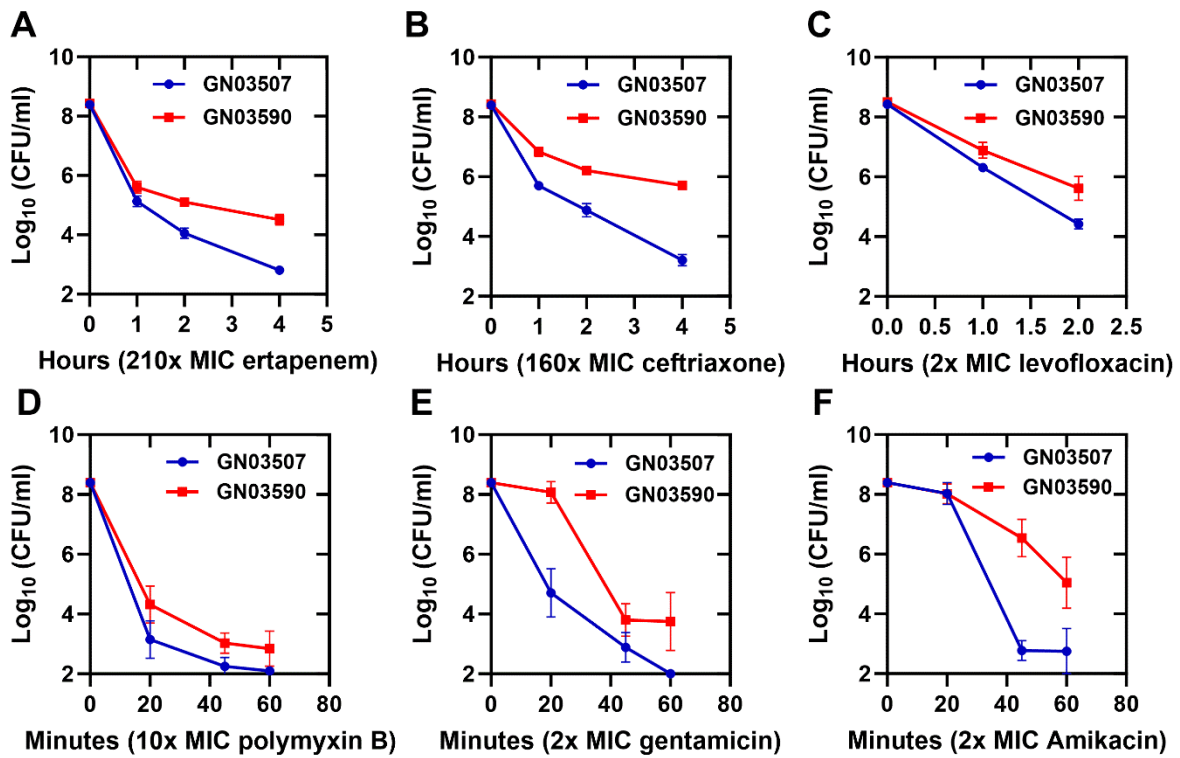
Supplementary Figure S1: A. *K. pneumoniae* *pfkA* mutant can no longer utilize glucose as sole carbon source. Growth of *Klebsiella pneumoniae* initial (GN02551) and relapsed (GN02708) isolate with *pfkA* frameshift. **B. Growth of GN03507 and GN03590 in Luria-Broth media.** **C. High persister phenotype of GN03590-R is not due to prolonged lag phase.** We sought to minimize residual stationary phase cells by performing the persister assay using a log-phase inoculum. Overnight cultures of GN03507-I and GN03590-R were diluted 1:1000 into fresh LB and grown to OD₆₀₀ = 1.0. These cultures were further backdiluted 1:100 to OD₆₀₀ = 0.01 to minimize residual stationary phase cells from the initial inoculum. Cultures then grown from OD₆₀₀ = 0.01 to OD₆₀₀ = 0.4 before addition of 160x MIC meropenem. Data are summary of six biological replicates. Error bars represent standard deviation.

