Supplementary Material

Clinical and genomic evolution of carbapenem-resistant *Klebsiella pneumoniae* bloodstream infections over two time periods at a tertiary care hospital in South India - a prospective cohort study

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Article Note

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Microbiology methods for genomic analysis supplement

DNA was extracted from 18- to 24-hour cultures using Wizard Genomic DNA purification Kit (Promega) as per manufacturer's instructions. The DNA was quantified using Nanodrop (Thermo Fisher) and subjected to short-read sequencing using Ilumina HiSeq as per the manufacturer's instructions. Sequencing reads with a PHRED quality score below 20 were discarded, and adapters were trimmed using cutadapt v1.8.1 and assessed with FastQC v0.11.4 (1,2). Draft genome sequence data generated using Illumina were assembled using SPAdes (v.3.13.0) (3). The genome sequences were polished using high-quality Illumina reads, as described previously using Pilon (4). The assembled genomes were subjected to quality assessments using CheckM v1.0.5 and Quast v4.5 (5,6). *K. pneumoniae* NTUH-K2044 (GenBank accession number AP006725) was used as the reference genome since it is a well-characterized type-strain of hypervirulent *K. pneumoniae* ST23.CRhvKp in the study was defined as the presence of rmpA2 and/or aerobactin.

Genome assemblies were submitted to NCBI GenBank and annotated using the NCBI Prokaryotic Genome Annotation Pipeline [PGAP v.4.1] (7). The antimicrobial resistance profiles of the assembled genome sequences were identified using Resfinder v.4.1 available from the CGE server (8). Similarly, the presence of plasmids in the genomes was identified and characterized using PlasmidFinder (v.1.3) available at the CGE server (9). MLST and virulence loci (yersiniabactin, aerobactin, and other siderophore production systems) were identified using Kleborate (v.2.0.0) (10). The presence of virulence factors was confirmed using the virulence database at Pasteur Institute for *K. pneumoniae* (11). Pairwise distances between the nine isolates were determined with *K. pneumoniae* NTUH-K2044 as the reference using SNP-dists v 0.6.3 (12) from the raw reads by aligning the short reads of each isolate against the reference. Single nucleotide

polymorphism (SNP) based phylogenetic trees of the ST14, ST15, ST16, and ST231 isolates which were isolated five years apart were constructed using CSI phylogeny (https://cge.cbs.dtu.dk/se).

