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Genomic insights into *Klebsiella pneumoniae*: Virulence, resistance, and transmission in South and Southeast Asia

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ABSTRACT

Background: *Klebsiella pneumoniae* has long posed a significant challenge in clinical settings worldwide, particularly due to its carbapenemase production and multidrug-resistant (MDR) characteristics. While extensive genomic studies of *K. pneumoniae* have been conducted globally, research in Asia, particularly South Asia, remains limited.

Objectives: This study aims to address and compare the genomic characteristics of *K. pneumoniae* isolates from South Asia and Southeast Asia, including virulence, antimicrobial resistance (AMR), plasmids, and mobile genetic elements (MGE) profiles, as well as potential transmission dynamics.

Methods: A total of 463 *K. pneumoniae* genomes were included from collected samples and public databases. All genomes underwent comprehensive analysis, including pan-genome profiling, multi-locus sequence typing (MLST), annotation of virulence factors, AMR genes, plasmids, and MGEs, as well as SNP distance–based analysis to infer transmission dynamics, using established bioinformatic tools.

Results: *K. pneumoniae* isolates exhibited diverse virulence determinants. Hypervirulent isolates were primarily associated with ST23 and ST86, and commonly harbour *aerobactin*, *salmochelin*, and *rmpA*. The majority of isolates were predicted to be MDR, with those from Southeast Asia showing a higher relative abundance of AMR genes associated with the antibiotic classes examined in this study. Among all isolates, the predominant carbapenemase-associated gene was bla_{NDM-1}. *Col440I_1* was the most prevalent plasmid replicon, although it did not co-occur with any AMR genes. Association between the IncFII_1_pKP9 plasmid replicon and resistance genes *sul-5,* bla_{CTX-M}, and bla_{TEM} was found. *ISSen9* was the dominant MGE, frequently co-occurring with the plasmid replicons *IncFIB(K)_1_Kpn3* and *IncFII_1_pKP91*. Transmission analysis indicated that the highest isolate similarity occurred within MLST and country. However, clustering based on plasmid replicon profiles revealed that some clusters comprised isolates from multiple countries.

Conclusion: This study provides a comprehensive analysis of the genomic characteristics and transmission patterns of *K. pneumoniae* in South and Southeast Asia, contributing to our understanding of its virulence and

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resistance mechanisms. These findings further suggest that plasmid replicons may play a critical role in shaping transmission dynamics and provide valuable insights to inform future AMR surveillance and control strategies.

1. Background

Klebsiella pneumoniae (*K. pneumoniae*) is a Gram-negative bacterium responsible for a range of infections, including pneumonia, bloodstream infections, meningitis, and urinary tract infections. It has emerged as a significant global public health threat due to its remarkable adaptability and its ability to acquire resistance and virulence genes via plasmids and mobile genetic elements (MGEs) (Liu et al., 2022). In recent years, the rise of hypervirulent strains has intensified this threat. These strains are distinguished by key virulence factors, including a hypermucoid capsule encoded by *rmpA* and *rmpA2*, alongside siderophores such as aerobactin, yersiniabactin, and salmochelin. These elements significantly enhance pathogenicity and invasiveness, frequently leading to severe and life-threatening infections (Farzana et al., 2019; Spadar et al., 2022; Zhu et al., 2021). Additionally, the carbapenem-resistant *K. pneumoniae* (CRKP) is also becoming a global concern, with the World Health Organization (WHO) identifying them as a priority pathogen (World Health Organization, 2024). The rapid global dissemination of *K. pneumoniae* is driven by human travel, international trade, and interconnected healthcare networks, which have facilitated the widespread prevalence of multidrug-resistant (MDR) and hypervirulent strains, especially in regions like South and Southeast Asia (Heng et al., 2024; Lee et al., 2016; Liu et al., 2024). This inter-regional spread underscores the urgent need for a comprehensive understanding of the genetic landscape of *K. pneumoniae*. Such insights are critical to guiding public health strategies, strengthening infection control measures, and developing effective therapeutic approaches.

Genome analysis is a powerful tool for understanding the mechanisms underpinning *K. pneumoniae*'s virulence, resistance, and transmission. Several approaches contribute to its characterisation, with multi-locus sequence typing (MLST) being a widely utilised method for distinguishing *K. pneumoniae* strains. (Diancourt et al., 2005). While most sequence types (STs) are not confined to specific regions or countries, ST11 has been identified as the dominant strain in China (Heng et al., 2024; Liao et al., 2020). Additionally, hypervirulent *K. pneumoniae* is frequently associated with ST23 and ST86 (Neumann et al., 2023), suggesting the potential of MLST to serve as a determinant for both regional and hypervirulent strains. Beyond MLST and virulence gene profiling, analysing antibiotic resistance genes (ARGs), plasmids, and MGEs offers valuable insights into antibiotic usage patterns and the resistance status of *K. pneumoniae*. Notably, many strains are MDR and exhibit resistance to carbapenems (Heng et al., 2024; Rocha et al., 2022; Spadar et al., 2023; Wang et al., 2024). Decoding the genetic composition of *K. pneumoniae* strains enables tracking the evolution of resistance, identifying unique regional virulence profiles, and understanding transmission dynamics.

This study focuses on the genomic characteristics of *K. pneumoniae* isolates from South Asia (n = 168) and Southeast Asia (n = 296), including their plasmids and MGEs. The findings aim to support the development of targeted interventions, guide treatment decisions, and control the spread of this pathogen across Southern Asia, and beyond.

2. Methods

2.1. Samples collection and K. pneumoniae isolation

A total of 114 suspected *K. pneumoniae* isolates were collected from clinical samples in Pakistan (n = 61) and Bangladesh (n = 53). Of these, 107 were confirmed as *K. pneumoniae* (Pakistan: n = 55, Bangladesh: n = 52). Other *Klebsiella* species identified included *K. variicola* (n = 2), *K. aerogenes* (n = 1), and *K. quasipneumoniae* in Pakistan (n = 3) and Bangladesh (n = 1). In Pakistan, samples were collected from urine, pus, wound swabs, blood, and other body fluids of patients attending Mardan Medical Complex (Mardan) and Ayub Medical Complex (Abbottabad) between 2022 and 2023. Ethical approval was obtained from the KMU Institutional Review Board (Ref: KMU/IPDM/IEC/202229). In Bangladesh, clinical *K. pneumoniae* isolates were cultured from urine and pus specimens collected from patients between 2018 and 2021, as part of a capacity-strengthening project approved by the Research Review Committee and Ethics Review Committee of the icddr,b (protocol number PR-23045). Informed consent was secured from all participants before sample collection. Samples were aseptically inoculated onto MacConkey agar plates (Oxoid, UK), and CHROMagar Orientation agar plates (Oxoid, UK) for preliminary identification of *Klebsiella* species (Mazumder et al., 2023), incubated overnight at 37 °C, and *Klebsiella* colonies were selected based on characteristic morphology. Further biochemical characterisation was carried out as described previously (Mazumder et al., 2022). The verified *K. pneumoniae* isolates were preserved in 80 % glycerol at −80 °C until further analysis.

