

Combining functional genomics and whole genome sequencing to detect antibiotic resistance genes in bacterial strains co-occurring simultaneously in a Brazilian hospital

by

Tiago Cabral Borelli^{1§}, Gabriel Lencioni Lovate^{1§}, Ana Flavia Tonelli Scaranello¹, Lucas Ferreira Ribeiro¹, Livia Zaramela², Felipe Marcelo Pereira-dos-Santos³, Rafael Silva-Rocha^{3#} and Maria-Eugenia Guazzaroni^{1#}

¹Department of Biology, Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, University of São Paulo, São Paulo, SP, Brazil

²Department of Pediatrics, University of California San Diego, San Diego, California, USA

³Department of Cell and Molecular Biology, Faculdade de Medicina de Ribeirão Preto, University of São Paulo, São Paulo, SP, Brazil

[§]Both authors contributed equally to this work

[#]Both authors contributed equally to this work

*Correspondence to: Maria-Eugenia Guazzaroni, meguazzaroni@ffclrp.usp.br
Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Universidade de São Paulo.
Av. Bandeirantes, 3.900. CEP: 14049-901, Ribeirão Preto, São Paulo, Brazil.

Supporting Materials

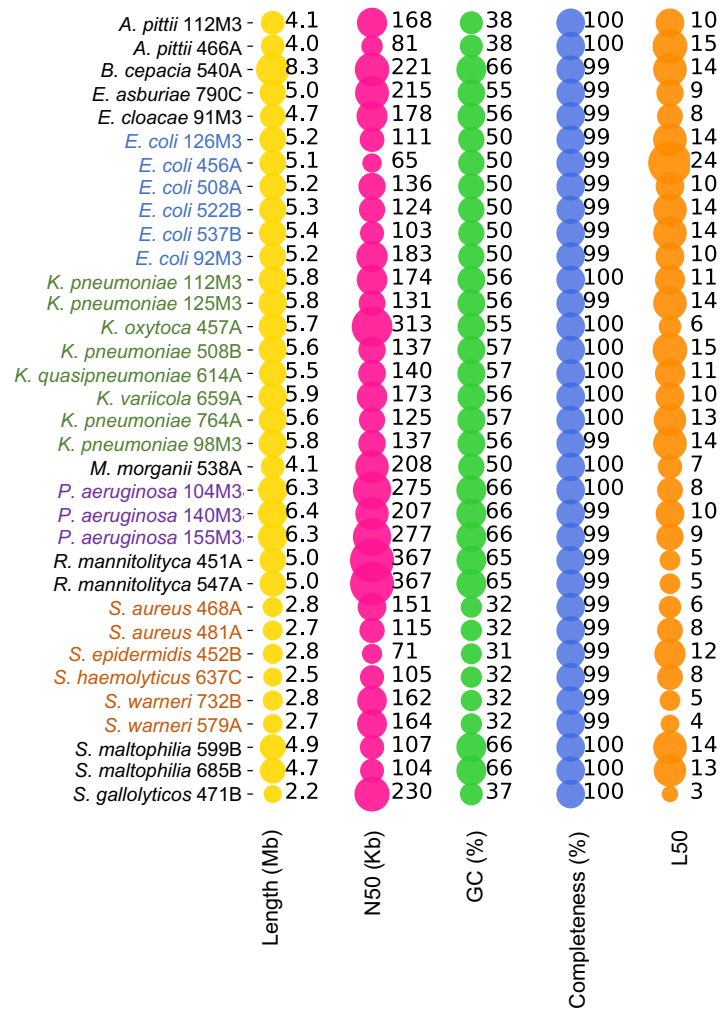


Figure S1. General features of genomes sequenced in this study.

