

Supplementary Information

Supplementary Methods

The calculation and definition of antimicrobial resistance score

The resistance score was calculated by Kleborate software v2.3.2 and the definition was: 0, without extended-spectrum beta-lactamase (ESBL) and carbapenemase; 1, positive for ESBL and no carbapenemase; 2, positive for carbapenemase without colistin resistance; and 3, positive for carbapenemase with colistin resistance as previously described¹. An EBSL positive isolate was defined by a resistance score equal to one and a carbapenem resistance phenotype was defined as a resistance score of more than one (> 1). Additionally, multi-drug resistance (MDR) Kp was defined by possessing AMR genes conferring resistance to more than two different classes of antimicrobials.

Supplementary Results

Clinical characteristics of the cohort

In the overall cohort, 56.2% (662/1179) of patients were male, with a median age of 72.0 years (IQR 59.0-84.0) (Supplementary Fig. 1A). Clinical isolates originated from six different specimen types. The majority were from respiratory tract (605/1179, 51.3%), followed by urogenital tract (276/1179, 23.4%), bloodstream (141/1179, 12.0%), abdominal cavity (64/1179, 5.4%), and skin and soft tissues (40/1179, 3.4%). An additional 63 isolates were collected from other specimen types. Males showed a significantly higher percentage of high CCI ($\text{CCI} \geq 3$) with more comorbid conditions compared to the females (55.9% vs 43.5%, $P < 0.001$). Cardiovascular diseases (73.0% vs 54.9%, $P < 0.001$), digestive diseases (39.4% vs 32.1%, $P = 0.001$), urologic diseases (36.4% vs 26.3%, $P < 0.001$), cerebrovascular diseases (41.1% vs 32.1%, $P = 0.001$), and nonsolid tumor (4.5% vs 1.9%, $P = 0.022$) were significantly more common in males compared to females. Additionally, patients across different specimen types also showed significant differences in CCI ($P < 0.001$). Those with respiratory tract infections had the highest CCI (3.6 ± 2.6), while

patients with skin and soft tissue infections showed the lowest CCI (1.8 ± 1.8) (Figure 1B). The overall ICU admission rate was 29.2% (345/1179). Over half of the infections were nosocomial (692/1179, 58.7%), of which 61.4% were originated from the respiratory tract. The 22.9% (270/1179) of infections belonged to healthcare-associated (HCAI) and 18.4% (217/1179) of infections were community-acquired (CAI). The mortality rates at 14, 28, and 90 days were 15.4% (148/960), 20.5% (197/960), and 27.1% (260/960), respectively. Meanwhile, poor prognosis was observed in 16.5% (195/1179) of patients at 14 days, 22.1% (261/1179) at 28 days, and 33.0% (389/1179) at 90 days. Both showed an increasing incidence over time.

Virulence-associated factors among three cohorts

The mean virulence score was 2.0 ± 1.9 in the overall cohort. There was a significant difference in virulence score among groups with different number of virulence factors (VFs) ($P < 0.001$). The hvKp-p isolates showed the highest virulence score (4.0 ± 0.9), followed by cKp-p isolates with 1 to 4 VFs (3.7 ± 1.4) and cKp-p isolates with 0 VF (0.5 ± 0.5) (Supplementary Fig. 2B).

Overall prevalence of *iucA*-positive Kp was 42.24% (498/1179). *IucA*-positive isolates were detected in all specimen type and were more common in the respiratory tract infection (280/498, 56.2%). The prevalence of *iroB*-positive (35.3%, 416/1179), *peg-344*-positive (38.5%, 454/1179), and *rmpA*-positive (35.6%, 420/1179) isolates was similar. Interestingly, *rmpA2*-positive Kp were only detected in 157 (13.3%) isolates (Supplementary Fig. 2C).

Antimicrobial resistance among three cohorts

The overall prevalence of ESBL genes among the Kp isolates was 18.4% (217/1179), but varied among specimen types from 12.5% in the abdominal cavity to 28.6% in the urogenital tract. The overall prevalence of CRKP genes was 22.9% (271/1179) and the *bla_{KPC}* genes were detected in 257 isolates (21.7%). Furthermore, the prevalence of MDR also differed between specimen types and ranged from 32.6%

in bloodstream to 54.7% in urogenital tract (Supplementary Fig. 2D). While one of the hvKp-p isolates was identified as MDR according to previous definition¹, the majority of cKp-p isolates were classified as MDR (0.8% vs 53.1%; P<0.001) (Supplementary Table 8 and Supplementary Fig. 2E). Furthermore, no hvKp-p isolates carried *bla*_{KPC-like} and CRKP (Figure 3G).

Reference

1. Liu, C., *et al.* An increased prevalence of carbapenem-resistant hypervirulent *Klebsiella pneumoniae* associated with the COVID-19 pandemic. *Drug resistance updates : reviews and commentaries in antimicrobial and anticancer chemotherapy* **77**, 101124 (2024).

Supplementary Tables

Supplementary Table 1. Baseline characteristics of hvKp-p and cKp-p cohorts after PSM.

