

Supplementary Material

Title: Phylogenetic and genomic diversity in isolates from the globally distributed *Acinetobacter baumannii* ST25 lineage

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Supplementary Table S1. Assembly stats and accession numbers for genomes sequenced in this study as well as reference genomes

Genome	Contigs	Assembly size	SRA accession	Assembly		Average depth of coverage	<i>in silico</i> MLST type
				accession			
LUH7841	16	3801220	SRS853050	JZBX00000000	42x	402	
LUH6220	63	3966581	SRS853048	JZBW00000000	52x	25	
NM3	54	4100904	SRS853047	JZBV00000000	49x	25	
741019	64	4055024	SRR1813762	JZBZ00000000	46x	25	
161/07	63	4282986	SRS853053	JZCA00000000	46x	25	
4390	157	3919530	SRS853051	JZBY00000000	42x	25	
RUH1486	62	3888398	SRS853044	JZBU00000000	50x	25	
5256	50	4158006	N/A	AHAI00000000	N/A	25	
NIPH 146	1	3933401	N/A	NZ_KB849308.1	N/A	25	
4190	398	4056516	N/A	AEPAA00000000	N/A	25	
UMB003	101	4140835	N/A	AEPMA00000000	N/A	25	
naval 18	55	4470438	N/A	AFDA00000000	N/A	25	
OIFC143	8	4441327	N/A	AFDL00000000	N/A	25	
107m	45	3880766	N/A	CBSGG00000000	N/A	25	
1429530	31	3985365	N/A	JEVMA00000000	N/A	25	
984213	449	4177035	N/A	JEVX00000000	N/A	25	
C179	85	4384434	N/A	AVOD00000000	N/A	25	
C186	89	4392843	N/A	AVOB00000000	N/A	25	
2008-15-69	186	4255122	N/A	AMHNN00000000	N/A	25	

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Supplementary Table S3. Accession information for plasmids screened in the current study

Plasmid name	Accession
p1ABAYE	NC_010401
p1ABSDF	NC_010395
p1ABST25	NZ_AEPA01000395
p1ABST78	NZ_AEOZ01000236
p1BJAB07104	NC_021727
p1BJAB0868	NC_021730
p2ABAYE	NC_010402
p2ABSDF	NC_010396
p2ABST25	NZ_AEPA01000396
p2BJAB07104	NC_021728
p2BJAB0868	NC_021731
p3ABAYE	NC_010404
p3ABSDF	NC_010398
p3BJAB0868	NC_021732
p4ABAYE	NC_010403
pAB0057	NC_011585
pAB1	NC_009083
pAB2	NC_009084
pABUH2a-5.6	AYFZ01000080
pABUH2b-5.4	AYFH01000057
pABUH3a-8.2	AYFH01000048
pABUH3b-7.8	AYFZ01000083
pAbNDM-1	NC_019985
pBJAB0715	NC_021734
pCanadaBC5-8.7	NZ_AFDN01000003
pD1279779	NC_020525
pMDR-ZJ06	NC_017172
pNaval18-131	NZ_AFDA02000009
pNaval18-5.7	NZ_AFDA02000010
pNaval18-6.1	NZ_AFDA02000006
pNaval18-7.0	NZ_AFDA02000011
pNaval18-74	NZ_AFDA02000007
pNaval18-8.4	NZ_AFDA02000008
pOIFC032-101	NZ_AFCZ02000003
pOIFC032-8.6	NZ_AFCZ02000004
pOIFC143-128	NZ_AFDL01000008
pOIFC143-2.3	NZ_AFDL01000005
pOIFC143-6.2	NZ_AFDL01000007
pOIFC143-70	NZ_AFDL01000006
pZW85p2	NC_023031