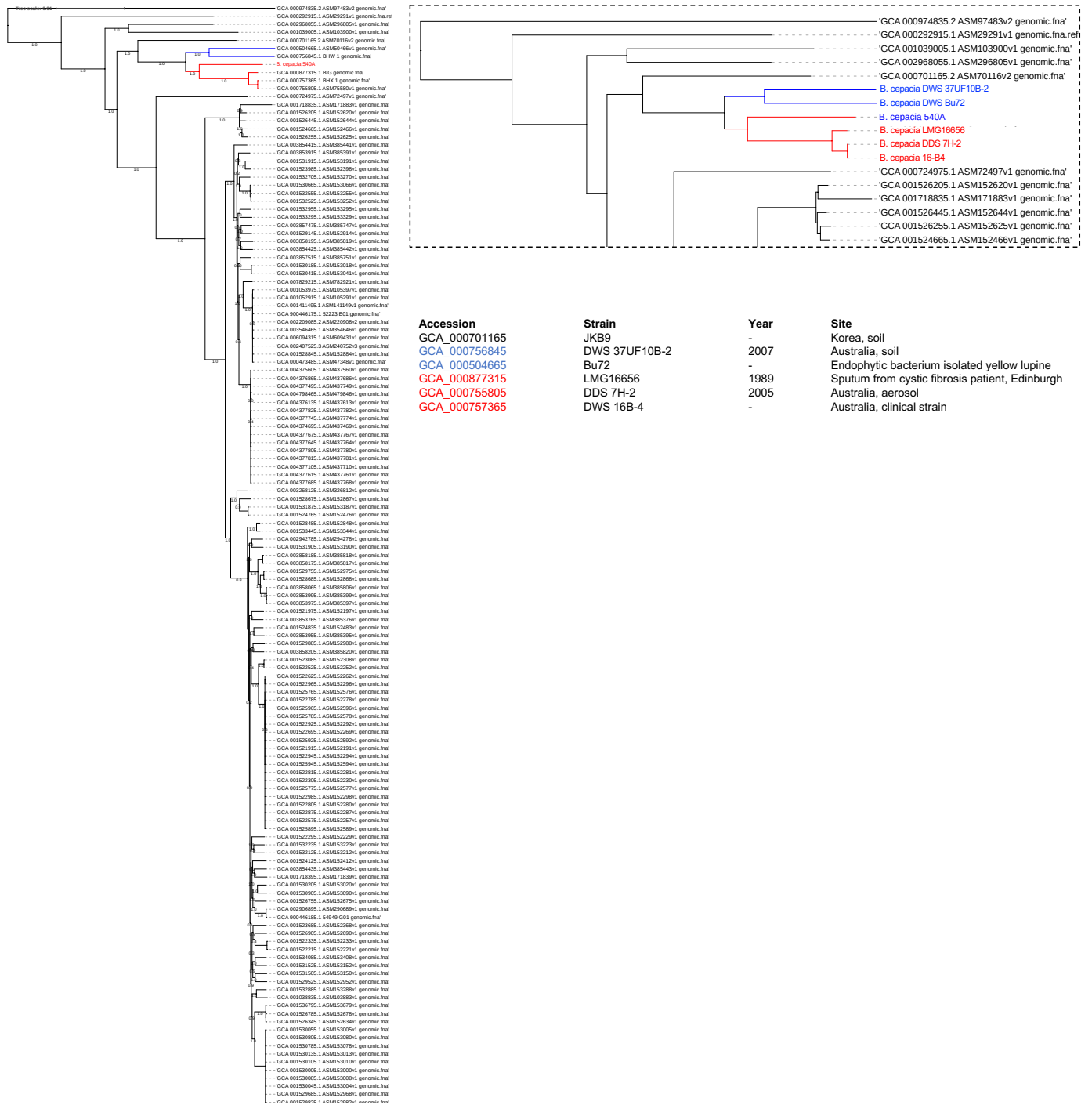


Figure S2. Phylogenetic tree of *B. cepacia* 540A and total number of available *B. cepacia* genomes (166) in NCBI at March 2020. NCBI accession number are indicated for *B. cepacia* strains compared in the figure. Blue branches are two *B. cepacia* endophytic strains isolated in Australia, while red branches are *B. cepacia* isolated from patients. Phylogenomic analyses were performed using Parsnp and Gingr (56) and phylogenetic trees visualized using iTOL (57).