Items	hvKp-p cohort (n=126)		cKp-p cohort (n=126)		P value
Basic demographics					
Age (yr) (means ± SD)	63.4±18.0		63.4±23.6		0.995
Male	52/126	41.3%	54/126	42.9%	0.799
Underlying disease					
Diabetes mellitus	49/126	38.9%	47/126	37.3%	0.795
Cardiovascular disease	73/126	57.9%	82/126	65.1%	0.244
Pulmonary disease	25/126	19.8%	21/126	16.7%	0.514
Digestive disease	35/126	27.8%	35/126	27.8%	1.000
Urinary disease	26/126	20.6%	26/126	20.6%	1.000
Rheumatic disease	5/126	4.0%	5/126	4.0%	1.000
Cerebrovascular disease	41/126	32.5%	44/126	34.9%	0.689
Cancer	22/126	17.5%	25/126	19.8%	0.628
Non-solid tumor	4/126	3.2%	2/126	1.6%	0.409
CCI≥3	49/126	38.9%	49/126	38.9%	1.000
Acquisition site					
CAI	30/126	23.8%	30/126	23.8%	1.000
HAI	58/126	46.0%	58/126	46.0%	1.000
HCAI	38/126	30.2%	38/126	30.2%	1.000
Social economics					
Superior medical insurance	95/110	86.4%	101/116	87.1%	0.876
Urban residence	106/125	84.8%	103/124	83.1%	0.709
ICU admission	25/126	19.8%	25/126	19.8%	1.000

The P value <0.05 was shown in the bold values. Subheadings are presented in bold. CCI: Charlson comorbidity index; CAI:

Community-acquired infection; HAI: Hospital-acquired infection; HCAI: Health care-associated infection. Two-sided Chi-square and t-tests were performed.

Supplementary Table 2. Baseline characteristics of hvKp-p and cKp-p sub-cohort 1* before and after PSM.

	Unmatched cohorts					Matched cohorts				
Items	hvKp-p cohort		cKp-p sub-cohort 1*		P value	hvKp-p cohort		cKp-p sub-cohort 1		P value
	(n=127)		(n=436)			(n=115)		(n=115)		
Basic demographics										
Age (yr) (means ± SD)	63.3±18.0		67.9±19.1		0.015	64.0±18.2		66.1±19.6		0.408
Male	74/127	58.3%	274/436	62.8%	0.350	70/115	60.9%	69/115	60.0%	0.893
Underlying disease										
Diabetes mellitus	50/127	39.4%	183/436	42.0%	0.600	44/115	38.3%	37/115	32.2%	0.334
Cardiovascular disease	73/127	57.5%	298/436	68.3%	0.023	69/115	60.0%	60/115	52.2%	0.232
Pulmonary disease	25/127	19.7%	81/436	18.6%	0.779	23/115	20.0%	23/115	20.0%	1.000
Digestive disease	35/127	27.6%	148/436	33.9%	0.176	31/115	27.0%	35/115	30.4%	0.560
Urinary disease	26/127	20.5%	146/436	33.5%	0.005	25/115	21.7%	21/115	18.3%	0.510
Rheumatic disease	5/127	3.9%	24/436	5.5%	0.482	5/115	4.3%	8/115	7.0%	0.392
Cerebrovascular disease	41/127	32.3%	143/436	32.8%	0.913	40/115	34.8%	29/115	25.2%	0.113
Cancer	22/127	17.3%	100/436	22.9%	0.177	20/115	17.4%	30/115	26.1%	0.110
Non-solid tumor	4/127	3.1%	11/436	2.5%	0.700	4/115	3.5%	3/115	2.6%	0.701
CCI≥3	49/127	38.6%	222/436	50.9%	0.014	47/115	40.9%	44/115	38.3%	0.686
Acquisition site										
CAI	31/127	24.4%	92/436	21.1%	0.427	30/115	26.1%	38/115	33.0%	0.248
HAI	58/127	45.7%	248/436	56.9%	0.026	53/115	46.1%	57/115	49.6%	0.597
HCAI	38/127	29.9%	96/436	22.0%	0.066	32/115	27.8%	20/115	17.4%	0.059
Social economics										
Superior medical insurance	95/110	86.4%	351/404	86.9%	0.887	85/99	85.9%	95/106	89.6%	0.411
Urban residence	107/126	84.9%	366/431	84.9%	1.000	98/114	86.0%	87/113	77.0%	0.082
ICU admission	25/127	19.7%	116/436	26.6%	0.113	24/115	20.9%	20/115	17.4%	0.502

The P value <0.05 was shown in the bold values. Subheadings are presented in bold. *: cKp-p sub-cohort 1 consist of isolates that possess any combination of less than 5 of the biomarkers *iucA*, *iroB*, *rmpA*, *rmpA2*, and *peg-344*. Two-sided Chi-square and t-tests were performed.

Supplementary Table 3. Clinical difference between hvKp-p and cKp-p sub-cohort 1 after PSM.