53 **Supplementary Figure S2: PtsI deficiency in *Klebsiella pneumoniae* KPPR1 confers multidrug tolerance.**

54 Panels A-E: Time-kill curves measuring persister formation with different antibiotics. Panel F. Summary

55 of panels A-E. Gentamicin used at 2x MIC for 4 hours, Polymyxin B 10x MIC for 1 hour, ceftriaxone 80x

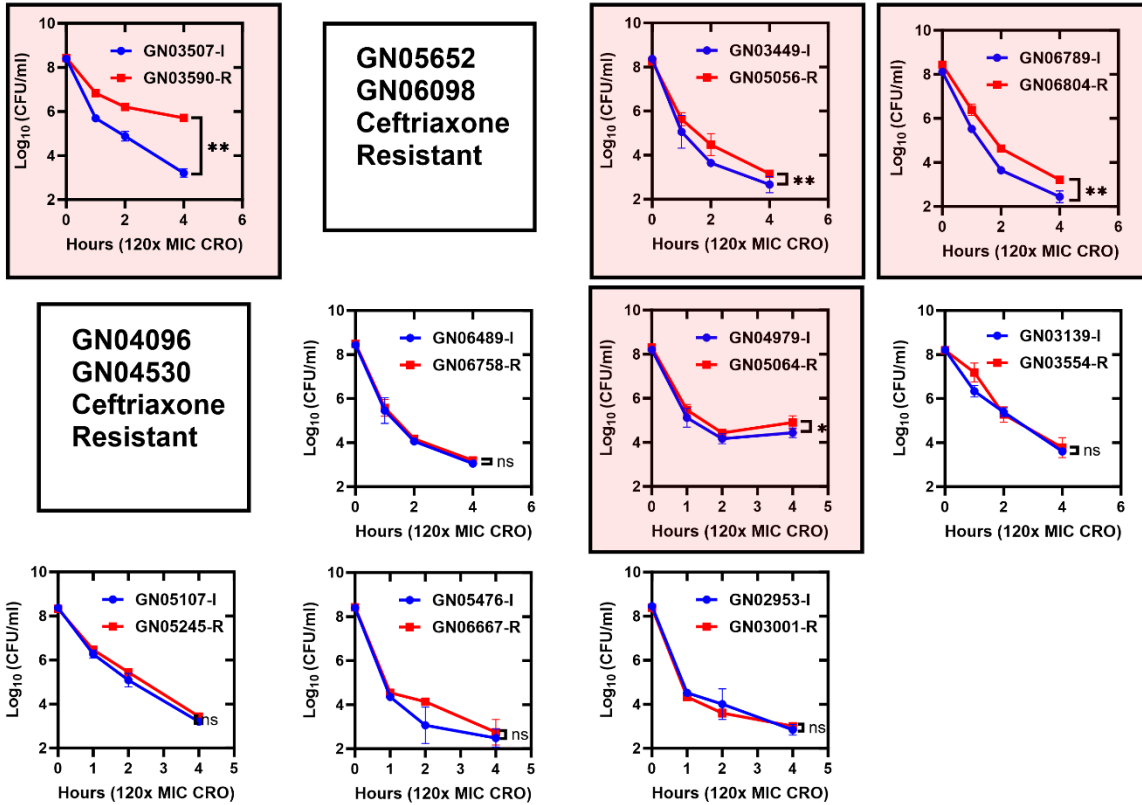
56 MIC for 4 hours, levofloxacin 2x MIC for 2 hours, meropenem 25x MIC for 4 hours. Statistical significance
57 was determined by Mann-Whitney test. Results considered significant if $p < 0.05$.



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59 **Supplementary Figure S3: Time-kill curves of GN03507 and GN03590 exposed to different antibiotics.**
60 X-axis represents the time exposed to each antibiotic.

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63 **Supplementary Figure S4: Ceftriaxone persister screen of initial and relapsed *E. coli* isolates.** Screen
 64 performed with 120-200x MIC ceftriaxone (CRO) added to mid-exponential *E. coli* grown in LB broth. At
 65 specified time point, aliquot removed, washed and enumerated. Isolate pairs highlighted in red show
 66 increased persisters, isolate pair in green with reduced persisters. Two isolate pairs (GN04096-GN04530
 67 and GN05652-GN06098) with one of both isolates resistant to ceftriaxone were excluded. Data are
 68 summary of six biological replicates. Error bars represent standard deviation. Significance determined by
 69 Mann-Whitney test of the 4 hour or 22 hour time point (significance level: $p < 0.05$).