Before sequencing, all confirmed *K. pneumoniae* isolates were subcultured on nutrient agar to obtain pure culture, and genomic DNA was extracted using the DNeasy Blood & Tissue QIAcube Kit (Qiagen, Hilden, Germany) following the manufacturer's protocol. DNA quantification was determined using the Qubit 4.0 Fluorometer (Life Technologies). The DNA quality control was assessed by NanoDrop spectrophotometer (Thermo Fisher Scientific, US), Quantus Fluorometer (Promega, US), and 1 % agarose gel electrophoresis. The paired-end libraries preparation was constructed from 220 to 250 ng of genomic DNA utilising the Illumina DNA Prep Kit (Illumina) as per the manufacturer's instructions. The normalised and pooled library was subjected to 150-base paired-end reads sequencing using the Mid-output v2.5 sequencing kit. Sequencing was performed at the icddr,b Genome Center and through The Applied Genome Centre (LSHTM) using the NextSeq500 platform.

2.2. Comparative sequence data

K. pneumoniae sequences from various regions across Asia were retrieved and randomly selected from the European Nucleotide Archive (ENA) database. Sequences were sourced from India (PRJEB29740, PRJNA548120; n = 61), and were analysed alongside isolates from Pakistan and Bangladesh sequenced in this study as part of the South Asia group (total n = 167). For the Southeast Asia group (total n = 296), sequences included those from: Myanmar (PRJDB5126, PRJDB8975; n = 42), the Philippines (PRJEB17615; n = 59), Singapore (PRJNA342893, PRJNA547865; n = 56), Thailand (PRJDB4948, PRJDB5929, PRJDB7066, PRJNA389557; n = 66) and Vietnam (PRJDB5317, PRJDB6407; n = 73). All selected sequences were derived from human-origin isolates. Detailed information on the *K. pneumoniae* sequences retrieved from the ENA database is provided (Supplementary Table 1).

2.3. Bioinformatics analysis

All sequences were quality checked with fastqc software (v0.12.1) (Andrew, 2010) and any adaptors were trimmed using trimmomatic (v0.39) (Bolger et al., 2014). The resulting sequences with mean quality scores > 20 then underwent *de novo* assembly using SPAdes software (v4.0.0) (Bankevich et al., 2012), followed by quality assessment with QUAST (v5.2.0) (Mikheenko et al., 2018). Assemblies were analysed to classify isolates into Klebsiella species, identify virulence factors and ARGs, and assign MLSTs (Diancourt et al., 2005) using Kleborate

(v2.3.2) (Lam et al., 2021). Isolates were classified as MDR if they carried resistance genes conferring resistance to more than two antibiotic classes, as determined by Kleborate. Plasmids were identified and extracted from chromosomal sequences using Mob-Suite (v3.1.8) (Robertson and Nash, 2018). Both assemblies and extracted plasmids were annotated with Prokka (v1.14.6) (Seemann, 2014). Core and pan-genome analyses were performed using Roary (v3.12.0) (Page et al., 2015) and a core genome alignment was generated for phylogenetic reconstruction with RAxML (v8.2.12) (Kozlov et al., 2019). The resulting tree was visualised and annotated in iTOL (v7) (Letunic and Bork, 2024). Abricate (v1.0.1) (https://github.com/tseemann/abricate) was used to detect plasmid replicons (PlasmidFinder) (Carattoli et al., 2014), identify ARGs (ResFinder) (Zankari et al., 2012) and AMRFinderPlus (Feldgarden et al., 2019), and screen for virulence genes (VFDB) (Chen et al., 2016). MGEs within plasmids were identified using MobileElementFinder (v1.1.2) (Durrant et al., 2020). Data visualisation was performed in R and Python 3.0.

2.4. Clustering and transmission inference

Plasmid clustering analysis was performed using principal component analysis (PCA) based on plasmid replicon counts. Outliers detected in the PCA plot were removed, and K-means clustering was applied to refine sample groupings. To investigate potential transmission between countries and regions, variant call format (VCF) files were aligned and merged using the Fastq2Matrix pipeline, with *K. pneumoniae* MGH78578 as the reference genome (NCBI accession: NC_009648, Bioproject: PRJNA224116). For SNP distance analysis, the merged VCF files were used as input for the *snp-dists* tool (https://github.com/tseemann/snp-dists) to generate a SNP distance matrix. The SNP distance matrix was analysed to determine clustering thresholds using a Gaussian Mixture Model (GMM) (Ward et al., 2025). Transmission events were visualised using Transmission Graph Viewer (TGV) (Phelan et al., 2024), applying a threshold of 10 SNPs.

2.5. Statistical analysis

The distribution of ARGs, plasmid replicons, and MGE counts was assessed using the Shapiro-Wilk test. Non-parametric tests were applied to compare differences between regions (Mann-Whitney *U* test) and across countries (Kruskal-Wallis test). A p-value < 0.05 was considered statistically significant. All statistical analyses were conducted using Python 3.0.

3. Results

3.1. K. pneumoniae characteristics

Across all *K. pneumoniae* sequences from South Asia (n = 167) and Southeast Asia (n = 296), a total of 96 MLSTs were identified (Table 1; Supplementary Material S1 Table). ST147 was the most prevalent sequence type (n = 77/463, 16.63 %) and was dominant in Bangladesh (n = 12/52, 23.08 %), India (n = 18/60, 30.0 %), Myanmar (n = 13/42, 30.95 %), the Philippines (n = 11/59, 18.64 %), and Singapore (n = 7/56, 12.5 %), making it the most common ST across both regions. In contrast, the dominant STs in Pakistan, Thailand, and Vietnam were ST14 (n = 6/55, 10.91 %), ST231 (n = 15/66, 22.73 %), and ST15 (n = 34/73, 46.58 %), respectively (Supplementary Material S1 Table, S1 Fig). Among the countries analysed, the Philippines exhibited the highest MLST diversity, with 31 unique sequence types, whereas Vietnam had the lowest (12 STs). Hypervirulent clones-related STs were identified including ST23 (n = 7/463, 1.51 %; Pakistan: n = 2, Philippines: n = 3, Singapore: n = 2) and ST86 (Singapore: n = 1/463, 0.22 %) (Supplementary Material S1 Table). The most common O and K loci were O1/O2v1 (n = 235, 50.76 %) and KL64 (n = 103, 18.29 %), respectively (Table 1; Supplementary Material S1 Table). A phylogenetic tree based on core genome alignments showed that *K. pneumoniae* isolates clustered primarily by MLST, as expected (Fig. 1).