Items	hvKp-p cohort (n=115)		cKp-p sub-cohort 1 (n=115)		P value
Risk factor					
Alcohol use	28/115	24.3%	21/115	18.3%	0.260
Immunosuppression	25/115	21.7%	26/115	22.6%	0.874
Severe infection					
Sepsis	71/115	61.7%	47/115	40.9%	0.002
Septic shock	27/115	23.5%	21/115	18.3%	0.330
Treatment interventions					
Oxygen therapy	47/115	40.9%	35/115	30.4%	0.099
Respiratory support	35/115	30.4%	22/115	19.1%	0.047

Non-invasive mechanical ventilation	11/115	9.6%	11/115	9.6%	1.000
Invasive mechanical ventilation	25/115	21.7%	16/115	13.9%	0.121
ECMO	2/115	1.7%	0/115	0.0%	0.155
Vasoactive agents	24/115	20.9%	22/115	19.1%	0.742
Dopamine	2/115	1.7%	5/115	4.3%	0.250
Norepinephrine	18/115	15.7%	12/115	10.4%	0.240
Metaraminol	6/115	5.2%	8/115	7.0%	0.581
Renal replacement therapy	5/115	4.3%	2/115	1.7%	0.250
Debridement	8/115	7.0%	7/115	6.1%	0.789
Drainage	17/115	14.8%	27/115	23.5%	0.094
Outcomes					
Mean days of hospital stay	12.2 ± 18.9		11.2 ± 11.9		0.619
Initial treatment failure	25/115	21.7%	20/115	17.4%	0.406
14-day poor prognosis	22/115	19.1%	14/115	12.2%	0.147
14-day mortality*	17/99	17.2%	9/99	9.1%	0.092
28-day poor prognosis	27/115	23.5%	20/115	17.4%	0.252
28-day mortality*	19/99	19.2%	15/99	15.2%	0.451
90-day poor prognosis	35/115	30.4%	25/115	21.7%	0.133
90-day mortality*	20/99	20.2%	17/99	17.2%	0.584
Re-admission	5/115	4.3%	12/115	10.4%	0.078
Re-infection	6/115	5.2%	12/115	10.4%	0.141

Supplementary Table 4. Baseline characteristics of hvKp-p and cKp-p sub-cohort 2* before and after PSM.

CAI	31/127	24.4%	94/616	15.3%	0.012	29/118	24.6%	21/118	17.8%	0.203
HAI	58/127	45.7%	386/616	62.7%	<0.001	57/118	48.3%	63/118	53.4%	0.435
HCAI	38/127	29.9%	136/616	22.1%	0.057	32/118	27.1%	34/118	28.8%	0.772
Social economics										
Superior medical insurance	95/110	86.4%	477/570	83.7%	0.481	88/103	85.4%	89/112	79.5%	0.251
Urban residence	107/126	84.9%	525/600	87.5%	0.433	102/117	87.2%	95/115	82.6%	0.331
ICU admission	25/127	19.7%	204/616	33.1%	0.003	24/118	20.3%	23/118	19.5%	0.871

The P value <0.05 was shown in the bold values. Subheadings are presented in bold. *: cKp-p sub-cohort 2 consists of isolates that possess none of these biomarkers. A two-sided Chi-square test was performed.

Supplementary Table 5. Genomic characteristics of the enrolled isolates.

Items	hvKp-p cohort (n=127)		cKp-p cohort (n=1052)		P value
Sequence type, ST					
ST11	0/127	0.0%	241/1052	22.9%	<0.001
ST15	0/127	0.0%	32/1052	3.0%	0.046
ST23	39/127	30.7%	98/1052	9.3%	<0.001
ST29	6/127	4.7%	16/1052	1.5%	0.012
ST37	0/127	0.0%	32/1052	3.0%	0.046
ST65	7/127	5.5%	32/1052	3.0%	0.142
ST86	10/127	7.9%	17/1052	1.6%	<0.001
ST307	0/127	0.0%	61/1052	5.8%	0.005
ST412	24/127	18.9%	14/1052	1.3%	<0.001
Other STs, n(%)	41/127	32.3%	509/1052	48.4%	<0.001
Capsule K locus, KL					
KL1	45/127	35.4%	111/1052	10.6%	<0.001
KL2	31/127	24.4%	86/1052	8.2%	<0.001
KL19	0/127	0.0%	38/1052	3.6%	0.029
KL25	0/127	0.0%	35/1052	3.3%	0.037
KL47	0/127	0.0%	116/1052	11.0%	<0.001
KL57	35/127	27.6%	28/1052	2.7%	<0.001
KL64	1/127	0.8%	121/1052	11.5%	<0.001
KL102	0/127	0.0%	82/1052	7.8%	0.001
Other K loci, n(%)	15/127	11.8%	435/1052	41.3%	<0.001
O-Antigen type					
O1	78/127	61.4%	390/1052	37.1%	<0.001
O2	3/127	2.4%	99/1052	9.4%	0.008
O2a	4/127	3.1%	100/1052	9.5%	0.017
O2afg	10/127	7.9%	111/1052	10.6%	0.348
O3/3a	3/127	2.4%	22/1052	2.1%	0.841
O3b	25/127	19.7%	88/1052	8.4%	<0.001
O5	0/127	0.0%	37/1052	3.5%	0.032
O101	1/127	0.8%	133/1052	12.6%	<0.001
Other O type, n(%)	3/127	2.4%	72/1052	6.8%	0.051
Plasmid type					

