Supplementary Material

Supplementary Text 1

Results: Distribution of isolates according to source and detection ward

Over the study period, sixty-three isolates of ESBL-Kp were included from 61 different hospitalized patients and two environmental sites. The isolates were detected in the intensive care unit (ICU, *n*=21), the nephrology department (NPHD, *n*=12), two different SARS-CoV-2 intensive care units (SICU 1 and SICU 2, *n*=10), the geriatric department (GD, *n*=7), the cardiac care unit (CCU, *n*=5), the obstetric ward (OW, *n*=2), the cardiology department (CD, *n*=1), the infectious disease department (IDD, *n*=1), the neurology department (ND, *n*=1), the neonatal intensive care unit (NICU, *n*=1), the oncohematology department (OHD, *n*=1), and the surgery department (SD, *n*=1).

Patient isolates sources were rectal swab (*n*=44), urine (*n*=12), sputum (*n*=3), wound exudate (*n*=1), and blood culture (*n*=1). Environmental isolates (*n*=2) were collected from a sink at the ICU and from a mattress at the SD **(Supplementary Table 1)**.

Supplementary Text 2

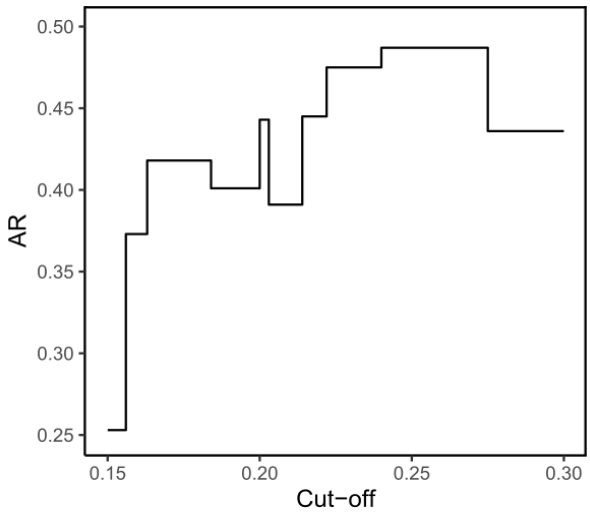
Results: Mean allele distances between isolates of a genomic cluster

According to whole-genome sequencing (WGS) results, 11 different clusters were detected. The mean allele distances between isolates from a genomic cluster ranged from 1 to 9.5. Specifically, six genomic clusters (III-VIII) included two isolates; the allele distances were 2, 10, 7, 1, 3, and 15 between isolates of clusters III, IV, V, VI, VII, and VIII, respectively. Additionally, five clusters (I, II, IX, X, and XI) comprised more than two isolates and presented an average allele distance of 9.5 (range, 0-18; *n*=16), 3.2 (range, 0-6; *n*=4), 2 (range, 1-3; *n*=3), 0.5 (range, 0-1; *n*=4), and 7 (range, 11-17; *n*=3), respectively.

Supplementary Text 3

Results: Description of FTIR clusters that affects more than one hospital location over the study period

As WGS, FTIR also detected clusters in more than one hospital location: (i) cluster 1 isolates were detected in the CCU, NPHD, ICU, and SICU 1; (ii) cluster 2 isolates were present in the CCU, ND and SD; (iii) cluster 4 isolates were present in the ICU, NPHD, OHD, and SICU 1; (iv) cluster 5 isolates were detected in the ICU and CCU; (v) cluster 6 isolates were detected in the SICU 1 and CD; (vi) cluster 10 isolates were detected in the GD and ICU (**Supplementary Table 1**). However, four (1, 4, 6, and 10) out of these six clusters were not fully concordant with WGS clustering (**Table 1**). Cluster 1 included only 13 out of the 16 isolates that formed genomic cluster I. Cluster 4 grouped together the two isolates of genomic cluster IV, however, FTIR also grouped within cluster 4 five genomic singletons and nine additional isolates of different genomic clusters. Cluster 6 included two out of the three isolates forming genomic cluster XI. Finally, cluster 10 grouped together two genomic singletons. Conversely, clusters 2 and 5 perfectly matched clusters II and V, respectively.



Supplementary Figure 1. Final internal validation of FTIR clustering cut-off value. The most restrictive cut-off value that maximized the Adjusted Rand index (AR) was 0.240 for the clustering of the study dataset (N=63) of extended-spectrum β-lactamase-producing *Klebsiella pneumoniae* isolates considering WGS as the reference method. The tested cut-off range corresponds to the unofficial range suggested by the manufacturer (0.20-0.25) with an additional 0.05 range on each side (0.15-0.30).

Supplementary Table 1. Comparison of the three clustering methods for each ESBL-Kp isolate.