Supplementary Table S4. Virulence associated genes screened across ST25 genomes in this study

gene	annotation	accession	Publication (PMID)
<i>zur</i>	ferric uptake regulator family protein	A1S_0145	23236280
<i>hcp</i>	type VI secretion system effector, Hcp1 family	A1S_1296	23365692
<i>bap</i>	biofilm associated protein	ABAYE0792	22083703
<i>entA</i>	2,3-dihydroxybenzoate-2,3-dehydrogenase	A1S_2579	22570720
<i>pmt</i>	transporter-like protein gene	HM595762	22518144
<i>csuE</i>	protein CsuE	AAP43040	14663080
<i>entE</i>	enterobactin synthase subunit E	ABAYE1096	20853905
<i>sc1</i>	siderophore biosynthesis protein	ABAYE2004	18350144
<i>bfmR</i>	two-component regulatory system response regulator	YP_045456	18957593
<i>envZ</i>	Osmolarity sensor protein EnvZ	ABBFA_000285	22911967
<i>epsA</i>	EPS I polysaccharide export outer membrane protein	ABBFA_003459	22911967
<i>ompF</i>	Outer membrane porin F precursor	ABBFA_000621	22911967
<i>ostA</i>	Organic solvent tolerance protein OstA	ABBFA_001929	22911967
<i>pbpG</i>	Penicillin-binding protein 7/8	ABBFA_003295	22911967
<i>ptk</i>	Tyrosine-protein kinase Ptk	ABBFA_003461	22911967
<i>rstA</i>	Transcriptional regulatory protein RstA	ABBFA_002866	22911967
<i>spsC</i>	Perosamine synthase	ABBFA_003451	22911967

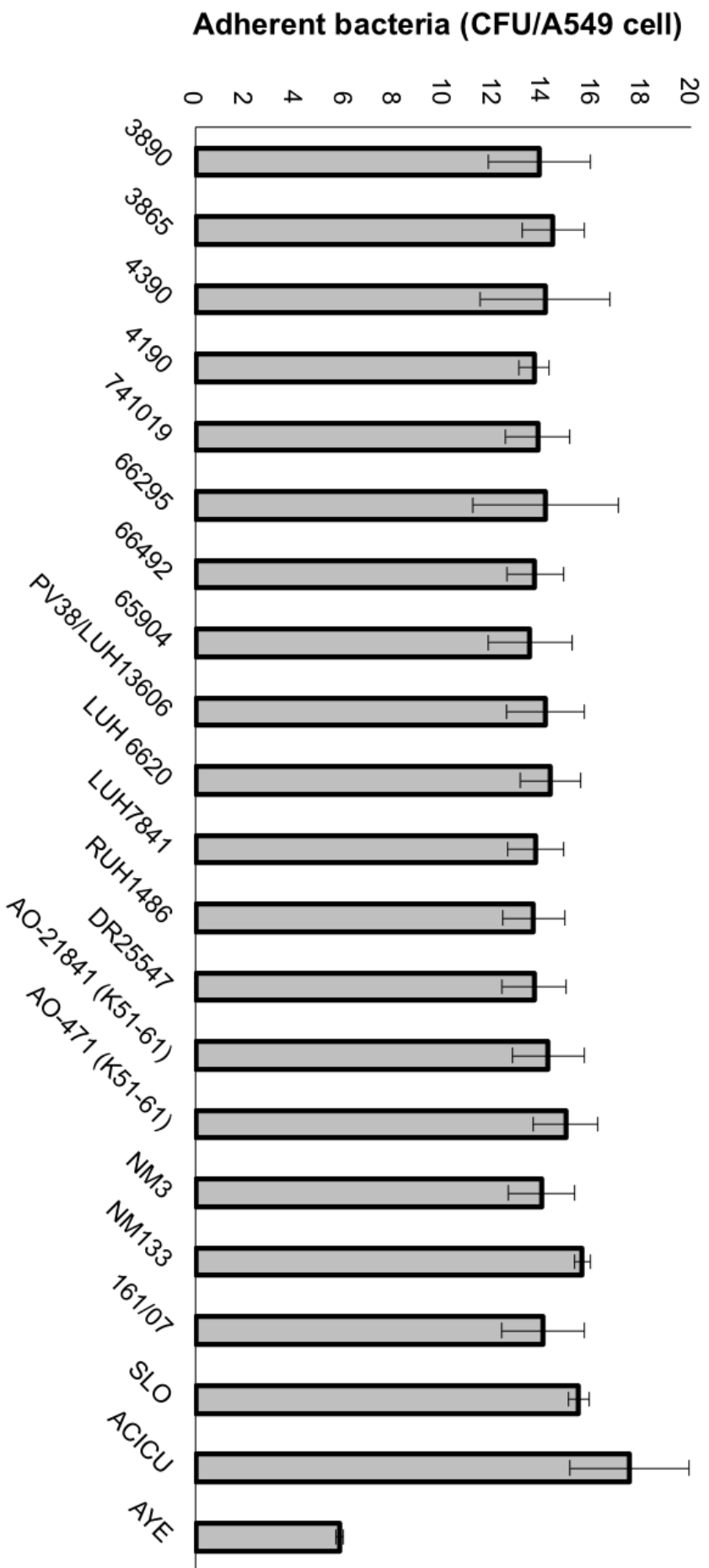
Supplementary Table S5. Antibiotic susceptibility profiles of *A. baumannii* strains included in this study.