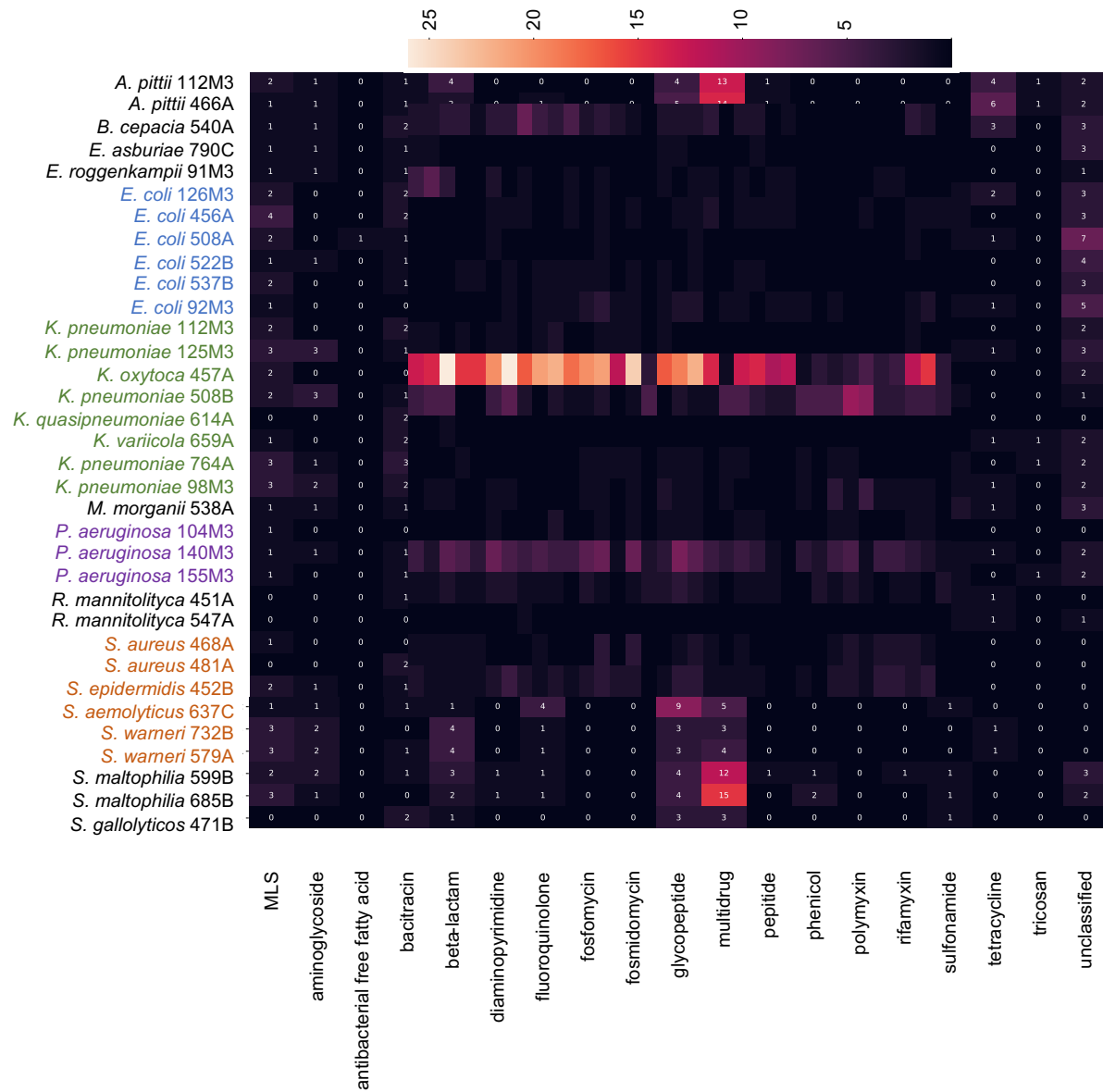


Figure S3. Heatmap showing the presence of ARGs identified by DeepARG in nearly sequenced genomes.