CLSI, EUCAST and FDA guidelines used in the study

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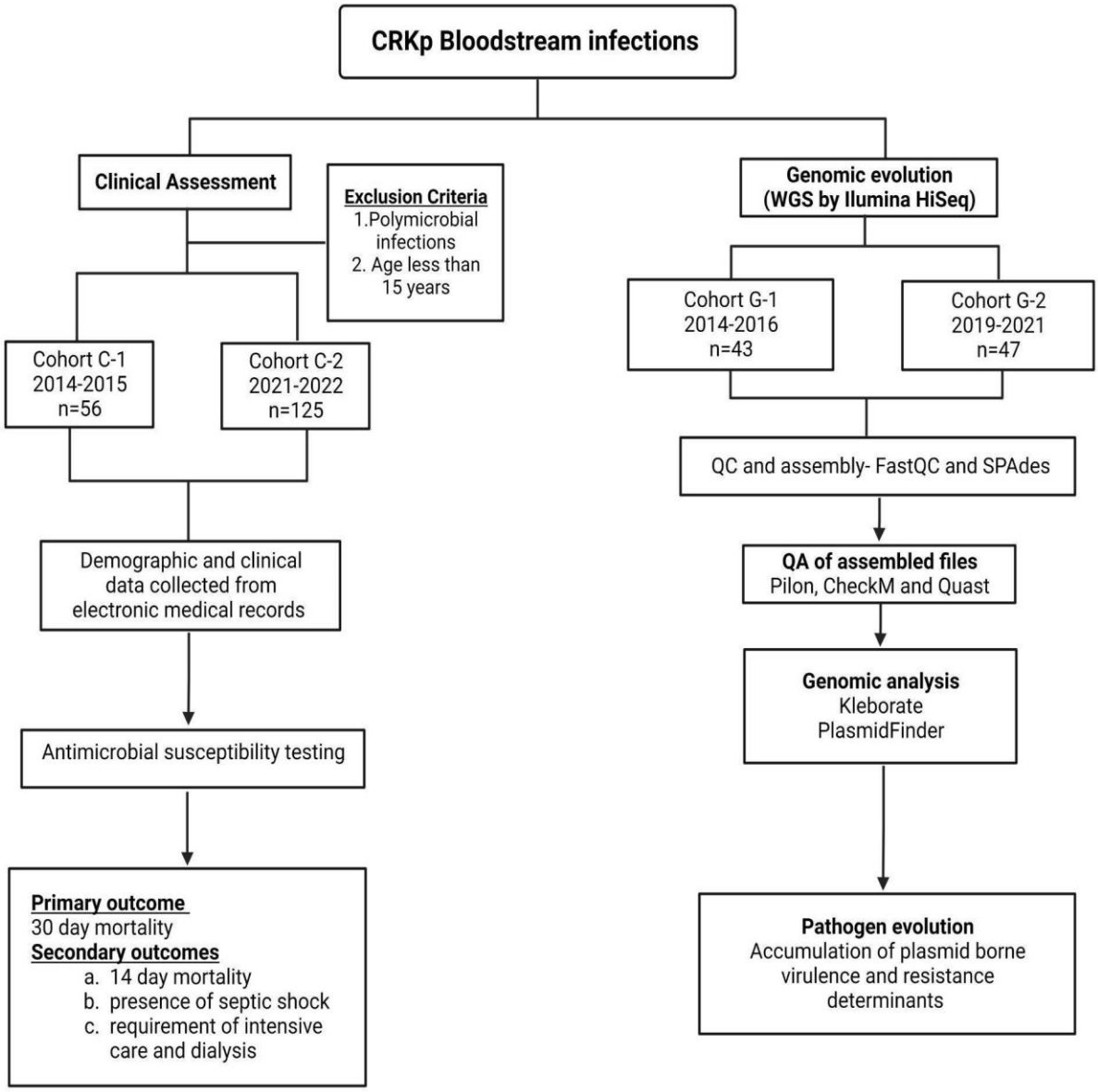

Figure S1: Study algorithm with clinical and genomic methodologies

Table S1:

a) Clinical details of study participants

	Baseline characteristics			
Characteristics	**Total**	**Cohort C-1**	**Cohort C-2**	**p-value**
	n=181(%)	**n=56(%)**	**n=125(%)**	
Risk Factors				
Hematological	72 (39.8)	32 (57.1)	40 (32)	0.001
Non hematological	109 (60.2)	24 (42.9)	85 (68)	
Trauma within 30 days before bacteremia	4 (2.2)	2 (3.6)	2 (1.6)	0.59
Duration of bacteremia:				
Persistent bacteremia	28 (15.47)	12 (21.43)	16 (12.8)	
Cleared bacteremia	117 (64.64)	37 (66.07)	80 (64)	0.03
Death within 48 hours	21 (11.6)	7 (12.5)	14 (11.2)	
No FUBC	15 (8.29)	0	15 (12)	
Therapy types				
Treatment type:				
Combination therapy	62 (34.3)	10 (17.9)	52 (41.6)	
Monotherapy	101 (55.8)	46 (82.1)	55 (44)	<0.001
Not treated appropriately	18 (9.9)	0	18 (14.4)	

Type of Monotherapy (n=101):				
Polymyxin	80 (79.21)	37 (80.43)	43 (78.18)	
CAZ-AVI	12 (11.88)	0	12 (21.82)	<0.001
Other antibiotics	9 (8.91)	9 (19.57)	0	
Type of Combination therapy (n=62):				
Polymyxin based	12 (19.35)	5 (50)	7 (13.5)	
CAZ-AVI based	9 (14.58)	0	9 (17.3)	<0.001
Polymyxin and CAZ-AVI based	36 (58.06)	0	36 (69.2)	
Other antibiotics	5 (8.06)	5 (50)	0	