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79 Table S1: Non-synonymous SNPs arising between initial and relapsed *E. coli* isolates

Initial Isolate	Recurrent Isolate	Species	Mutation type	Gene	Product	Amino acid Substitution	Function
GN06789	GN06804	<i>E. coli</i>	Missense	<i>pfkB</i>	ATP-dependent 6-phosphofructokinase isozyme 2	M225L	CHO metabolism
			Missense	<i>ompR</i>	Transcriptional regulatory protein OmpR	K88Q	Drug resistance
			Frameshift	<i>pal</i>	Peptidoglycan-associated lipoprotein	L9fs	Virulence
GN05652	GN06098	<i>E. coli</i>	Missense	<i>gpml</i>	2,3-bisphosphoglycerate-independent phosphoglycerate mutase	G410V	CHO metabolism
			Missense	<i>mtl</i>	PTS system mannitol-specific EIICB component	A122V	CHO metabolism
			Frameshift	<i>mnmC</i>	tRNA 5-methylaminomethyl-2-thiouridine biosynthesis bifunctional protein MnmC	S30fs	Amino acid/Protein synthesis
			Missense	<i>yedI</i>	Inner membrane protein YedI	A122V	Unknown
			Missense	<i>rimI</i>	[Ribosomal protein S18]-alanine N-acetyltransferase	Stop130Y	Amino acid/Protein synthesis
			Missense	<i>oxyR</i>	Hydrogen peroxide-inducible genes activator	A100T	Stress response
			Conservative deletion	<i>traD</i>	Coupling protein TraD	Q638-P640	Conjugation
GN03507	GN03590	<i>E. coli</i>	Missense	<i>argD</i>	Acetylmithine/succinylidiaminopimelate aminotransferase	P85L	Amino acid/Protein synthesis
			Missense	<i>ptsI</i>	Phosphoenolpyruvate-protein phosphotransferase	V488F	CHO metabolism
GN04096	GN04530	<i>E. coli</i>	Disruptive insertion	<i>gatC</i>	PTS system galactitol-specific EIIC component	P85L	CHO metabolism
			Missense	<i>lapB</i>	Lipopolysaccharide assembly protein B	R375W	Cell wall synthesis
			Frameshift	<i>nfsA</i>	Oxygen-insensitive NADPH nitroreductase	G158fs	Drug resistance
			Missense	<i>rob</i>	Right origin-binding protein	K108E	Drug resistance
			Missense	<i>rob</i>	Right origin-binding protein	G245R	Drug resistance
			Missense	<i>purD</i>	Phosphoribosylamine--glycine ligase	G368S	DNA synthesis
			Frameshift	<i>repB</i>	RepFIB replication protein A	Q89fs	Plasmid replication
GN03139	GN03531	<i>E. coli</i>	Missense	<i>lapB</i>	Lipopolysaccharide assembly protein B	H72Y	Stress response
			Missense	<i>intA</i>	Prophage integrase IntA	V181L	Unknown
			Missense	<i>cynS</i>	Cyanate hydratase	N66D	Unknown
			Missense	<i>blc</i>	Outer membrane lipoprotein Blc	F53V	Lipoprotein
			Missense	<i>uvrA</i>	UvrABC system protein A	L168Q	Stress response
			Disruptive deletion	<i>rpIW</i>	50S ribosomal protein L23	D56del	Protein Synthesis
			Missense	<i>ygaV</i>	putative HTH-type transcriptional regulator YgaV	D73G	Unknown