Col	0/127	0.0%	8/1052	0.8%	0.324
Col156	0/127	0.0%	32/1052	3.0%	0.046
ColRNAI	10/127	7.9%	422/1052	40.1%	<0.001
IncFII	13/127	10.2%	559/1052	53.1%	<0.001
IncHI1B	80/127	63.0%	320/1052	30.4%	<0.001
IncR	1/127	0.8%	238/1052	22.6%	<0.001
IncQ1	0/127	0.0%	22/1052	2.1%	0.100
<i>Peg-589</i>	126/127	99.2%	349/1052	33.2%	<0.001

The P value <0.05 was shown in the bold values. Subheadings are presented in bold. A two-sided Chi-square test was performed. Gene names are presented in italics.

Supplementary Table 6. Virulence gene combinations of cKp-p isolates.

<i>iucA</i>	<i>iroB</i>	<i>peg-344</i>	<i>rmpA</i>	<i>rmpA2</i>	Total
0	0	0	0	0	616
1	1	1	1	0	204
1	0	0	0	0	79
0	1	1	1	0	48
1	0	1	1	0	40
1	0	0	0	1	24
0	1	1	0	0	16
1	1	1	0	0	11
1	0	1	0	0	4
1	1	1	0	1	4
1	1	0	0	0	2
1	1	0	0	1	2
0	1	0	0	0	1
1	1	0	1	0	1

Gene names are presented in italics.

Supplementary Table 7. Virulence gene combinations of cKp-p isolates.

Virulence gene combinations	Total
0	616
1	80
2	46
3	102
4	208

Virulence gene combinations: The classification of virulence gene combinations is based on the number of positive detections for five virulence genes: *iucA*, *iroB*, *peg-344*, *rmpA*, and *rmpA2*. Gene names are presented in italics.

Supplementary Table 8. Antimicrobial resistance.

Items	hvKp-p cohort (n=127)		cKp-p cohort (n=1052)		P value
AMR score (mean± SD)	0.0±0.1		0.7±0.9		<0.001
Resistance*	2/127	1.6%	486/1052	46.2%	<0.001

ESBL ^a	2/127	1.6%	215/1052	20.4%	<0.001
CRKP ^b	0/127	0.0%	271/1052	25.8%	<0.001
KPC	0/127	0.0%	257/1052	24.4%	<0.001
MDR ^c	1/127	0.8%	559/1052	53.1%	<0.001

The P value <0.05 was shown in the bold values. *: Antimicrobial resistance was defined by the resistance score; a: extended-spectrum beta-lactamase (ESBL) - positive isolates were defined by a resistance score of 1; b: Carbapenem-resistant Klebsiella pneumoniae (CRKP) was defined by a resistance score ≥ 2 ; c: Multi-drug-resistant (MDR) was defined by possessing antimicrobial resistance (AMR) genes conferring resistance to more than two antimicrobial classes. Two-sided Chi-square and t-tests were performed.

Supplementary Table 9. Clinical and molecular characteristics of the ESBL-producing hvKp-p isolates.

Items	Patient 1	Patient 2
Clinical characteristics		
Date of collection (yr)	2022	2022
Department	Internal Medicine	Emergency Department
Gender	Female	Female
Age (yr)	81-85	86-90
Alcohol use	-	-
Immunosuppression	-	-
CCI	6	1
Medical insurance	Superior medical insurance	Superior medical insurance
Permanent residence	Urban residence	Urban residence
Infection type	Urinary infection	Bacteremia
Acquisition site	Hospital-acquired infection	Community-acquired infection
ICU admission	-	-
Respiratory Support	-	Non-invasive mechanical ventilation
Vasoactive agents	-	-
Renal replacement therapy	-	-
Debridement	-	-
Drainage	-	-
Sepsis	+	+
Septic shock	-	+
Length of stay (days)	6	1
Initial treatment failure	-	+
Clinical outcomes	Survived over 90 days	Died within 14 days
Re-infection	-	-
Re-admission	-	-
Genomic characteristics		
Sequence type	ST374	ST23
Capsule K locus	KL2	KL1
O-Antigen type	O1	O1
<i>Peg-589</i>	-	+