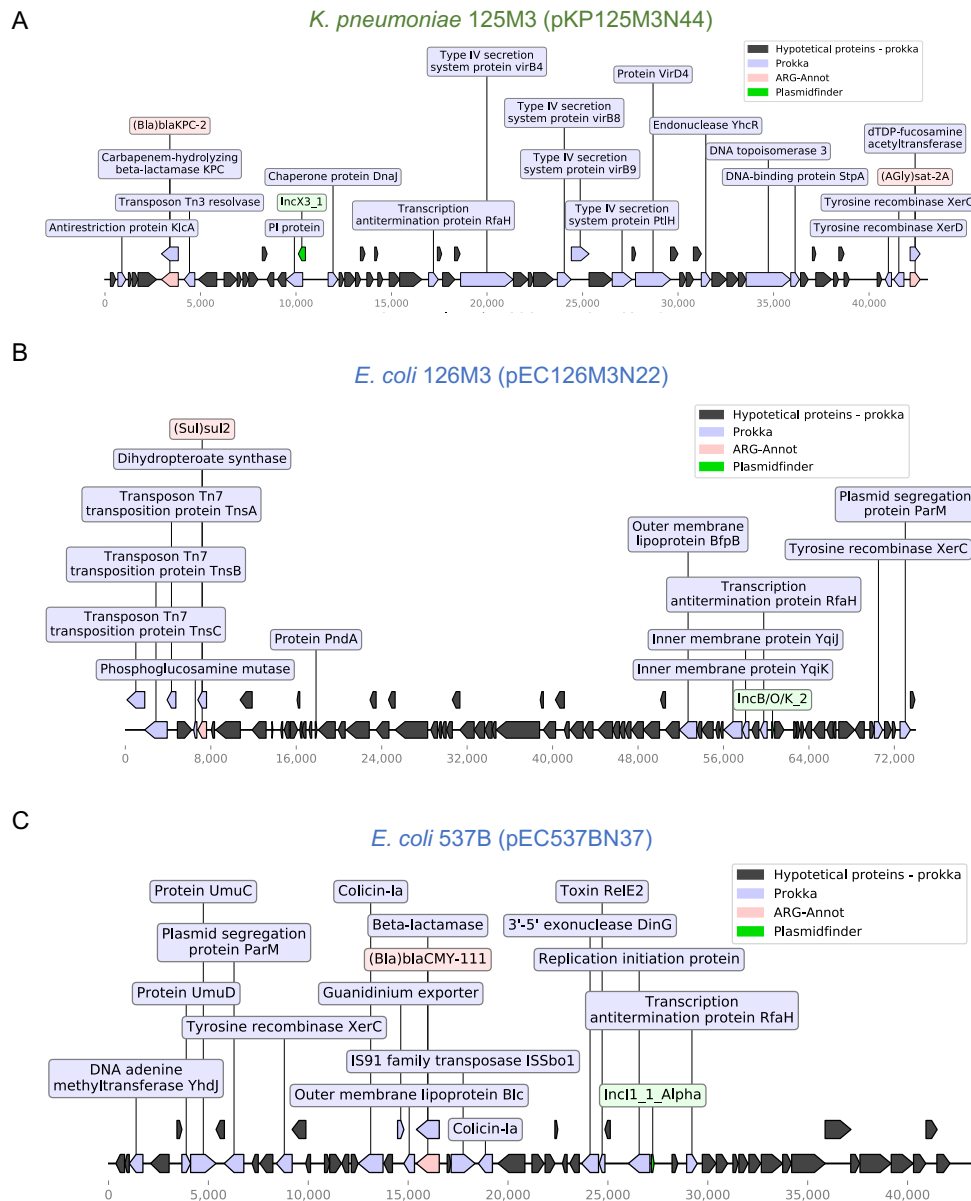
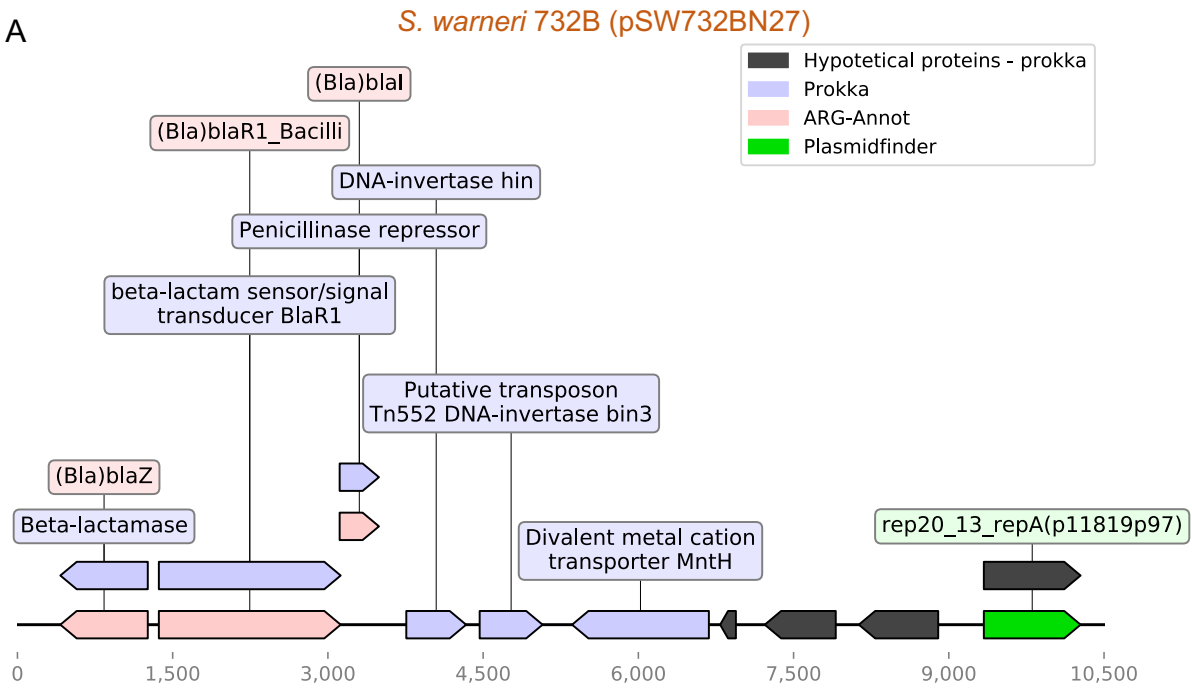
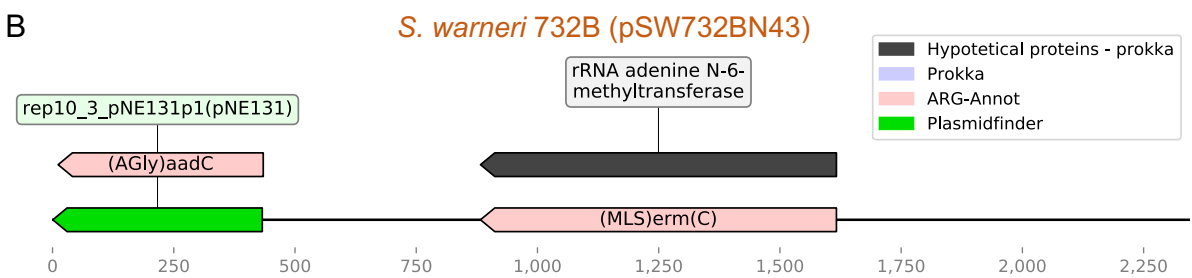