			Missense	<i>fliT</i>	Flagellar protein FliT	V93I	Flagella
GN04979	GN05064	<i>E. coli</i>	Missense	<i>nuoF</i>	NADH-quinone oxidoreductase subunit F	Stop106E	CHO metabolism
			Frameshift	<i>yurK</i>	putative HTH-type transcriptional regulator YurK	Ser200fs	Unknown
GN03449	GN05056	<i>E. coli</i>	Disruptive Deletion	<i>rplW</i>	50S ribosomal protein L23	E56-V57 del	Protein Synthesis
			Missense	<i>ygaV</i>	putative HTH-type transcriptional regulator YgaV	D73G	Unknown
			Missense	<i>fliT</i>	Flagellar protein	V93I	Flagella
GN02953	GN03001	<i>E. coli</i>	No mutations in coding sequences				
GN06489	GN06758	<i>E. coli</i>	No mutations in coding sequences				
GN05476	GN06667	<i>E. coli</i>	No mutations in coding sequences				
GN05107	GN05245	<i>E. coli</i>	Sequencing incomplete – unable to call SNPs				

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99 Table S2: Non-synonymous SNPs arising between initial and relapsed *Klebsiella species* isolates

Initial Isolate	Recurrent Isolate	Species	Mutation type	Gene	Product	Amino acid Substitution	Function
GN05714	GN06034	<i>Klebsiella spp.</i>	Missense	<i>ompC</i>	Outer membrane porin C	Q313K	Drug resistance
			Missense	<i>fyuA</i>	Pesticin receptor	G514R	Unknown
			Missense	<i>sufB</i>	FeS cluster assembly protein SufB	V132L	Stress response
			Missense	<i>ydjE</i>	Inner membrane metabolite transport protein YdjE	G78V	Transport
			Missense	<i>lon</i>	Lon protease	A672V	CHO metabolism
GN03826	GN04029	<i>Klebsiella spp.</i>	Missense	<i>dgcP</i>	Diguanylate cyclase	P12T	Stress response
GN02551	GN02708	<i>Klebsiella spp.</i>	Disruptive deletion	<i>lldR</i>	Putative L-lactate dehydrogenase operon regulatory protein	H75P, E78-81del	Unknown
			Frameshift	<i>lepA</i>	Elongation factor 4	R90fs	Protein synthesis
			Missense	<i>gyrA</i>	DNA gyrase subunit A	S83Y	Drug resistance
			Missense	<i>sufB</i>	FeS cluster assembly protein SufB	Q491stop	Stress response
			Frameshift	<i>pfkA</i>	ATP-dependent 6-phosphofructokinase isozyme 1	R262fs	Glucose metabolism

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112 Table S3: Non-synonymous SNPs arising between initial and relapsed *Serratia marcescens* isolates

Initial Isolate	Recurrent Isolate	Species	Mutation type	Gene	Product	Amino acid substitution	Function
GN02777	GN02991	<i>S. marcescens</i>	Missense	<i>livF</i>	High-affinity branched-chain amino acid transport ATP-binding protein LivF	A193V	Transport
			Missense	<i>crr</i>	PTS system glucose-specific EIIA component	I71T	CHO metabolism
GN02673	GN03438	<i>S. marcescens</i>	Frameshift	<i>wecA</i>	Undecaprenyl-phosphate alpha-N-acetylglucosaminyl 1-phosphate transferase	Ile144fs	Cell wall synthesis
GN02673	GN03465	<i>S. marcescens</i>	Missense	<i>rpoB</i>	DNA-directed RNA polymerase subunit beta	P497S	RNA synthesis
			Frameshift	<i>tetC</i>	Transposon Tn10 TetC protein	Leu137fs	Drug resistance
			Missense	<i>arcA</i>	Aerobic respiration control protein	H171Y	Virulence
			Missense	<i>ftsW</i>	putative peptidoglycan glycosyltransferase FtsW	R34C	Cell division
			Frameshift	<i>pcm</i>	Protein-L-isoaspartate O-methyltransferase	Y62fs	Stress response
			Frameshift	<i>lon</i>	Lon protease	L506fs	Stress response
			Frameshift	<i>dacA</i>	D-alanyl-D-alanine carboxypeptidase DacA	K395fs	Cell wall synthesis
			Disruptive deletion	<i>clpA</i>	ATP-dependent Clp protease ATP-binding subunit ClpA	R431del	Stress response
			Disruptive deletion	<i>rpsA</i>	30S ribosomal protein S1	V75del	Protein synthesis
			Frameshift	<i>sufD</i>	FeS cluster assembly protein SufD	W35fs	Stress response
			Missense	<i>ampR</i>	HTH-type transcriptional activator AmpR	G89R	Drug resistance
			Deletion	<i>topA</i>	DNA topoisomerase 1	Leu761del	DNA synthesis
			Frameshift	<i>ompC</i>	Outer membrane porin C	S147fs	Drug resistance
			Missense	<i>gyrA</i>	DNA gyrase subunit A	D82N	DNA synthesis
			GN02903	GN02955	<i>S. marcescens</i>	Frameshift	<i>nuoC</i>
Deletion	<i>rpsL</i>	30S ribosomal protein S12				L24del	Protein synthesis
Frameshift	<i>nuoF</i>					Y156fs	Electron transport