Strain	IPM	MER	COL	RIF	SAM	SXT	MIC ($\mu\text{g/ml}$) of antibiotic									
							TIGEC	TETRA	CAZ	TZP	FEP	LEVOFL	CIP	GEN	TOB	
3890	16	6	0.75	8	8	0.75	0.19	6	6	96	6	0.38	1	>256	64	
4390	32	8	0.75	4	16	>32	0.125	8	192	>256	>256	12	>32	64	16	
66492	>32	>32	0.75	6	128	>32	0.38	24	>256	>256	>256	>32	>32	192	128	
AO-471 (K51-49)	>32	>32	0.5	3	48	>32	0.25	4	>256	>256	>256	8	>32	>256	128	
741019	>32	>32	0.75	1.5	32	1	0.38	4	>256	>256	>256	2	>32	>256	>256	
66295	>32	>32	0.75	>32	48	0.5	0.19	4	>256	>256	>256	24	>32	1	1	
NM133	>32	>32	0.75	>32	256	>32	0.38	12	>256	>256	>256	6	>32	>256	>256	
65904	>32	>32	0.75	4	64	>32	0.19	6	>256	>256	>256	12	>32	192	64	
AO-21841 (K51-61)	>32	>32	0.38	2	>256	>32	0.38	4	>256	>256	>256	0.094	>32	>256	128	
RUH 1486	0.25	0.5	0.5	3	4	0.125	0.125	4	4	12	4	0.094	0.5	1	1	
NM3	>32	>32	0.75	3	24	16	0.25	4	8	>256	>256	4	>32	>256	192	
LUH 6220	>32	32	0.5	8	0.5	0.5	0.5	32	8	>256	>256	8	16	>256	64	
PV38/LUH 13606	>32	>32	0.75	12	16	1	0.25	12	12	>256	>256	3	8	2	2	
3865	>32	>32	0.75	>32	>256	32	0.25	6	192	>256	>256	12	>32	>256	128	
SLO	>32	>32	0.5	3	>256	12	0.25	8	>256	>256	>256	16	>32	>256	128	
161/07	>32	>32	0.5	3	>256	16	0.38	6	>256	>256	>256	12	>32	>256	128	
LUH 7841	0.19	0.5	0.5	6	3	0.75	0.094	2	2	2	1.5	0.19	1	1.5	1	
4190	>32	>32	0.75	4	24	0.094	0.38	32	>256	>256	>256	8	>32	3	2	
DR25547 (LUH 14601)	>32	>32	1	2	48	0.19	0.5	8	>256	>256	>256	8	>32	>256	>256	

Abbreviations: MIC, Minimum inhibitory concentration; IPM, imipenem; MER, meropenem; COL, colistin; RIF, rifampin; SAM, sulbactam-ampicillin; SXT, trimetoprim/sulfametoxazole; TIGEC, tigecycline; TETRA, tetracycline; CAZ, ceftazidime; TZP, piperacillin/tazobactam; FEP, cefepime; LEVOFL, levofloxacin; CIP, ciprofloxacin; GEN, gentamicin; TOB, tobramycin.