Table 1
Klebsiella pneumoniae study isolates (n = 463).

Characteristic	Overall (n = 463)		South Asia (n = 167)		Southeast Asia (n = 296)	
	N	%	N	%	N	%
Country/region						
South Asia	167	36.07	167	100	-	-
Bangladesh	52	11.23	52	31.13	-	-
India	60	12.96	61	36.53	-	-
Pakistan	55	11.88	55	32.93	-	-
Southeast Asia	296	63.93	-	-	296	100.00
Myanmar	42	9.07	-	-	42	14.19
Philippines	59	12.74	-	-	59	19.93
Singapore	56	12.09	-	-	56	18.92
Thailand	66	14.25	-	-	66	22.29
Vietnam	73	15.77	-	-	73	24.66
O locus						
O1/O2v1	235	50.76	97	58.08	138	46.62
O1/O2v2	117	25.05	39	23.25	77	26.01
O3b	49	10.58	11	6.59	38	12.84
O4	28	6.05	7	4.19	21	7.09
OL101	13	2.81	6	3.59	7	2.36
O3/O3a	12	2.59	4	2.40	8	2.70
Other	10	2.16	3	1.80	7	2.36
K locus						
KL64	103	18.29	50	29.94	53	17.91
KL51	56	9.95	19	11.38	37	12.50
KL2	24	4.26	5	2.99	19	6.42
KL62	24	4.26	6	3.59	18	6.08
KL15	19	3.37	2	1.20	17	5.74
KL10	17	3.02	1	0.60	16	5.41
KL17	17	3.02	5	2.99	12	4.05
KL24	12	2.13	6	3.59	6	2.03
Other (56 with freq < 10)	191	33.93	73	43.71	118	39.86
Multidrug resistant (MDR)	439	94.82	149	89.22	290	97.97
Virulence determinants						
Yersiniabactin	285	61.56	115	68.86	170	57.43
Aerobactin	53	11.45	27	16.17	26	8.78
Salmochelin	8	1.73	3	1.80	5	1.69
Colibactin	8	1.73	2	1.20	6	2.03
Hypervirulent rmp genes	7	1.51	2	1.20	5	1.69
rmpA	15	3.24	10	5.98	5	1.69
rmpA2	18	4.09	7	3.89	11	3.72
Multilocus Sequence Typing						
ST147	77	16.63	32	19.16	45	15.20
ST15	46	9.94	3	1.80	43	14.53
ST231	39	8.42	21	12.57	18	6.08
ST14	27	5.83	9	5.39	18	6.08
ST11	22	4.75	4	2.40	18	6.08
ST16	22	4.75	2	1.20	20	6.76
ST101	19	4.10	3	1.80	16	5.41
ST340	11	2.38	11	6.59	0	0.00
ST37	11	2.38	8	4.79	3	1.01
Other (87 with frequency <10)	189	40.82	85	51.19	104	35.14

3.2. Core- and pan-genome analysis of K. pneumoniae

The *K. pneumoniae* pan-genome across all countries comprised 49,868 genes, with a core genome of 3441 genes. In South Asia, 3064 core genes were shared, alongside a pan-genome of 8221 genes. Among South Asian countries, India exhibited the highest number of core genes (3763) and the lowest number of pan genes (12,104), whereas Bangladesh had the largest pan-genome (30,812 genes) and a high number of core genes (3632). Pakistan showed the lowest number of core genes (3377) and a pan-genome of 15,034 genes (Supplementary Material S2A Fig). In Southeast Asia, the regional core genome comprised 2716 genes, with 7865 genes in the pan-genome. Thailand had the highest number of core genes (3721) and the smallest pan-