Abbreviations: ICU, intensive care unit; FUBC, follow-up blood culture; IQR, interquartile range

b) Risk factors for 30-day mortality among study participants

	Outcome		Univariate analysis	
Characteristics	**Alive**	**Death**	**HR**	**p-value**
	n (%)	**n (%)**	**(95% CI)**	
Risk Factors: Hematological	42 (46.15)	30 (33.33)	0.72 (0.46, 1.12)	0.14
Trauma within 30 days before bacteremia	3 (3.3)	1 (1.11)	0.45 (0.06, 3.24)	0.43
Duration of bacteremia:				
Persistent bacteremia	13 (14.29)	15 (16.67)	1.63 (0.91, 2.94)	0.10
Cleared bacteremia	74 (81.32)	44 (48.89)	Ref	
Death within 48 hours	0	20 (22.22)		
No FUBC	4 (4.40)	11 (12.22)		
Therapy types				
Treatment type:				
Combination therapy	34 (37.36)	28 (31.11)	0.85 (0.54, 1.35)	0.50
Monotherapy	49 (53.85)	52 (57.78)	Ref	
Not treated appropriately	8 (8.79)	10 (11.11)	1.29 (0.66, 2.54)	0.46

	Outcome		Univariate analysis	
Characteristics	**Alive**	**Death**	**HR**	**p-value**
	n (%)	**n (%)**	**(95% CI)**	
Type of Monotherapy (n=101):				
Polymyxin	41 (83.67)	39 (75)	Ref	
CAZ-AVI	4 (8.16)	8 (15.38)	1.33 (0.62, 2.85)	0.47
Other antibiotics	4 (8.16)	5 (9.62)	1.34 (0.53, 3.40)	0.53
Type of Combination therapy (n=62):				
Polymyxin based	5 (14.71)	7 (25)	Ref	
CAZ-AVI based	4 (11.76)	5 (17.86)	0.84 (0.27, 2.65)	0.77
Polymyxin and CAZ-AVI based	23 (67.65)	13 (46.43)	0.47 (0.19, 1.17)	0.10
Other antibiotics	2 (5.88)	3 (10.71)	0.99 (0.26, 3.85)	0.99