Figure S4. Schematic representation of genes in identified plasmids exposing the coexistence of ARGs with transposon elements. **A)** Plasmid pKP125M3N44 (43 kb) from *K. pneumoniae* 125M3. This plasmid is identical to plasmid pKP98M3N42 from *K. pneumoniae* 98M3 (Fig. 4A). **B)** Plasmid pEC126M3N22 (73,9 kb) from *E. coli* 126M3. **C)** Plasmid pEC537BN37 (43,3 kb) from *E. coli* 537B. Legends represents the colors code for the identified genes.

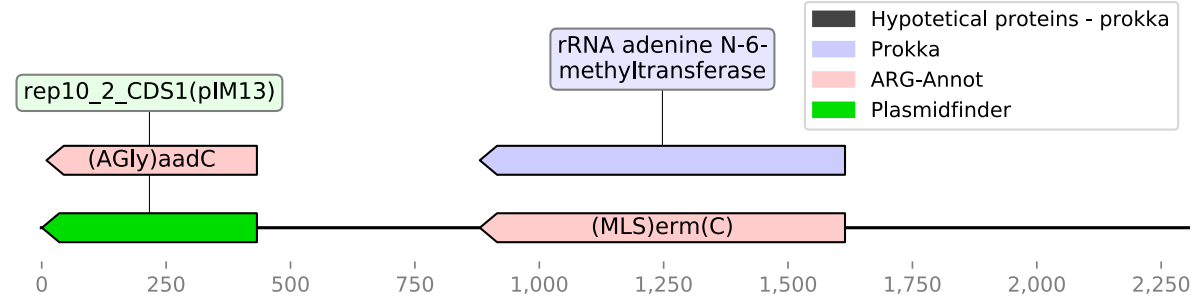
A



B



Staphylococcus epidermidis



NODE 56 length 2305 cov 620.290118

Species	Isolate	ARG	Origin	Predicted resistance	Phenotype (resistant to)
<i>E. coli</i>	126M3	<i>bla</i> _{OXA-1}	Acquired	Cephalosporin	Ceftazidime Cefotaxime Cefepime
				Penam (penicillin)	Ampicillin
				Carbapenem	Ertapenem Meropenem
		<i>bla</i> _{KPC-2}	Acquired	Cephalosporin	Cefotaxime Ceftazidime Cefepime
				Monobactam	Not tested