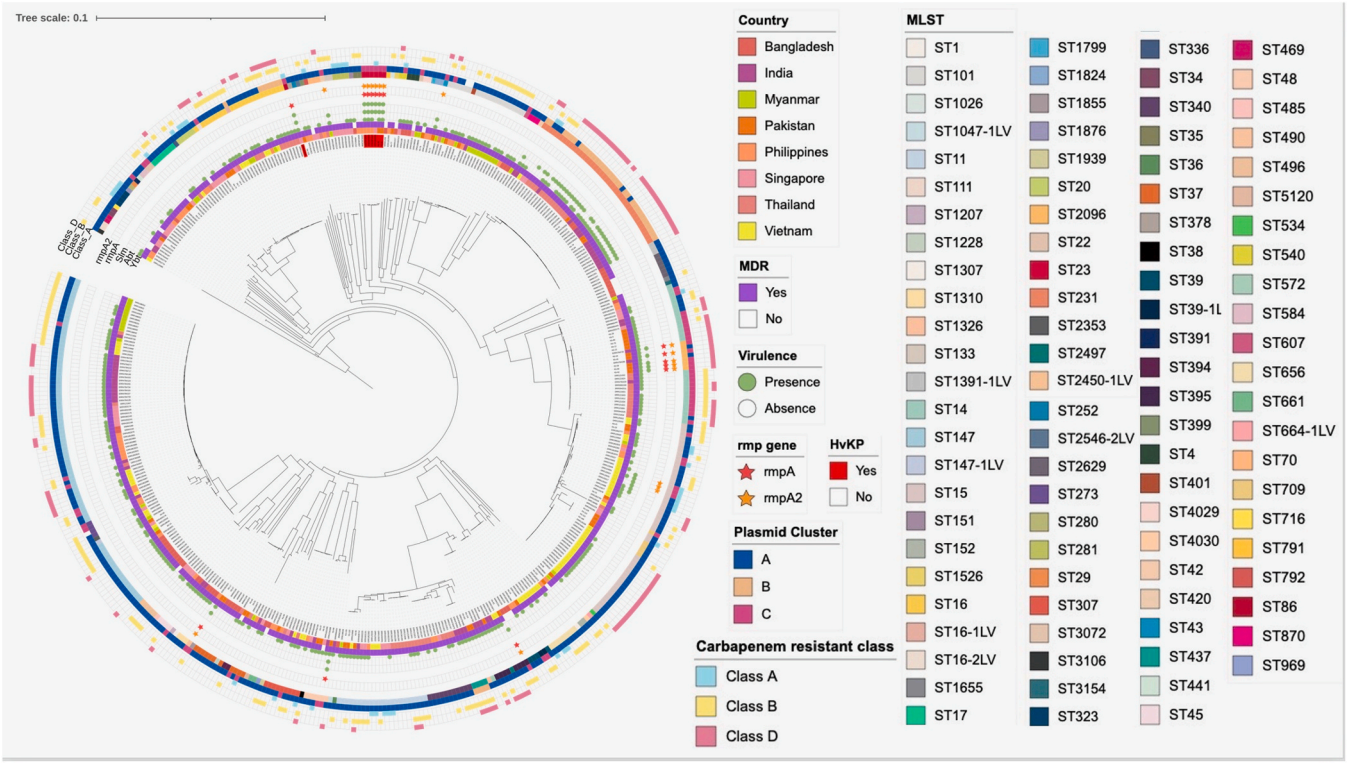

Fig. 1. Phylogenetic tree of *K. pneumoniae* created with a core genome alignment. HvKp is hypervirulent *K. pneumoniae*.

genome (13,140 genes), while Singapore had the lowest core gene count (3277) and the largest pan-genome (16,265 genes) (Supplementary Material S2B Fig).

3.3. Virulence genes found in K. pneumoniae isolates

Yersiniabactin ($n = 285$, 61.56 %) was the most prevalent virulence determinant among all isolates, followed by aerobactin (n = 53, 11.45 %), salmochelin (n = 8, 1.73 %), and colibactin (n = 8, 1.73 %) (Table 1). Notably, all isolates from Bangladesh harboured only yersiniabactin, with no other virulence factors detected (Fig. 1; Supplementary Material S1 Table). Salmochelin was absent in isolates from Myanmar, Thailand, and Vietnam (Fig. 1; Supplementary Material S1 Table). The *rmpA* (n = 15/463, 3.24 %) and *rmpA2* (n = 18/463, 3.89 %) genes were predominantly found in isolates from Pakistan (n = 9/55, 16.36 %). The *rmpA2* gene was present in most hypervirulent-related ST isolates (n = 6/7, 85.71 %). Notably, isolates which were hypervirulent-related STs consistently exhibited a combination of salmochelin, aerobactin, and *rmpA*, a characteristic feature of these isolates (Fig. 1; Supplementary Material S1 Table).

3.4. Antimicrobial resistance genes found in K. pneumoniae

The majority of *K. pneumoniae* isolates were predicted as MDR (n = 439/463, 94.82 %) (Table 1; Fig. 1). Isolates from Southeast Asia generally exhibited a higher ARG count per isolate (mean 18.01, median 18) than those from South Asia (mean 14.86, median 15), with Myanmar having the highest ARG count overall (mean 21.71, median 21). Pakistan was the country in South Asia with the highest ARG count (mean 17.00, median 19) (Supplementary Material S2 Table, S3 Fig). Based on ARG classification by antibiotic class, isolates from Southeast Asia showed a higher relative abundance compared to South Asian isolates in almost every class except Macrolide, Lincosamides, and Streptogramins (MLS) (South Asia 9.0±14.59 %; Southeast Asia 5.39±9.43 %) (Supplementary Material S3 Table). Notably, ARGs related to Peptide antibiotics were found only in Southeast Asian isolates (0.62±1.42 %) (Supplementary Material S3 Table). The number of ARGs per isolate differed significantly across individual countries (Kruskal-Wallis test: $p < 0.001$) and between South Asia and Southeast Asia (Mann-Whitney U test: $p < 0.001$) (Supplementary Material S3a Fig).

A total of 166 unique ARGs were identified, with Southeast Asia harbouring the majority ($n = 146/166$, 87.95 %) (Supplementary Material S2 Table). Singapore had the highest number of unique ARGs (n = 92/166, 55.42 %), whereas Myanmar had the fewest (n = 66/166, 39.76 %) (Supplementary Material S2 Table). Relative abundance analysis showed that $bla_{\text{CTX-M-15}}$ (53.6–95.2 %), *oqxA_1* (92.7–100 %), and *oqxB_1* (71.7–100 %) were highly prevalent across all countries (Supplementary Material S3b Fig). Clustering analysis of ARG patterns revealed that isolates from South Asia and Southeast Asia generally grouped by region, except for Thailand, which clustered with South Asian isolates (Supplementary Material S3c Fig).

3.5. Carbapenem resistant genes prevalence in K. pneumoniae

A total of 10 carbapenem resistant genes were found in *K. pneumoniae* isolates including bla_{KPC-2}, bla_{IMP-14}, bla_{NDM-1}, bla_{NDM-4}, bla_{NDM-5}, bla_{NDM-7}, $bla_{OXA-181}$, $bla_{OXA-232}$, bla_{OXA-23}, and bla_{OXA-48}. The predominant gene found among all isolates was bla_{NDM-1} (n = 109, 23.54 %), while bla_{IMP-14} (n = 1, 0.22 %) and bla_{OXA-23} (n = 1, 0.22 %) were each identified in only one isolate from Thailand and Bangladesh, respectively (Supplementary Material S4 Table). Southeast Asian *K. pneumoniae* isolates harboured nine carbapenemase genes (bla_{KPC-2}, bla_{IMP-14}, bla_{NDM-1}, bla_{NDM-4}, bla_{NDM-5}, bla_{NDM-7}, $bla_{OXA-181}$, $bla_{OXA-232}$, and bla_{OXA-48}), whereas South Asian isolates carried six (bla_{NDM-1}, bla_{NDM-5}, $bla_{OXA-181}$, $bla_{OXA-232}$, bla_{OXA-23}, and bla_{OXA-48}) (Supplementary Material S4 Table). Notably, South Asian isolates carried no class A carbapenem-resistant genes. The Philippines was the only country where the isolates carried only one carbapenem-resistant gene, bla_{NDM-1} (n = 11/59, 18.64 %), which is classified as class B (Supplementary

Material S4 Table).