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118 Table S4: Non-synonymous SNPs arising between initial and relapsed *Pseudomonas aeruginosa* isolates

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Initial Isolate	Recurrent Isolate	Species	Mutation type	Gene	Product	Amino acid substitution	Function
GN06288	GN06816	<i>P. aeruginosa</i>	Insertion	<i>tdh</i>	L-threonine 3-dehydrogenase	Cys245dup	Amino acid catabolism
GN06251	GN06858	<i>P. aeruginosa</i>	Missense	<i>gyrA</i>	DNA gyrase subunit A	T83I	DNA synthesis
			Frameshift	<i>oprD</i>	Porin D	D178fs	Drug resistance
			Frameshift	<i>oprD</i>	Porin D	N199fs	Drug resistance
			Frameshift	<i>mscS</i>	Small-conductance mechanosensitive channel	V47I	Stress Response
			Frameshift	<i>ampD</i>	1,6-anhydro-N-acetylmuramyl-L-alanine amidase AmpD	K165E	Drug resistance
GN04821	GN04922	<i>P. aeruginosa</i>	Frameshift	<i>livF</i>	High-affinity branched-chain amino acid transport ATP-binding protein LivF	M48I	Transport
			Missense	<i>mltB</i>	Membrane-bound lytic murein transglycosylase B	W7stop	Cell Wall Synthesis

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132 Table S5: Bacterial Strains and Minimum Inhibitory Concentrations of Different Isolates to antibiotics
 133 used in this study

Species	Isolate	Source	Initial or Relapse	MIC (µg/ml)						
				MEM	EPM	CRO	PMB	LEV	AMK	GM
<i>E. coli</i>	GN03507	(1)	Initial	0.03	0.016	0.125	0.5	0.1	15	15
<i>E. coli</i>	GN03590	(1)	Relapse	0.03	0.016	0.125	0.5	0.1	15	15
<i>E. coli</i>	GN02953	(1)	Initial	0.05	NT	3.125	NT	25	NT	NT
<i>E. coli</i>	GN03001	(1)	Relapse	0.05	NT	3.125	NT	25	NT	NT
<i>E. coli</i>	GN03139	(1)	Initial	0.06	NT	0.32	NT	50	NT	NT
<i>E. coli</i>	GN03551	(1)	Relapse	0.06	NT	0.32	NT	50	NT	NT
<i>E. coli</i>	GN03449	(1)	Initial	0.125	NT	0.078	NT	6.25	NT	NT
<i>E. coli</i>	GN05056	(1)	Relapse	0.125	NT	3.125	NT	25	NT	NT
<i>E. coli</i>	GN04096	(1)	Initial	0.06	NT	>200	NT	50	NT	NT
<i>E. coli</i>	GN04530	(1)	Relapse	0.06	NT	>200	NT	50	NT	NT
<i>E. coli</i>	GN04979	(1)	Initial	0.06	NT	0.078	NT	0.03	NT	NT
<i>E. coli</i>	GN05064	(1)	Relapse	0.06	NT	0.039	NT	0.03	NT	NT
<i>E. coli</i>	GN05476	(1)	Initial	0.012	NT	0.048	NT	0.05	NT	NT
<i>E. coli</i>	GN06667	(1)	Relapse	0.012	NT	0.048	NT	0.05	NT	NT
<i>E. coli</i>	GN05652	(1)	Initial	0.03	NT	0.156	NT	6.25	NT	NT
<i>E. coli</i>	GN06098	(1)	Relapse	0.03	NT	>200	NT	12.5	NT	NT
<i>E. coli</i>	GN05107	(1)	Initial	0.02	NT	0.156	NT	12.5	NT	NT
<i>E. coli</i>	GN05245	(1)	Relapse	0.02	NT	0.156	NT	25	NT	NT
<i>E. coli</i>	GN06489	(1)	Initial	0.05	NT	0.125	NT	0.063	NT	NT
<i>E. coli</i>	GN06758	(1)	Relapse	0.025	NT	0.125	NT	0.063	NT	NT
<i>E. coli</i>	GN06789	(1)	Initial	0.06	NT	0.156	NT	12.5	NT	NT
<i>E. coli</i>	GN06804	(1)	Relapse	0.06	NT	0.078	NT	25	NT	NT
<i>K. pneumoniae</i>	KPPR1	(2)	N/A	0.125	NT	0.06	NT	0.13	NT	2
<i>K. pneumoniae</i>	KPPR1 <i>pts::kan</i>	(2)	N/A	0.25	NT	0.06	NT	0.13	NT	2