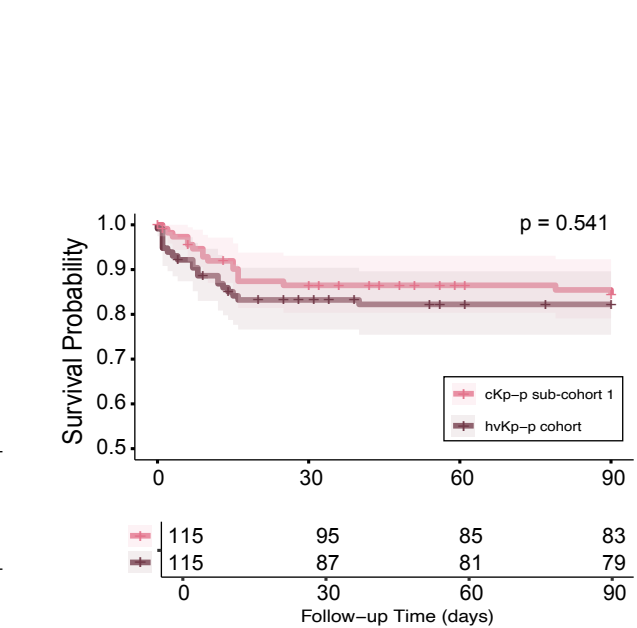
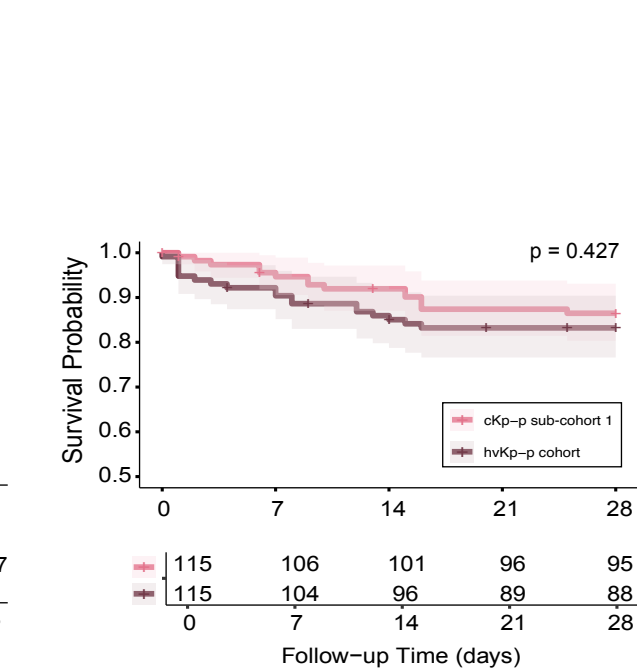
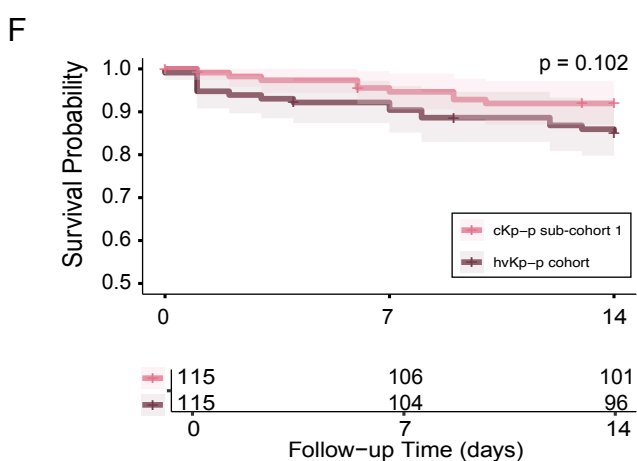
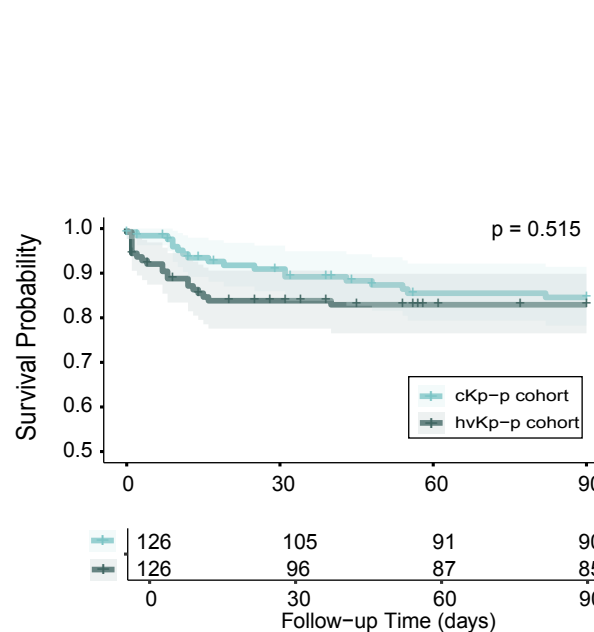
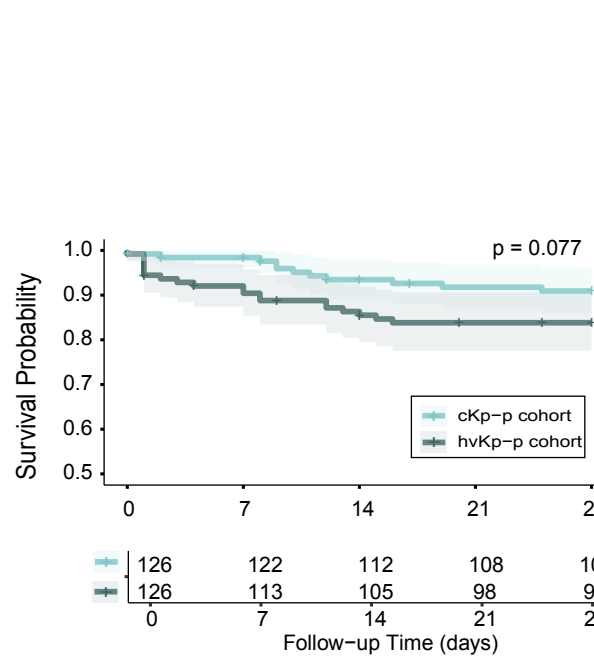
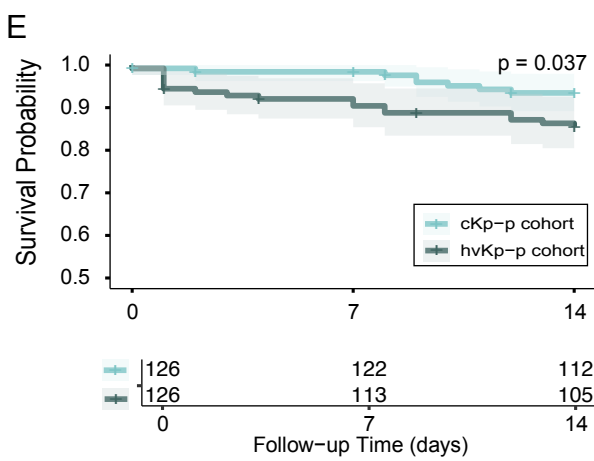