The class A carbapenem resistant gene, *bla*$_{KPC-2}$, was found mostly in ST15 (n = 10, 23.81 %) (Fig. 1, Supplementary Material S4 Table). Class B resistance genes were identified across various STs, with *bla*$_{NDM-1}$ notably present in multiple STs. ST147 was the ST that exhibited the highest number of class B (*bla*$_{NDM-1}$ n = 27, 24.77 %; *bla*$_{NDM-4}$ n = 4, 19.05 %; and *bla*$_{NDM-5}$ n = 2, 5.41 %) and class D (*bla*$_{OXA-181}$ n = 16, 55.17 %; *bla*$_{OXA-232}$ n = 2, 2.70 %; and *bla*$_{OXA-48}$ n = 6, 22.22 %) resistant genes (Fig. 1, Supplementary Material S4 Table). The least frequently detected genes were the class B *bla*$_{IMP-14}$ and class D *bla*$_{OXA-23}$, found in ST70 and ST540, respectively (Fig. 1, Supplementary Material S4 Table).

3.6. Plasmid replicons found in K. pneumoniae

Among 463 *K. pneumoniae* isolates, a total of 58 plasmid replicons were identified (Supplementary Material S5 Table). The prevalence of unique plasmid replicons was higher in Southeast Asia (56/58, 96.55 %), with Thailand hosting the highest number (35/58, 60.34 %). However, Vietnam had the highest total plasmid count per sample (Supplementary Material S5 Table). A significant difference in plasmid replicon counts per sample was observed across countries after excluding outliers (isolates with ≥20 plasmid replicons; n = 4) (Kruskal-Wallis test: $p < 0.001$) (Supplementary Material S4a Fig). However, there was no significant difference between South and Southeast Asia (Mann-Whitney *U* test: $p = 0.552$) (Supplementary Material S4a Fig). The most prevalent plasmid replicon was *Col440I_1* (10.00 –15.38 %), which was also dominant in India (12.47 %), Thailand (12.50 %), and Vietnam (14.14 %) (Fig. 2). *IncFIB(K)_1_Kpn3* was the most common plasmid in Bangladesh (18.00 %), Pakistan (14.19 %), and Singapore (15.47 %), while *IncFII_1_pKP91* was dominant in Myanmar (17.52 %) and the Philippines (16.41 %) (Fig. 2). *ColKP3* was more abundant in isolates from India (11.00 %) and Thailand (8.02 %) compared to other countries (0.00–5.74 %) (Fig. 2). Analysis of plasmid replicon and ARG co-occurrence revealed the strongest association between *IncFII_1_pKP91* plasmid and multiple ARGs including *sul1_5, mph(A)_2*, and *bla*$_{CTX-M-15\_1}$. Notably, a strong association was also found between *ColKP3_1* and *bla*$_{OXA-232\_1}$. *Col440I_1*, the most dominant plasmid replicon, showed no co-occurrence with any ARGs (Supplementary Material S4b Fig).

Principal Component Analysis (PCA) revealed that most samples clustered together, with a distinct subgroup separating from the main distribution (Supplementary Material S4c Fig). Further analysis with K-means clustering identified three clusters (Supplementary Material S4d Fig), with Cluster A being the largest ($n = 348$) (Supplementary Material S6 Table). The distinct PCA subgroup corresponded to Cluster B, which contained the lowest number of isolates ($n = 44$), but had the highest number of plasmids per isolate (mean = 8.45). The cluster included isolates from Bangladesh ($n = 1$, 2.27 %), India ($n = 18$, 40.91 %), Myanmar ($n = 1$, 2.27 %), Pakistan ($n = 3$, 6.82 %), and Thailand ($n = 21$, 47.73 %) (Supplementary Material S6 Table). Cluster C contained no isolates from Bangladesh (Supplementary Material S6 Table). A comparison of exclusive plasmid replicons across clusters revealed that *Col* plasmids were predominant in Cluster A, whereas *Inc* plasmids were exclusively found in Clusters B and C (Supplementary Material S6 Table).

3.7. Mobile genetic elements (MGEs) found in K. pneumoniae

A total of 460 *K. pneumoniae* isolates carried MGEs. The identified MGEs included composite transposons, insertion sequences, miniature inverted-repeat transposable elements (MITEs), and unit transposons. Across all isolates, 322 unique MGEs were identified (Supplementary Material S7 Table). *ISSen9* ($n = 332/460$, 72.71 %) was the most common MGE, detected in isolates from all countries (Supplementary Material S7 Table). Singapore harboured the highest number of unique