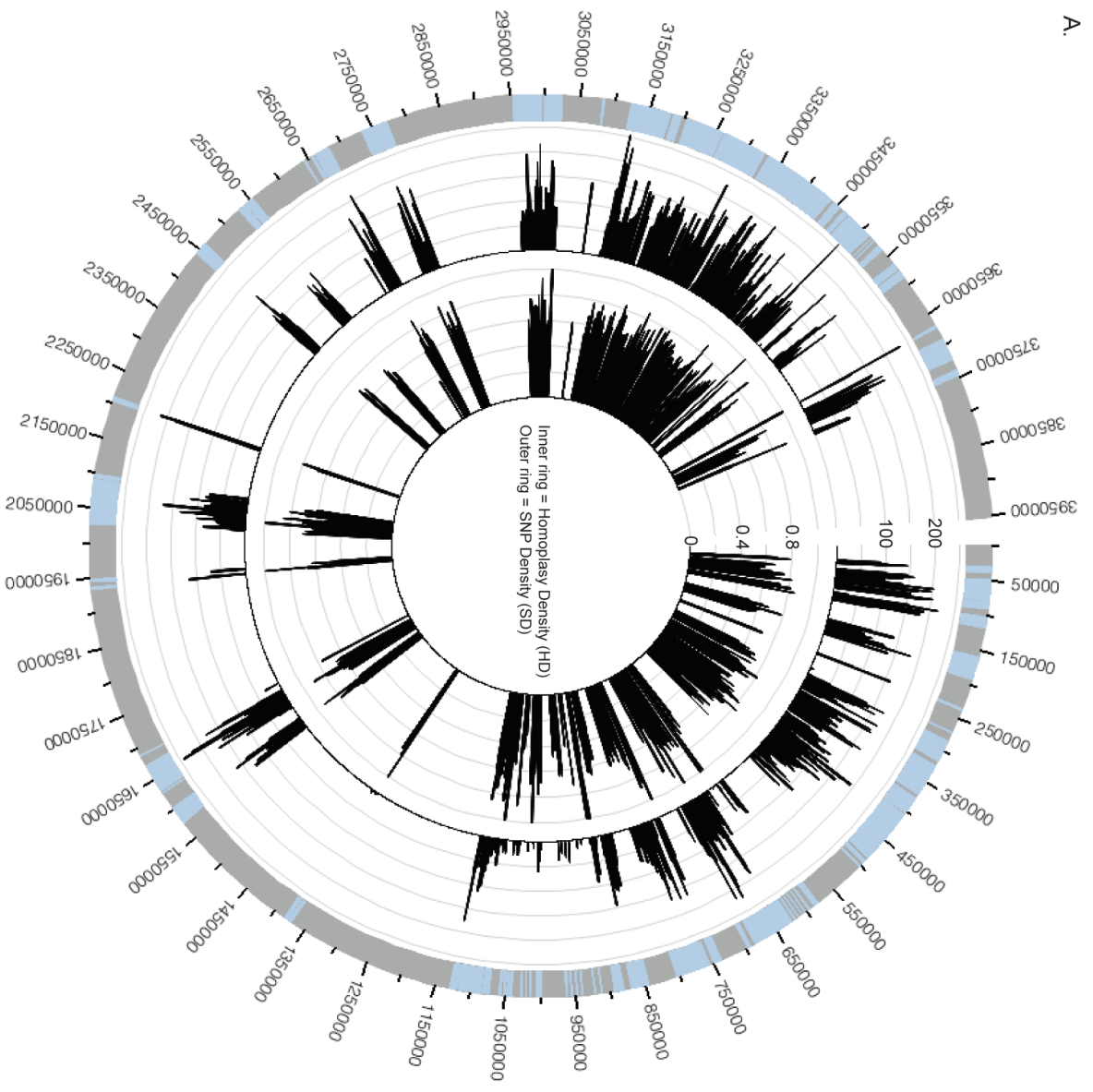
Supplementary Table S6. Regions in the reference chromosome associated with high SNP density in ST25 genomes