Isolate ID	Collection date	Isolate source	Hospital location	ST	Epidemiological cluster	FTIR cluster	WGS cluster
1	07/16/2018	RS	CCU	392	A	1	I
5	07/23/2018	RS	CCU	392	A	Singleton	I
13	08/02/2019	U	NPHD	392	Singleton	1	I
14	08/07/2019	U	NPHD	392	C	1	I
15	08/11/2019	RS	NPHD	392	C	1	I
16	08/21/2019	RS	NPHD	392	C	1	I
21	08/31/2020	RS	ICU	392	D	1	I
22	10/05/2020	U	NPHD	392	Singleton	1	I
31	02/15/2021	RS	SICU 1	392	F	1	I
33	02/22/2021	RS	SICU 1	392	F	1	I
35	03/03/2021	RS	OHD	392	Singleton	4	I
37	03/10/2021	U	NPHD	392	Singleton	1	I
41	03/29/2021	RS	SICU 2	392	G	Singleton	I
43	04/13/2021	RS	ICU	392	E	1	I
57	05/17/2021	RS	NPHD	392	H	1	I
58	05/17/2021	RS	NPHD	392	H	1	I
2	07/18/2018	RS	CCU	307	A	2	II
3	07/21/2018	U	CCU	307	A	2	II

4	07/23/2018	RS	ND	307	Singleton	2	II
62	10/11/2021	EI	SD	307	Singleton	2	II
6	08/16/2018	U	OW	147	B	3	III
7	08/17/2018	U	OW	147	B	3	III
17	07/20/2020	RS	ICU	307	D	4	IV
59	05/25/2021	RS	NPHD	307	H	4	IV
20	08/24/2020	RS	ICU	45	Singleton	5	V
40	03/25/2021	U	CCU	45	Singleton	5	V
23	10/08/2020	BC	ICU	307	D	4	VI
24	10/13/2020	RS	ICU	307	D	4	VI
26	10/28/2020	RS	ICU	307	D	4	VII
27	11/30/2020	RS	ICU	307	D	4	VII
30	02/15/2021	RS	SICU 1	307	F	4	VIII
61	10/07/2021	S	GD	307	Singleton	4	VIII
42	04/13/2021	RS	ICU	307	E	7	IX
47	04/26/2021	RS	ICU	307	E	7	IX
52	05/04/2021	RS	ICU	307	E	7	IX
44	04/19/2021	RS	SICU 2	2703	G	8	X
49	04/27/2021	RS	SICU 2	2703	G	8	X
53	05/10/2021	RS	SICU 2	2703	G	9	X

56	05/12/2021	U	SICU 2	2703	G	9	X
32	02/19/2021	RS	SICU 1	15	F	6	XI
34	02/26/2021	RS	CD	15	Singleton	6	XI
45	04/20/2021	U	GD	15	Singleton	Singleton	XI
8	05/16/2019	RS	NICU	628	Singleton	Singleton	Singleton
9	07/25/2019	S	GD	336	Singleton	Singleton	Singleton
10	07/29/2019	RS	GD	17	Singleton	Singleton	Singleton
11	07/29/2019	RS	GD	Unknown	Singleton	Singleton	Singleton
12	07/31/2019	U	NPHD	307	Singleton	4	Singleton
18	07/27/2020	RS	ICU	485	D	Singleton	Singleton
19	08/17/2020	RS	ICU	307	D	4	Singleton
25	10/13/2020	RS	ICU	54	D	Singleton	Singleton
28	12/07/2020	RS	ICU	147	E	3	Singleton
29	12/21/2020	RS	ICU	307	E	4	Singleton
36	03/10/2021	RS	ICU	307	Singleton	4	Singleton
38	03/16/2021	RS	ICU	307	E	4	Singleton
39	03/22/2021	RS	GD	792	Singleton	Singleton	Singleton
46	04/26/2021	S	IDD	15	Singleton	Singleton	Singleton
48	04/27/2021	WE	NPHD	268	Singleton	Singleton	Singleton
50	04/27/2021	RS	ICU	29	Singleton	Singleton	Singleton

51	05/04/2021	RS	ICU	14	E	Singleton	Singleton
54	05/10/2021	U	NPHD	391	H	Singleton	Singleton
55	05/10/2021	RS	SICU 2	556	G	Singleton	Singleton
60	07/02/2021	EI	ICU	307	Singleton	10	Singleton
63	10/11/2021	RS	GD	307	Singleton	10	Singleton

BC: Blood culture, CCU: Cardiac care unit, CD: Cardiology department, EI: Environmental isolate, GD: Geriatric department, ICU: Intensive care unit, IDD: Infectious disease department, ND: Neurology department, NICU: Neonatal intensive care unit, NPHD: Nephrology department, OHD: Oncohematology department, OW: obstetric ward, RS: Rectal swab, S: Sputum, SD: Surgery department, SICU 1: SARS-CoV-2 intensive care unit 1, SICU 2: SARS-CoV-2 intensive care unit 2, ST: Sequence type, U: Urine, WE: Wound exudate.