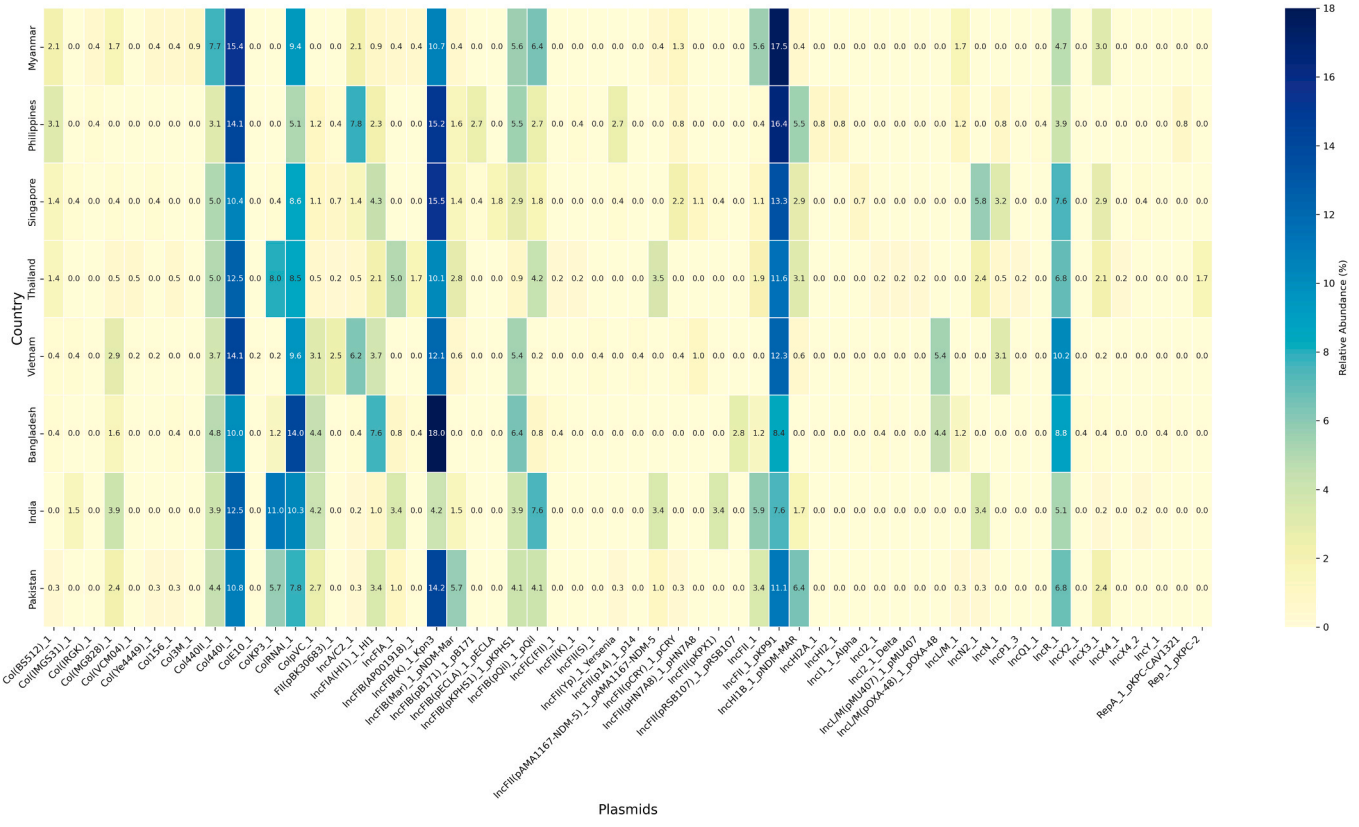

Fig. 2. Heatmap of plasmid replicon relative abundance across samples from all countries.

MGEs (n = 145), while India had the fewest (n = 77) (Supplementary Material S8 Table). The number of unique MGEs varied significantly across countries (Kruskal-Wallis test: $p < 0.001$) and between South Asia and Southeast Asia (Mann-Whitney U test: $p < 0.001$) (Supplementary Material S5a Fig). Many MGEs co-occurred with *IncFIB (K)_1_Kpn3* and *IncFII_1_pKP91*, displaying nearly identical distribution patterns. The most frequent co-occurring MGEs in both plasmid replicons were *ISSen9* (Supplementary Material S5b Fig).

3.8. *K. pneumoniae* cluster and transmission inference

The phylogenetic tree revealed clustering by MLST (Fig. 1), prompting us to investigate potential transmission clusters. A Gaussian Mixture Model (GMM) approach (Supplementary Material S6 Fig) suggested a SNP distance cut-off of 10, identifying 56 clusters comprising of 185 isolates (median size: 2, range: 2–14). Analysis of these clusters showed that isolates from the same country tended to cluster together and shared the same MLST (Fig. 3). Notably, transmission clusters containing ≥ 5 isolates reinforced this pattern of within-country and MLST-based clustering. Interestingly, two transmission clusters in Bangladesh (clusters 53 and 54) exhibited distinct transmission dynamics, with cluster 53 containing ST147 MDR predicted isolates and cluster 54 comprising ST2696 non-MDR predicted isolates (Supplementary Material S9 Table).

4. Discussion

K. pneumoniae is a major pathogenic bacterium responsible for hospital- and community-acquired pneumonia, meningitis, and urethritis worldwide. Characterising *K. pneumoniae* isolates is crucial for understanding their clinical impact and informing treatment and prevention strategies. Beyond *K. pneumoniae*, the *K. pneumoniae* species complex (KpSC), comprising closely related *Klebsiella* species, is also a significant public health concern. In this study, KpSC species detected in Pakistan included *K. variicola*, *K. aerogenes*, and *K. quasipneumoniae*, whereas only *K. quasipneumoniae* was identified in Bangladesh. However, given the relatively small sample size and convenient nature of the sampling, the absence of other KpSC species in Bangladesh cannot be conclusively determined. Previous studies have reported an outbreak of hypervirulent and MDR *K. variicola* ST771 in a neonatal unit in Bangladesh (Farzana et al., 2019). *K. variicola* is increasingly recognised as an emerging pathogen, particularly in immunocompromised patients (Rodríguez-Medina et al., 2019). However, its virulence and AMR profiles remain incompletely characterised. Further research on non-*pneumoniae* KpSC species is warranted to advance understanding of their clinical relevance, epidemiological dynamics, and public health significance.

The number of core and pan genes in *K. pneumoniae* isolates from South and Southeast Asia showed no substantial differences, suggesting similar adaptive pressures. Many of the commonly detected pan genes were ARGs, reflecting exposure to comparable antibiotic selection pressures in clinical settings. This may be attributed to shifting trends in antibiotic usage for treating *K. pneumoniae* infections, alternating between early and later-generation antibiotics (Karampatakis et al., 2023), thereby exposing the bacteria to a diverse range of antimicrobial agents. India and Thailand exhibited the highest number of core genes and the lowest number of pan genes within their respective regions, suggesting limited genetic exchange. In contrast, isolates from Singapore and Bangladesh carried the highest number of pan genes, indicating adaptation to more diverse or dynamic environments. A previous study also described an open pan-genome for clinical *K. pneumoniae* isolates, highlighting their potential to acquire additional genes through