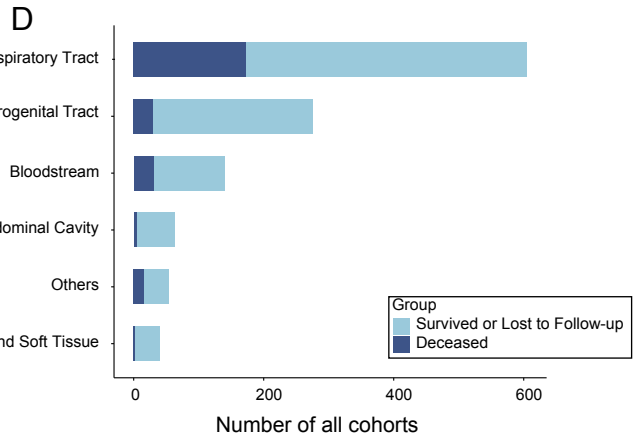
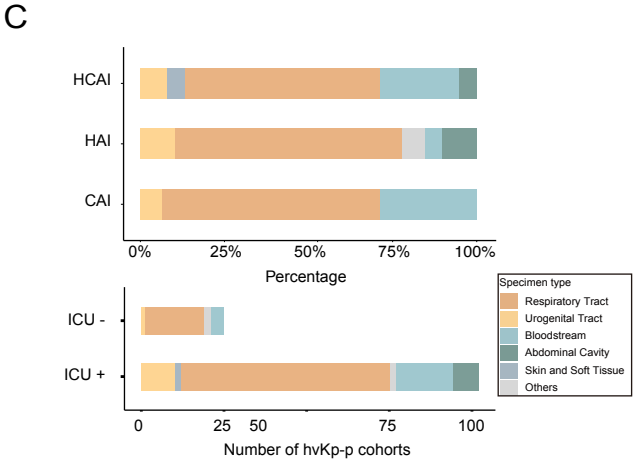
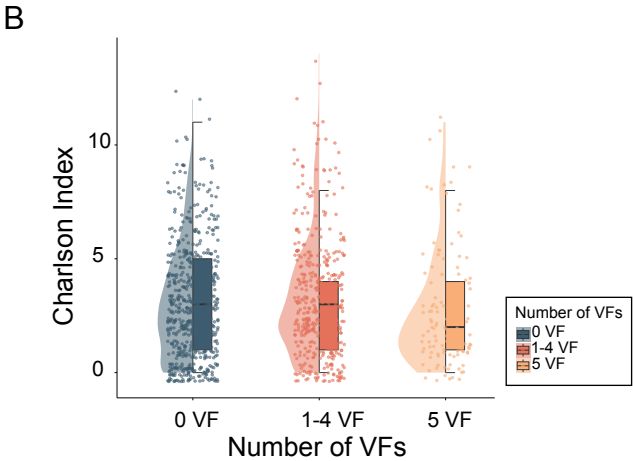
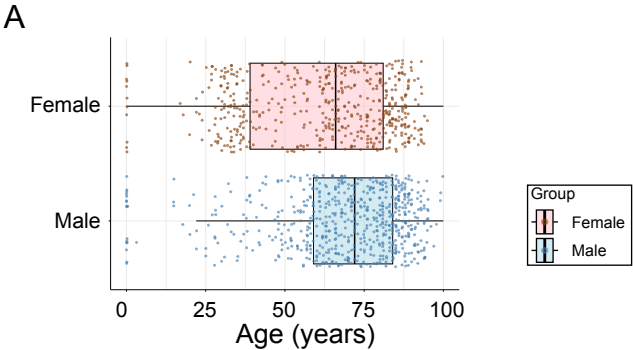
-: negative; +: positive. Gene names are presented in italics. Subheadings are presented in bold.