Chromosome	start coord	end coord	# Parsimony- informative SNPs	#Homoplasious SNPs	Homoplasy density ratio	locus tag(s)	Annotation
NC_011595	15001	16001	27	27	1.00	ABBFA_000013	Anhydro-N-acetyl/muramic acid kinase(AnhMUrNAc kinase)
NC_011595	196001	197001	53	45	0.85	ABBFA_000174,ABBFA_000175	transcriptional regulator IrpB, Alkanesulfonate monooxygenase
NC_011595	533001	534001	25	25	1.00	ABBFA_000509,ABBFA_000510	Acyl-CoA dehydrogenase, Enoyl-CoA hydratase,Enoyl-CoA hydratase
NC_011595	534001	535001	67	67	1.00	ABBFA_000510, ABBFA_000511	Enoyl-CoA hydratase, acetyl-CoA carboxylase
NC_011595	535001	536001	53	53	1.00	ABBFA_000511	acetyl-CoA carboxylase
NC_011595	627001	628001	21	21	1.00	ABBFA_000597,ABBFA_000598	Metallo-beta-lactamase, AhpC/TSA family protein
NC_011595	1176001	1177001	60	53	0.88	ABBFA_001079, ABBFA_001080	xanthine dehydrogenase, accessory protein XdhC
NC_011595	2280001	2281001	21	21	1.00	ABBFA_002139, ABBFA_002140	Major Facilitator Superfamily protein, enoyl-(acyl-carrier-protein) reductase

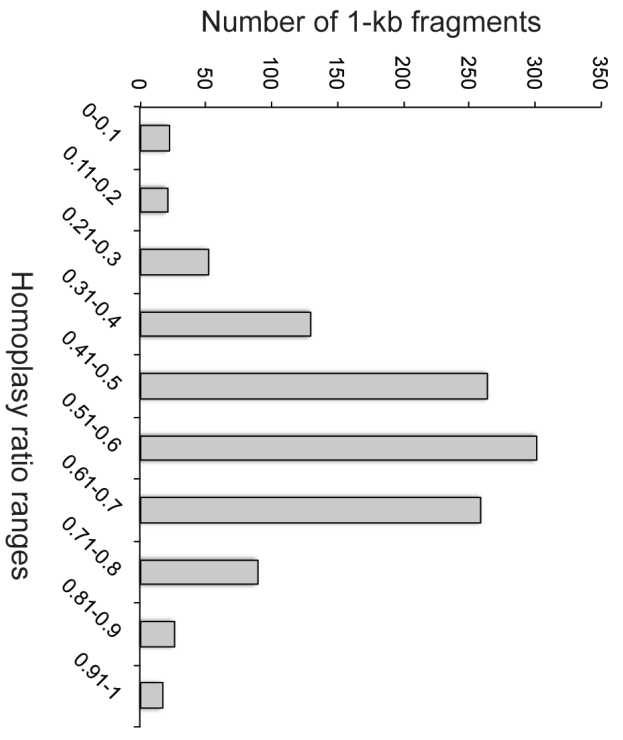


Supplementary Figure S1. Bacterial adherence of *A. baumannii* strains to A549 bronchial epithelial cells. Cell surface-associated bacteria after 60 min incubation at 37°C. Error bars represent the standard deviation between triplicates.