134 NT; Not tested, MEM; meropenem, EPM; ertapenem, CRO; ceftriaxone, PMB; polymyxin B, LEV;
 135 levofloxacin, AMK; amikacin, GM; gentamicin

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141 Table S6: Clinical microbiology susceptibilities (based on Clinical and Laboratory Standards Institute
 142 criteria) of initial and relapsed *E. coli* isolates to different antibiotics(3). Red indicates resistance, yellow
 143 indicates intermediate resistance and green represents susceptible. Persister column refers to change in
 144 persister abundance between initial and relapse isolates, described in Figure 2.

Isolate	Species	Persisters	AMP	PT	SAM	CZ	CRO	CPIM	MEM	GM	TOB	AMK	SMX	LEV
GN03507	<i>E. coli</i>	↑	Red	Green	Red	Green	Green	Green	Green	Green	Green	Green	Green	Green
GN03590	<i>E. coli</i>		Red	Green	Red	Green	Green	Green	Green	Green	Green	Green	Green	Green
GN02953	<i>E. coli</i>	No Change	Red	Green	Red	Red	Green	Green	Green	Red	Red	Green	Red	Red
GN03001	<i>E. coli</i>		Red	Green	Red	Red	Green	Green	Green	Red	Yellow	Green	Red	Red
GN03139	<i>E. coli</i>	No Change	Red	Green	Yellow	Green	Green	Green	Green	Red	Red	Green	Red	Red
GN03551	<i>E. coli</i>		Red	Yellow	Red	Red	Green	Green	Green	Red	Yellow	Green	Red	Red
GN03449	<i>E. coli</i>	↑	Red	Green	Yellow	Green	Green	Green	Green	Red	Yellow	Green	Green	Red
GN05056	<i>E. coli</i>		Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Red	Red
GN04096	<i>E. coli</i>	↓	Red	Red	Red	Red	Red	Red	Green	Red	Yellow	Green	Red	Red
GN04530	<i>E. coli</i>		Red	Red	Red	Red	Red	Red	Green	Green	Green	Green	Red	Red
GN04979	<i>E. coli</i>	No Change	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green
GN05064	<i>E. coli</i>		Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green
GN05476	<i>E. coli</i>	No Change	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green
GN06667	<i>E. coli</i>		Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green	Green
GN05652	<i>E. coli</i>	↑	Red	Green	Yellow	Green	Green	Green	Green	Red	Red	Green	Green	Red
GN06098	<i>E. coli</i>		Red	Red	Red	Red	Red	Red	Green	Green	Red	Red	Green	Red
GN05107	<i>E. coli</i>	No Change	Red	Green	Red	Red	Green	Green	Green	Red	Green	Green	Red	Red
GN05245	<i>E. coli</i>		Red	Green	Red	Yellow	Green	Green	Green	Red	Red	Green	Red	Red
GN06489	<i>E. coli</i>	No Change	Red	Green	Red	Green	Green	Green	Green	Green	Green	Green	Green	Green
GN06758	<i>E. coli</i>		Red	Green	Yellow	Yellow	Green	Green	Green	Green	Green	Green	Green	Green
GN06789	<i>E. coli</i>	↑	Red	Green	Red	Green	Green	Green	Green	Green	Green	Green	Red	Red
GN06804	<i>E. coli</i>		Red	Green	Red	Yellow	Green	Green	Green	Green	Green	Green	Red	Red