Supplementary Figures legends

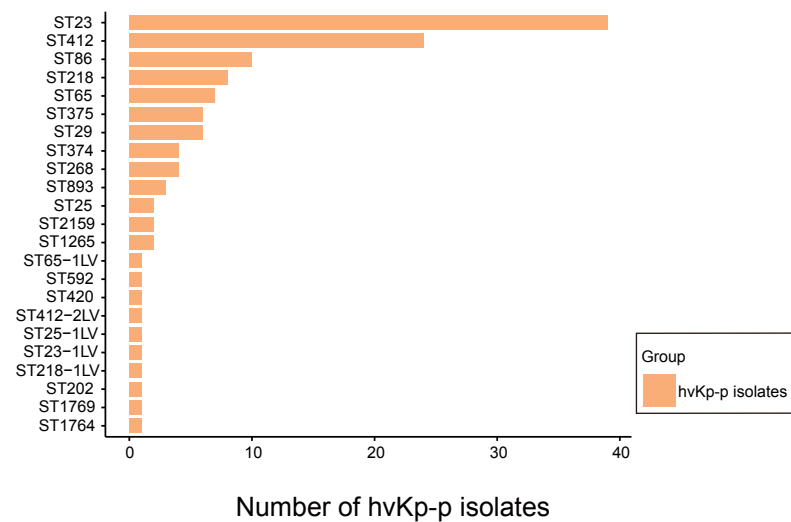
Supplementary Fig. 1 Clinical characteristics of patients with Kp infections. A) Age distribution: each point represents an individual patient. Females (n=517) shown in red, males (n=662) in blue. The center line represents the median, the box bounds the 25th and 75th percentiles, and the whiskers extend up to $1.5 \times$ IQR. B) Charlson Comorbidity Index (CCI) distribution colored by the number of virulence factors (VFs). 5VF (n=127): isolates harboring all five key virulence factors including *iucA*, *iroB*, *peg-344*, *rmpA* and *rmpA2*. 1-4 VF (n=436): isolates harboring any combination of fewer than five key virulence factors. 0 VF (n=616): isolates harboring none of the five key virulence factors. Each point represents an individual patient. The half-violin plots show the density of CCI and the half-boxplot also indicate the median, interquartile range (IQR) including upper and lower quartiles, whiskers with $1.5 \times$ interquartile range. C) Baseline characteristics of the hvKp-p cohorts. Specimen type (colored as Figure 1A) categorized by the site of acquisition and ICU admission status. D) Survival status by specimen type: Respiratory Tract (n=605), Urogenital Tract (n=276), Bloodstream (n=141), Abdominal Cavity (n=64), Skin and Soft Tissue (n=53), Others (n=40). Bars are colored in dark blue for deceased, and light blue for survived or lost to follow-up. E) Survival up to days 14, 28, and 90 for the hvKp-p and cKp-p cohort after PSM. The corresponding table showed number at risk over time. F) Survival up to days 14, 28, and 90 for the hvKp-p and cKp-p cohort 1 after PSM. The corresponding table showed number at risk over time. A two-tailed log-rank test was performed and p-values are indicated in the figures. Source data are provided as a Source Data file.

Supplementary Fig. 2 Molecular characteristics of Kp isolates. A) Sequence types (STs) of the hvKp-p cohort (n=127). B) Resistance scores (left panel) and virulence scores (right panel) by the number of VFs. A two-tailed Kruskal-Wallis test was performed, followed by Dunn's post-test with Bonferroni correction for multiple comparisons. Left panel: $P < 0.001$ for 0 VF vs 5 VF and 1-4 VF vs 5 VF. Right panel: $P < 0.001$ for 0 VF vs 5 VF and 0 VF vs 1-4 VF, $P = 0.001$ for 1-4 VF vs 5 VF. In the left panel: P values between 0 VF and 5VF < 0.001 , between 1-4VF and 5VF < 0.001 .