<i>K. pneumoniae</i>	522B	<i>bla</i> _{CTX-M-15}	Acquired	Penam (penicillin)	Ampicillin
				Cephalosporin	Cefotaxime Ceftazidime Cefepime
				Cephamycin	Cefoxitin
				Cephalosporin	Susceptible
	537B	<i>bla</i> _{CMY-111}	N (<i>Citrobacter freundii</i>)	Monobactam	Not tested
				Penam (penicillin)	Ampicillin
				Penem	Not tested
				Cephalosporin	Susceptible
	92M3	<i>bla</i> _{TEM-105}	Acquired	Penam (penicillin)	Ampicillin
				Penem	Not tested
				Cephalosporin	Susceptible
				Penam (penicillin)	Ampicillin
	508A	<i>bla</i> _{OXA-1}	Acquired	Carbapenem	Ertapenem Meropenem
				Cephalosporin	Cefotaxime Ceftazidime Cefepime
				Penam (penicillin)	Ampicillin
				Carbapenem	Ertapenem Meropenem
	112M3	<i>bla</i> _{SHV-187}	Natural (<i>K. pneumoniae</i>)	Cephalosporin	Cefotaxime Ceftazidime Cefepime
				Penam (penicillin)	Ampicillin
				Carbapenem	Ertapenem Meropenem
				Cephalosporin	Cefotaxime Ceftazidime Cefepime
<i>K. pneumoniae</i>	98M3	<i>bla</i> _{KPC-2}	Acquired	Penam (penicillin)	Ampicillin
				Carbapenem	Ertapenem Meropenem
				Cephalosporin	Cefotaxime Ceftazidime Cefepime
				Monobactam	Not tested
		<i>bla</i> _{CTX-M-19}	Acquired	Penam (penicillin)	Ampicillin
				Cephalosporin	Cefotaxime Ceftazidime Cefepime
				Cephalosporin	Cefotaxime Ceftazidime Cefepime
				Monobactam	Not tested
	764A	<i>bla</i> _{TEM-105}	Acquired	Penam (penicillin)	Ampicillin
				Penem	Not tested
				Cephalosporin	Cefotaxime Ceftazidime Cefepime
				Natural (<i>Enterobacter sp.</i> MGH 14)	Cefotaxime Ceftazidime Cefepime

508B	<i>bla</i> _{TEM-105}	Acquired	Carbapenem	Ertapenem Meropenem
			Penam (penicillin)	Ampicillin
			Cephalosporin	Cefotaxime Ceftazidime Cefepime
			Monobactam	Not tested
			Penam (penicillin)	Ampicillin
	<i>bla</i> _{CTX-M-15}	Acquired	Penem	Not tested
			Cephalosporin	Cefotaxime Ceftazidime Cefepime
	<i>bla</i> _{OXA-1}	Acquired	Cephalosporin	Cefotaxime Ceftazidime Cefepime
			Penam (penicillin)	Ampicillin
	<i>bla</i> _{SHV-11}	Acquired	Carbapenem	Susceptible
			Cephalosporin	Cefotaxime Ceftazidime Cefepime
			Penam (penicillin)	Ampicillin
			Cephalosporin	Cefotaxime Ceftazidime Cefepime
			Penam (penicillin)	Ampicillin
125M3	<i>bla</i> _{LAP-2}	Acquired	Aminoglycoside Fluoroquinolone Rifamycin Tetracycline	Gentamicin Ciprofloxacin Not tested Tigercycline
			Carbapenem	Ertapenem Meropenem
			Cephalosporin	Cefotaxime Ceftazidime Cefepime
	<i>bla</i> _{KPC-2}	Acquired	Penam (penicillin)	Ampicillin
			Carbapenem	Ertapenem Meropenem
	<i>bla</i> _{KPC-2}	Acquired	Cephalosporin	Cefotaxime Ceftazidime Cefepime
			Monobactam	Not tested