145 AMP; Ampicillin, PT; piperacillin-tazobactam, SAM; ampicillin-sulbactam, CZ; cefazolin, CRO; ceftriaxone,
 146 CPIM; cefepime, MEM; meropenem, GM; gentamicin, TOB; tobramycin, AMK; amikacin, SMX;
 147 trimethoprim-sulfamethoxazole, LEV; levofloxacin

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- 1 Table S7: Treatment regimens for each episode of *E. coli* bacteremia. Green indicates isolate is susceptible to antibiotic used, yellow indicates
- 2 intermediate resistance.

Isolate	Species	Initial or Relapse	Days since initial BSI	Source of bacteremia	First Antibiotic	Duration first antibiotic (days)	Second antibiotic	Duration second antibiotic (days)	Third antibiotic	Duration third antibiotic (days)	Total days effective therapy
GN03507	<i>E. coli</i>	Initial	-	Urine	Piperacillin-tazobactam	2	Ceftriaxone	14			16
GN03590	<i>E. coli</i>	Relapse	39	Skin/Soft tissue	Trimethoprim-sulfamethoxazole	1	Ertapenem	28			29
GN02953	<i>E. coli</i>	Initial	-	Urine	Piperacillin-tazobactam	6	Cefdinir	8			14
GN03001	<i>E. coli</i>	Relapse	21	Urine	Piperacillin-tazobactam	5	Ceftriaxone	15			20
GN03139	<i>E. coli</i>	Initial	-	Urine	Ceftazidime	3	Cephalexin	12			15
GN03551	<i>E. coli</i>	Relapse	271	Urine	Piperacillin-tazobactam	1	Ceftriaxone	11			11
GN03449	<i>E. coli</i>	Initial	-	Urine	Piperacillin-tazobactam	14	Cefazolin	1			15
GN05056	<i>E. coli</i>	Relapse	957	Urine	Ceftriaxone	4	Cephalexin	9			13
GN04096	<i>E. coli</i>	Initial	-	Unknown	Meropenem	6	Ertapenem	10			16
GN04530	<i>E. coli</i>	Relapse	219	Urine	Ertapenem	43					43
GN04979	<i>E. coli</i>	Initial	-	Urine	Ceftriaxone	7	Cephalexin	7			14
GN05064	<i>E. coli</i>	Relapse	40	Urine	Ciprofloxacin	4	Aztreonam	5			9
GN05476	<i>E. coli</i>	Initial	-	Urine	Ceftriaxone	1	Piperacillin-tazobactam	2	Ciprofloxacin	8	11
GN06667	<i>E. coli</i>	Relapse	606	Urine	Cefepime	2	Ciprofloxacin	12			14
GN05652	<i>E. coli</i>	Initial	-	GI	Piperacillin-tazobactam	5		0			5
GN06098	<i>E. coli</i>	Relapse	263	Urine	Meropenem	6	Ertapenem	6			12
GN05107	<i>E. coli</i>	Initial	-	Urine	Piperacillin-tazobactam	1	Ceftazidime	3	Ceftriaxone	10	14
GN05245	<i>E. coli</i>	Relapse	97	Urine	Piperacillin-tazobactam	4	Cefuroxime	14			18
GN06489	<i>E. coli</i>	Initial	-	Urine	Piperacillin-tazobactam	3	Ceftriaxone	5	Cephalexin	10	18
GN06758	<i>E. coli</i>	Relapse	114	Urine	Ceftriaxone	2	Ciprofloxacin	10			12
GN06789	<i>E. coli</i>	Initial	-	Urine	Piperacillin-tazobactam	4	Cephalexin	4			8
GN06804	<i>E. coli</i>	Relapse	8	Urine	Piperacillin-tazobactam	4	Ceftriaxone	30			34

Supplemental References

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