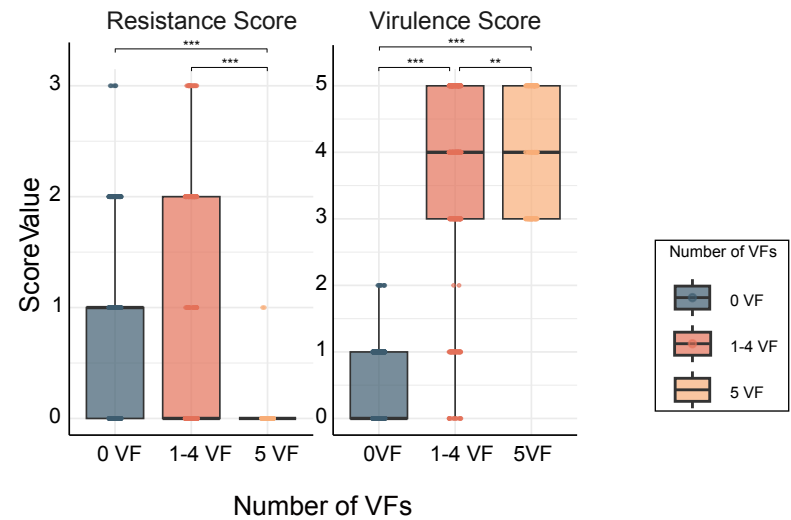
In the right panel: P values between 0 VF and 5VF <0.001, 0 VF and 1-4VF <0.001, between 1-4VF and 5VF =0.001. *: P value between 0.01 and 0.05, **: P value between 0.001 and 0.01, ***: P value < 0.001. C) Proportion of genomes with detection of five key genes (%iucA+, %iroB+, %peg-344+, %rmpA+, %rmpA2+) by specimen type. D-E) Prevalence of antimicrobial resistance determinants among Kp isolates. Density plots show the distributions of the number of resistance classes (n = total genomes by specimen type in Supplementary Fig. 2D and the number of VFs in Supplementary Fig. 2E). Grey shading indicates multi-drug resistance (≥ 3 resistance classes). Proportion of genomes where ESBL, CRKP and KPC were detected respectively (%ESBL+, %CRKP+, %KPC+). Source data are provided as a Source Data file.



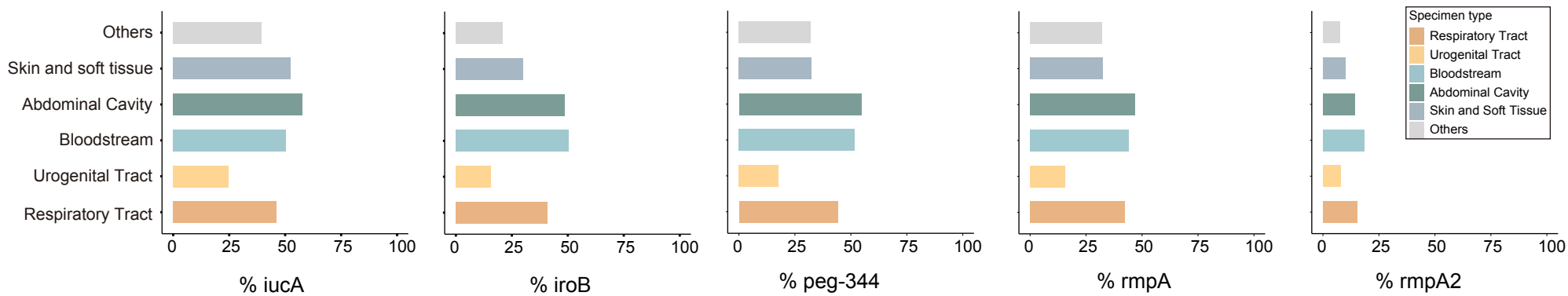
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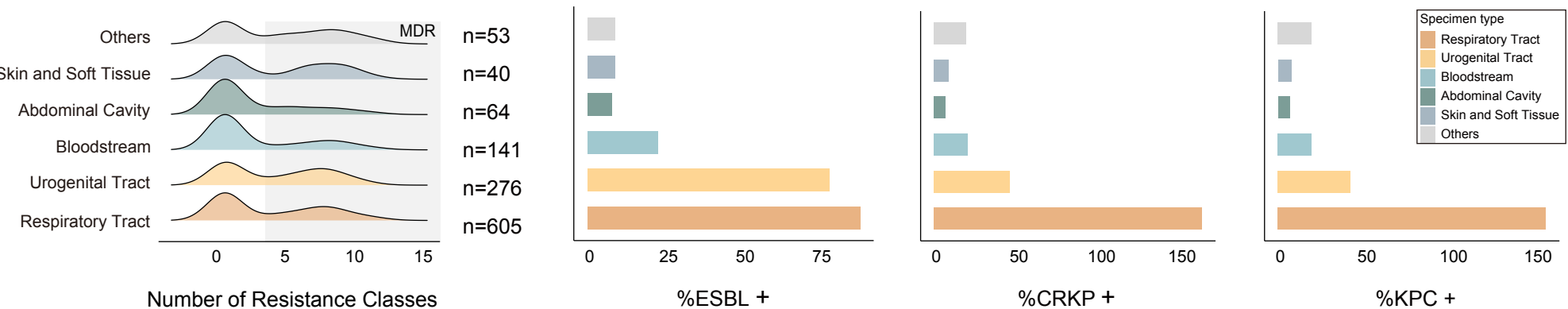
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