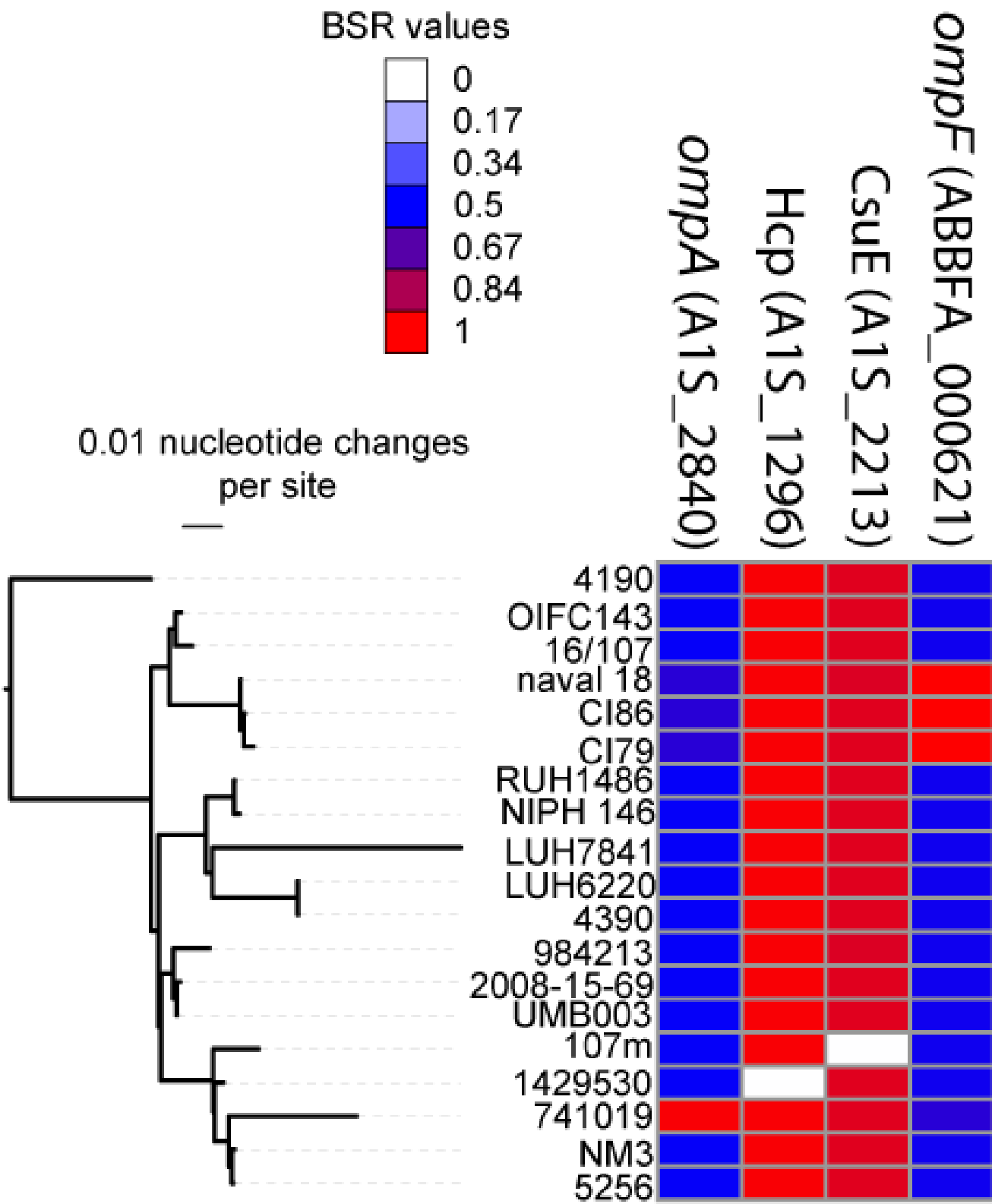
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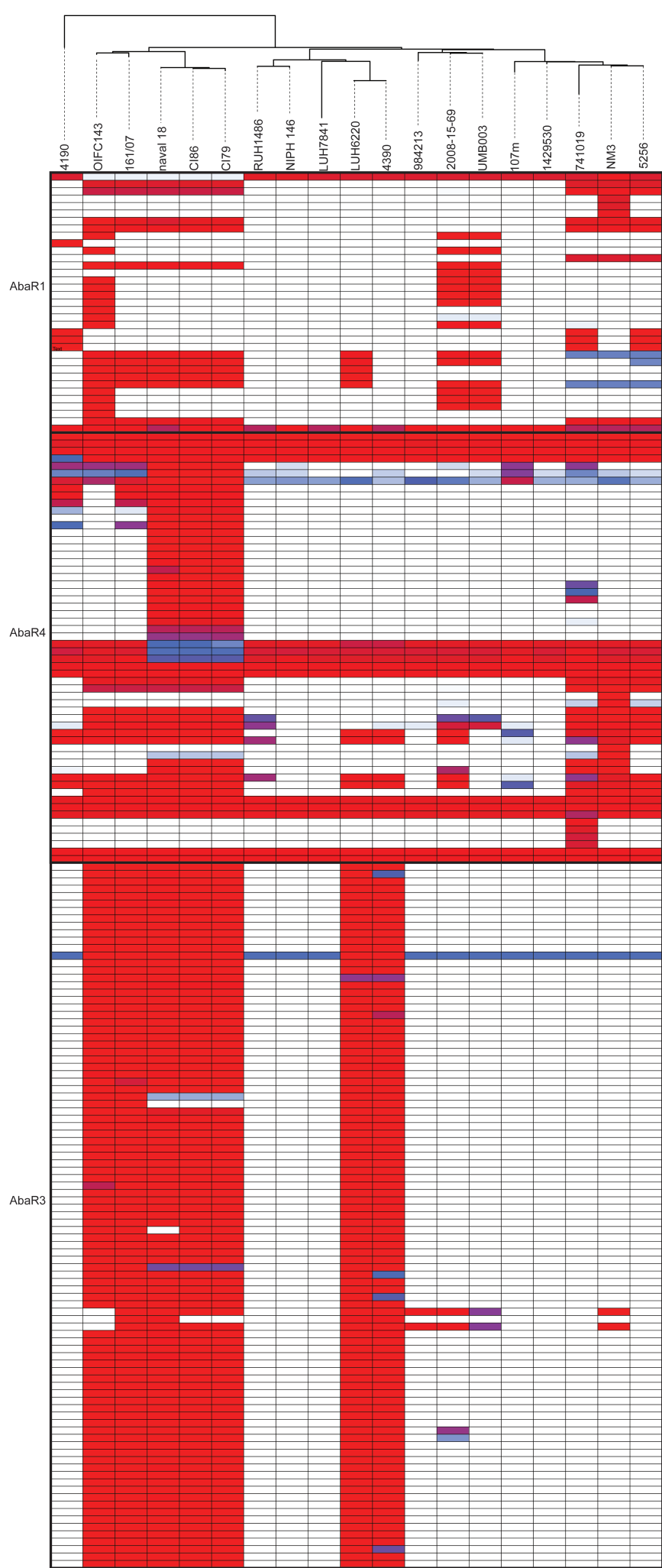
B.



Supplementary Figure S2. Homoplasmy density (HD) ratio analysis of queried *A. baumannii* genomes. Panel A: Parsimony informative (PI) single nucleotide polymorphisms (SNPs) were identified across 1-kb, non-overlapping windows (SNP density, or SD), compared to the reference genome of AB307-0294. Homoplasious SNPs were identified by a Retention Index value < 0.05. The HD was calculated by dividing the number of homoplasious SNPs by the total number of PI SNPs. The SD and HD values were visualized with Circos. Core genome regions were identified where there was a call in all query genomes compared to the reference genome. Panel B: The distribution of 1-kb, non-overlapping regions, based on the HD values.



Supplementary Figure S3. The ST25 phylogeny associated with a heatmap of differentially-conserved genes (Supplementary Table S4) associated with virulence. The heatmap was generated from LS-BSR output and was visualized with the interactive tree of life. GenBank accession numbers are listed for each gene.



Supplementary Figure S4. The ST25 phylogeny associated with a heatmap of differentially-conserved genes from previously-characterized antibiotic resistance islands. The heatmap was generated from LS-BSR output and was visualized with the interactive tree of life. GenBank accession numbers are listed for each gene.