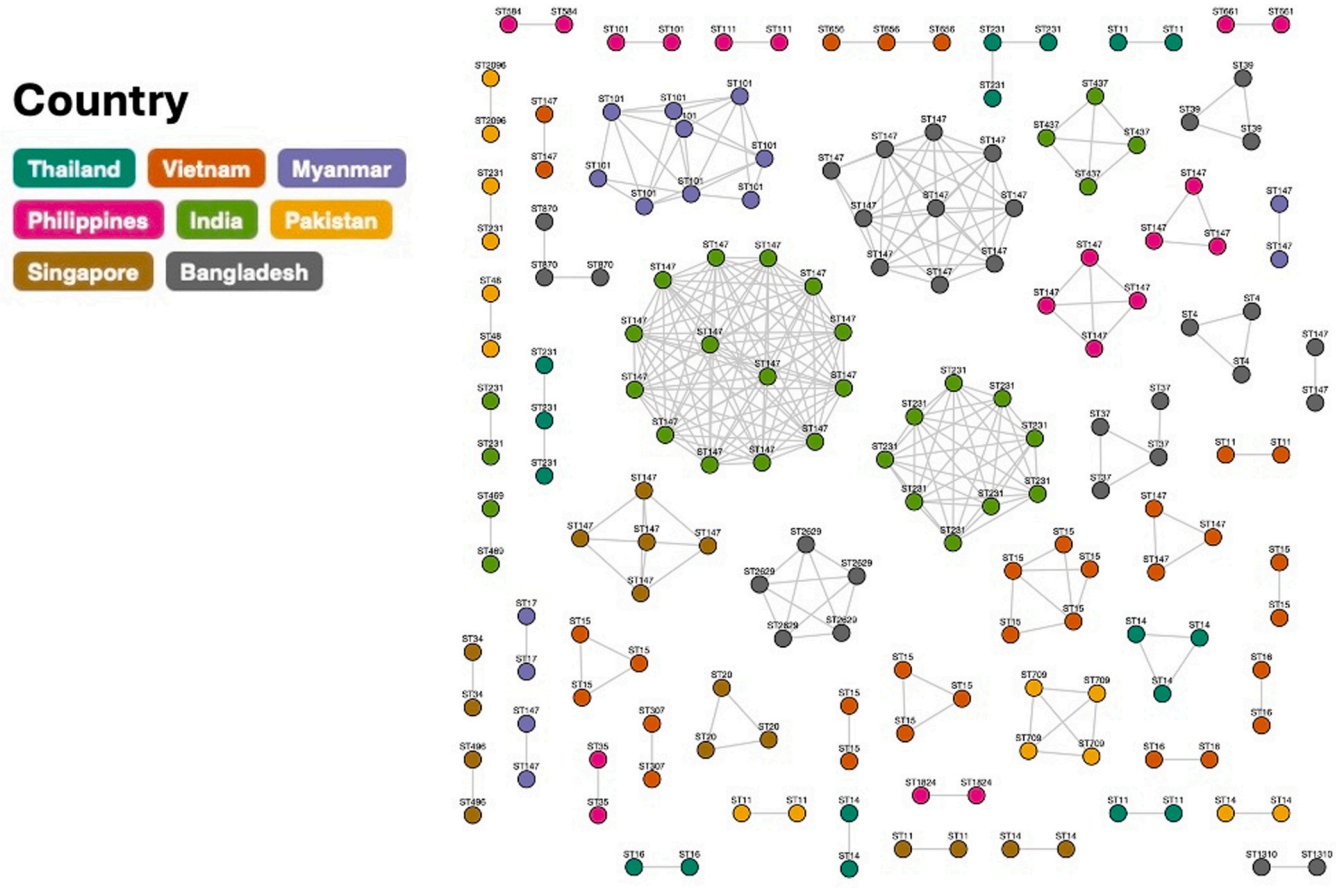

Fig. 3. Transmission network plots with 185 isolates in 56 clusters resulting from a SNP cut-off of 10.

insertion sequences and plasmids (Pustam et al., 2023).

Capsule production and siderophores are key virulence determinants of *K. pneumoniae*, aiding in host adhesion, immune evasion, and iron acquisition (Abbas et al., 2024). Among the four major siderophores (yersiniabactin, salmochelin, aerobactin, and enterobactin), yersiniabactin was the most prevalent (61.56 %), while salmochelin was the least (1.73 %). Notably, Bangladesh isolates lacked both aerobactin and salmochelin, consistent with previous genomic studies suggesting a low prevalence of plasmid-encoded siderophore genes in Bangladesh (Hussain et al., 2023). Myanmar, Thailand, and Vietnam isolates also lacked salmochelin, aligning with previous studies that reported its absence in Thailand but moderate prevalence in Vietnam (Wyres et al., 2020). Hypervirulent *K. pneumoniae* (hvKp), capable of causing more severe infections than classical *K. pneumoniae* (cKp), is characterised by the presence of *rmpA/rmpA2*, regulators of capsule hypermucoviscosity (Choby et al., 2020; Pomakova et al., 2012). In this study, *rmpA* and *rmpA2* were detected in 3.24 % and 4.09 % of isolates, respectively, with none found in Bangladesh or Thailand. Myanmar and Vietnam isolates lacked *rmpA*, suggesting a low prevalence of these hypervirulence-associated plasmids, especially in Southeast Asia isolates. Additionally, 85.71 % of hypervirulent-related STs isolates exhibited both *rmpA/rmpA2*. The data support previous findings that hvKp often requires a combination of virulence-associated genes, including aerobactin and salmochelin (Spadar et al., 2022; Zhu et al., 2021). Notably, in this study, ST23 isolates from Pakistan, India, the Philippines, and Singapore carried both siderophores and *rmpA/rmpA2*, while ST86 isolates from Singapore harboured *rmpA* and siderophores. Future studies should further investigate the genetic basis of hvKp to delineate its pathogenic potential.

Most *K. pneumoniae* isolates in this study were predicted as MDR, consistent with global reports (Kochan et al., 2022; Lee et al., 2016; Pham et al., 2023; Yang et al., 2023). Southeast Asia isolates exhibited a generally higher abundance of ARGs than South Asia isolates. Myanmar isolates had the highest number of resistance genes per isolate (mean 21.71), while Bangladesh had the lowest (mean 12.42). Among South Asian countries, Pakistan had the highest ARG count per sample (mean 17.00). Concerningly, Southeast Asian isolates exhibited the ARGs related to more classes of antibiotics than South Asian ones. A previous study on global antibiotic consumption trends reported a greater increase in antibiotic use in Southeast Asia compared to South Asia (Klein et al., 2024). However, MLS-related ARGs were more prevalent in South Asian isolates, which may be linked to the rising consumption of macrolide antibiotics in middle-income countries (Klein et al., 2024). Notably, Thailand clustered more closely with South Asian countries in terms of ARG profiles, warranting further investigation into shared antimicrobial selection pressures. The most common ARGs detected were $bla_{\text{CTX-M-15}}$, *oqxA_1*, and *oqxB_1*, indicating a high prevalence of extended-spectrum β-lactamase (ESBL)-producing *K. pneumoniae*. This is concerning, as previous studies reported the absence of $bla_{CTX\text{-}M}$ in Thailand in 2020 (Wyres et al., 2020), but our findings suggested otherwise.