Abbreviations: FUBC, follow-up blood culture; IQR, interquartile range

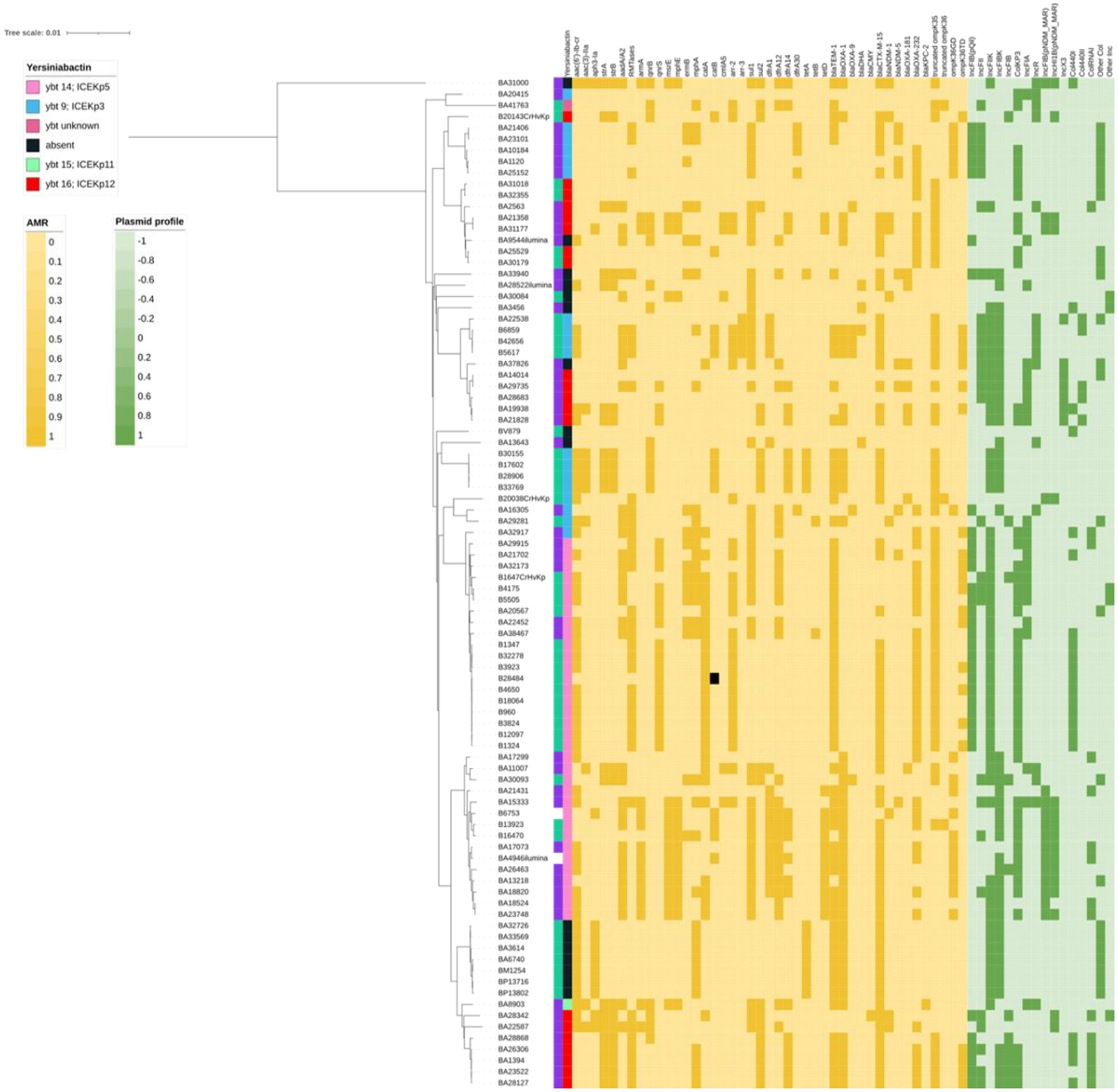

Figure S2: Phylogenetic tree of carbapenem resistant *K. pneumoniae* using Mash distances.

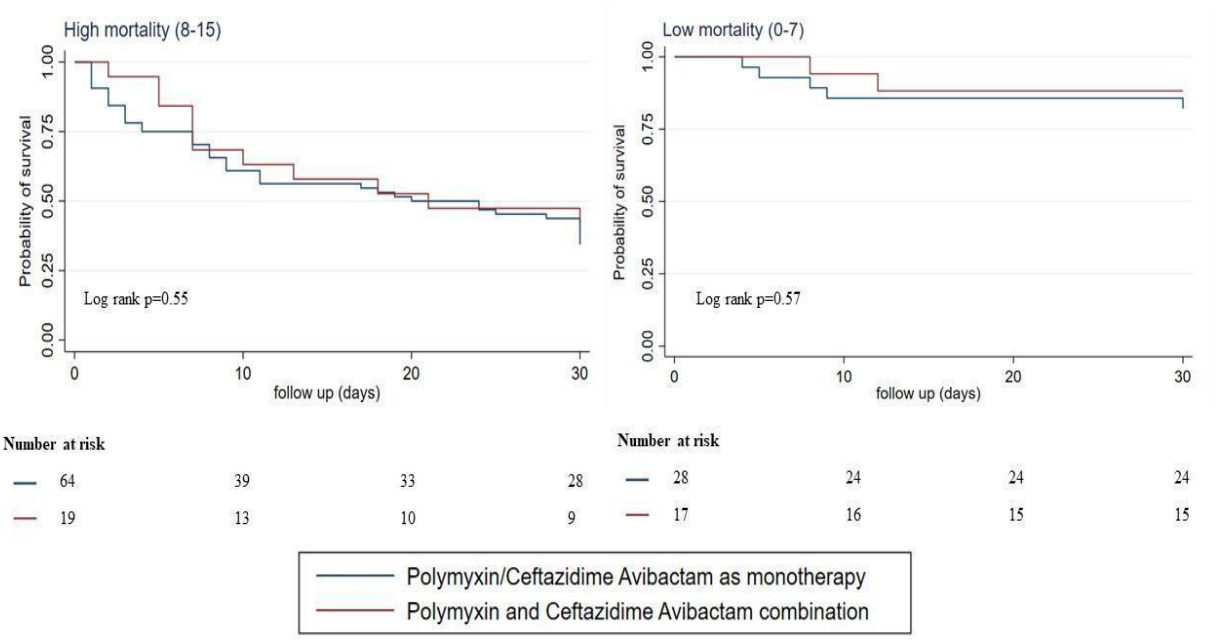

Figure S3: Survival curves among patients who received monotherapy or combination therapy at 30 days as stratified by INCREMENT score

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