				Penam (penicillin)	Ampicillin
		<i>bla</i> _{CTX-M-14}	Natural (<i>Kluyvera</i> sp. N03-0458)	Cephalosporin	Cefotaxime Ceftazidime Cefepime
				Cephalosporin	Cefotaxime Ceftazidime Cefepime
		<i>bla</i> _{TEM-105}	Acquired	Monobactam	Not tested
				Penam (penicillin)	Ampicillin
				Penem	Not tested
<i>K. variicola</i>	659A	<i>bla</i> _{LEN-24}	Natural (<i>K. pneumoniae</i>)	Penem	Not tested
				Penam (penicillin)	Ampicillin
<i>K. quasipneumoniae</i>	614A	<i>bla</i> _{OKP-B-9}	Natural (<i>K. pneumoniae</i>)	Cephalosporin	Susceptible
				Penam (penicillin)	Ampicillin
<i>K. oxytoca</i>	457A	<i>bla</i> _{OXY2-1}	Natural (<i>K. oxytoca</i>)	Cephalosporin	Cefotaxime Ceftazidime Cefepime
				Monobactam	Not tested
				Penam (penicillin)	Ampicillin

Table S2 – Phage-related contigs identified by Phaster.

Strain	Contig	Completeness Score*	Start-End
<i>K. pneumoniae</i> 508B	NODE_53_length_23265_cov_16.832643	Questionable (75)	9692-22726
<i>E. coli</i> 537B	NODE_52_length_27715_cov_8.380540	Intact (100)	703-9465
<i>K. pneumoniae</i> 98M3	NODE_52_length_23307_cov_9.564590	Questionable (75)	398-12901
<i>K. pneumoniae</i> 125M3	NODE_57_length_13879_cov_8.456749	Incomplete (30)	4970-13790

*The criteria for completeness scoring are based on the number of phases CDSs found in the phage region

Table S3 – Replicons identified by bacWSGTdb for *K. pneumonia* 508B and 98M3 genomes.

Strain	Replicon	Contig	Identity (%)	Start-End
<i>K. pneumoniae</i> 508B	IncC	NODE_15_length_136795_cov_22.099162*	100	28286 - 28702
	IncFIB(K)	NODE_66_length_8337_cov_24.095654	100	3603 - 4162
	Col440I	NODE_68_length_5245_cov_640.966187	93.64	286 - 395
	Col440II	NODE_68_length_5245_cov_640.966187	90.75	4599 - 4879
	Col440I	NODE_71_length_4632_cov_690.022060	97.37	4298 - 4411
	Col440I	NODE_72_length_4287_cov_589.476839	96.49	1426 - 1539
<i>K. pneumoniae</i> 98M3	IncFII(K)	NODE_27_length_87380_cov_12.014012	100	44394 - 44541
	IncC	NODE_39_length_51018_cov_14.408885	100	21125 - 21541
	IncX3	NODE_42_length_42978_cov_16.990438*	99.73	10037 - 10410
	ColRNAI	NODE_62_length_9393_cov_64.187218	100	4306 - 10410
	IncFIB(K)	NODE_67_length_6761_cov_15.677875	100	2698 - 3257

*Contigs with co-occurrence of antibiotic resistance genes

Table S4 – Most similar strains* identified by bacWSGTdb SNP strategy for *K. pneumonia* 98M3.

Isolate	Accession number	Country	Collection Year
HSP08	SBDB01	Brazil	2016
P72	SBDF01	Brazil	2015
HSP31	SBCZ01	Brazil	2016
P73	SBDE01	Brazil	2016
P27	SBDL01	Brazil	2015
P43	SBDI01	Brazil	2015
P39	SBDJ01	Brazil	2014
P38	SBDK01	Brazil	2016
P88	SBDC01	Brazil	2015
HSP15	SBDD01	Brazil	2016
HSP16	SBDA01	Brazil	2016
P48	SBDH01	Brazil	2016
P71	SBDG01	Brazil	2016
T38_P39_mcr1_tc	VIAI01	Thailand	2018
TUM16641	BFCA01	Japan	-
1849	MZZT01	Brazil	2013

* Only isolates with country information were included in the analysis.

Table S5 – Most similar strains* identified by bacWSGTdb SNP strategy for *K. pneumonia* 508B.

Isolate	Accession number	Country	Collection Year
k1534	FLCC01	United Kingdom	2007
k2037	FLFE01	United Kingdom	2009
k557	FLDM01	United Kingdom	2003
EuSCAPE_AT004	UKBH01	Austria	2014
EuSCAPE_CZ034	UKFW01	Czech Republic	2014
EuSCAPE_AT032	UKBF01	Austria	2013
EuSCAPE_CZ032	UKFP01	Czech Republic	2013

* Only isolates with country information were included in the analysis.