Carbapenem-resistant *K. pneumoniae* identified in this study were predominantly found in Southeast Asian isolates. Although class A carbapenemase genes were absent in South Asian isolates, sporadic cases of *K. pneumoniae* producing class A carbapenemases have been reported in India (Munoz-Price et al., 2013). Therefore, periodic surveillance studies in South Asia should be considered to support the prevention and control of these resistant strains. The findings on class B carbapenemase genes align with previous reports indicating that NDM-producing *K. pneumoniae* is endemic in India, Pakistan, and Bangladesh (Nordmann et al., 2011b). Similarly, results from Southeast Asian isolates correspond with studies from Vietnam, which reported the presence of $bla_{KPC\text{-}2}$, $bla_{NDM\text{-}1}$, $bla_{NDM\text{-}4}$, $bla_{OXA\text{-}181}$ and $bla_{OXA\text{-}48}$ (Nguyen et al., 2021; Tada et al., 2017). Isolates from the Philippines carried only one class B carbapenemase gene, $bla_{NDM\text{-}1}$, whereas a previous study had reported the presence of $bla_{\text{NDM-7}}$ in Manila isolates (Chou et al., 2016). This discrepancy may be due to limitations in sample selection. While the current findings confirm the presence of $bla_{\text{NDM-1}}$ in the Philippines, a nationwide surveillance study is recommended to comprehensively identify all circulating carbapenemase genes in the country.

Previous studies have reported the detection of $bla_{\text{KPC-2}}$ in ST258 and ST39 (Gomez et al., 2011; Karampatakis et al., 2022). However, in this study, $bla_{\text{KPC-2}}$ was predominantly identified in ST15, with no detection in ST258 or ST39. Although $bla_{\text{KPC-2}}$ has been frequently reported in *IncFII(pHN7A8)/IncR*-type and *IncHI1B/IncFIB(K)*-type plasmids (Han et al., 2024), our findings suggest that it was not associated with any of the plasmid replicons detected in this study. Class B and class D carbapenemases were most commonly found in ST147, whereas previous studies identified ST11 as the predominant sequence type among carbapenemase-producing strains (Izdebski et al., 2022; Nordmann et al., 2011a). This finding suggests a potential spread of these resistance genes to other STs, underscoring the need for further investigation into their transmission pathways.

Plasmid replicon analysis revealed no region-specific patterns. The most prevalent replicons were *Col440I_1*, *IncFIB(K)_1_Kpn3*, and *IncFII_1_pKP91*, consistent with previous studies from Bangladesh and India (Hussain et al., 2023; Nagaraj et al., 2021). *IncFIB(K)_1_Kpn3* was associated with $bla_{CTX\text{-}M}$, aligning with prior reports (Zhou et al., 2023). This study has also documented an association between *IncFII_1_pKP91* and *mph(A)*, *sul-5*, $bla_{CTX\text{-}M}$, and bla_{TEM}. Interestingly, *Col440I* did not co-occur with the top ARGs, contradicting previous reports linking it to $bla_{OXA\text{-}1}$ and $bla_{SHV\text{-}28}$ (Hussain et al., 2023), suggesting a need for further exploration of its resistance-carrying potential. The *ColKP3* replicon was highly abundant in Indian and Thai isolates, aligning with prior reports (Nagaraj et al., 2021; Takeuchi et al., 2022). This replicon was co-associated with $bla_{OXA\text{-}232}$, consistent with previous studies linking it to bla_{OXA} genes (Sands et al., 2021). Notably, *ColKP3* has been implicated in co-transferring last-line antibiotic resistance and aerobactin operons as part of an *IncFIBk-FII-X3-ColKP3* hybrid plasmid. However, the absence of *IncFIBk-FII* in this study suggests a low likelihood of hybrid plasmid formation in South and Southeast Asia. The most prevalent MGE identified was *ISSen9*, which frequently co-occurred with *IncFIB(K)_1_Kpn3* and *IncFII_1_pKP9*. While *ISSen9* has not previously been reported as a dominant MGE in *K. pneumoniae*, it has been implicated in the transfer of tet(X4) in *Escherichia coli* (Zhang et al., 2023). These findings emphasise the need for further genomic and MGE-focused studies in Asia to better understand their role in resistance gene dissemination.

SNP-based transmission analysis identified 56 clusters, each containing a single ST and country, aligning with phylogenetic analyses. This supports prior findings that *K. pneumoniae* clusters primarily by ST rather than geographic origin (Heng et al., 2024). Previous transmission studies also reported limited transmission within single sources or regions (Dereeper et al., 2022). However, plasmid replicon-based clustering revealed multiple-country clusters (Spadar et al., 2023), suggesting that plasmids could provide additional insights into transmission dynamics. Notably, the isolation year was not included in the transmission analysis due to limited metadata availability. Future investigations should integrate plasmid-based analyses with whole-genome approaches and detailed temporal data to provide a more comprehensive understanding of bacterial dissemination.

This study provides a comprehensive genomic characterisation of *K. pneumoniae* isolates from South and Southeast Asia, highlighting their MDR nature and the widespread presence of key plasmid replicons. Notably, we report for the first time an association between *IncFII_1_pKP9* and ARGs (*mph(A)*, *sul-5*, $bla_{CTX\text{-}M}$, and bla_{TEM}). While *K. pneumoniae* isolates primarily clustered by MLST, plasmid replicon-based clustering suggests potential cross-border transmission. These findings highlight the importance of incorporating plasmid replicon analyses in transmission studies to better understand the spread of *K. pneumoniae* and inform containment strategies. However, since sequences from India, Myanmar, the Philippines, Thailand, Singapore, and

Vietnam were obtained through convenience sampling from the ENA database and originate from different time points and study settings, this variation may introduce biases that limit the accuracy of inter-country transmission analyses. To validate transmission patterns and expand on our AMR findings, a larger-scale, systematically designed surveillance study is needed. This should incorporate standardised sampling, patient contact tracing, environmental sampling, and social network data across multiple regions and timeframes to provide a more comprehensive understanding of *K. pneumoniae* transmission dynamics. Despite the limitations, this study provided valuable insights into the potential transmission dynamics of *K. pneumoniae* across the Asian region, laying the groundwork for future research that could enhance public health interventions.

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Declaration of Competing interest

The authors declare that there are no conflicts of interest

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Repositories

The genome data *for Klebsiella pneumoniae* isolates generated in this study have been deposited in European Nucleotide Archive (ENA) database under project accession PRJEB87707.

Data statement

Supplementary figures and tables are available with the online version of this article.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ijmm.2025.151666.

Data availability

The data and code have been made available.

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