

Genomic Characterisation of Opportunistic Pathogen *Kluyvera* Reveals a Novel CTX-M Subgroup

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Table S1 The detail information of strains used in this study

No.	Strain	Sequence size (Mb)	GC content (%)	BioSample	geographic location	host	isolation time	isolation source	Species labelled (Originally identified)	Precise species
1	220	5.1	54.2	SAMN16845431	Argentina: Buenos Aires	Homo sapiens	1998	peritoneal fluid	<i>Kluyvera ascorbata</i>	<i>Kluyvera ascorbata</i>
2	280	4.90	54.3	SAMN16845432	Japan: Tokio	environment	1956	Sewage	<i>Kluyvera ascorbata</i>	<i>Kluyvera ascorbata</i>
3	3162	4.98	54.3	SAMN16845433	USA: SENTRY	Homo sapiens	-	sepsis	<i>Kluyvera ascorbata</i>	<i>Kluyvera ascorbata</i>
4	4663	4.88	54.2	SAMN16845434	USA: Charlottesville, VA	Homo sapiens	2004	sepsis	<i>Kluyvera ascorbata</i>	<i>Kluyvera ascorbata</i>
5	58	5.02	54.1	SAMN16845429	Argentina: Buenos Aires	Homo sapiens	2014	bile from acute cholecystitis	<i>Kluyvera ascorbata</i>	<i>Kluyvera ascorbata</i>
6	68	4.91	54.3	SAMN16845430	Argentina: Buenos Aires	Homo sapiens	1995	sputum	<i>Kluyvera ascorbata</i>	<i>Kluyvera ascorbata</i>
7	711	4.73	54.4	SAMN16845443	USA: Burlington, VA	Homo sapiens	1999	skin infection and soft tissues, abscess	<i>Kluyvera ascorbata</i>	<i>Kluyvera ascorbata</i>
8	8633	5.06	54.2	SAMN16845435	USA: Burlington, MA	Homo sapiens	1999	sepsis	<i>Kluyvera ascorbata</i>	<i>Kluyvera ascorbata</i>
9	ATCC 33433	4.93	54.3	SAMN02743264	USA:North Carolina	Homo sapiens	-	human sputum	<i>Kluyvera ascorbata</i>	<i>Kluyvera ascorbata</i>
10	FDA-CDC-AR_0144	4.98	54.1	SAMN04014985	-	-	-	-	<i>Kluyvera ascorbata</i>	<i>Kluyvera ascorbata</i>

11	LFC	4.92	54.2	SAMN16845437	Argentina: Buenos Aires	Homo sapiens	2014	lower urinary tract infection	<i>Kluyvera ascorbata</i>	<i>Kluyvera ascorbata</i>
12	NCTC973 7	5.13	54.1	SAMEA103980416	Uruguay: Montevide o	Homo sapiens	-	urine	<i>Kluyvera ascorbata</i>	<i>Kluyvera ascorbata</i>
13	oral taxon 865. F0526	5.25	54.2	SAMN00691209	-	-	-	-	<i>Kluyvera ascorbata</i>	<i>Kluyvera ascorbata</i>
14	OT2	5.37	53.9	SAMN10265530	Brazil:Porto Alegre	Homo sapiens	2016	abdominal collection	<i>Kluyvera ascorbata</i>	<i>Kluyvera ascorbata</i>
15	TP1631	5.37	54.0	SAMD00204448	Japan	-	2017	-	<i>Kluyvera ascorbata</i>	<i>Kluyvera ascorbata</i>
16	131SC8	5.47	53.7	SAMEA104032393	Spain	environ ment	2013	Sewage	<i>Kluyvera ascorbata</i>	<i>Kluyvera ascorbata</i>
17	121SC68	5.18	53.7	SAMEA104032383	Spain	environ ment	2012	Sewage	<i>Kluyvera cryocrescens</i>	<i>Kluyvera cryocrescens</i>
18	1919	4.97	53.7	SAMN16845439	USA: San Francisco, CA	Homo sapiens	2002	sputum (lower respiratory tract infection)	<i>Kluyvera cryocrescens</i>	<i>Kluyvera cryocrescens</i>
19	4701	4.80	54.0	SAMN16845440	USA: Milwaukee, WI	Homo sapiens	2002	sepsis from intravenous catheter	<i>Kluyvera cryocrescens</i>	<i>Kluyvera cryocrescens</i>
20	88CZ1	5.23	53.6	SAMEA104032394	Spain	environ ment	2011	Sewage	<i>Kluyvera cryocrescens</i>	<i>Kluyvera cryocrescens</i>
21	NBRC 102467	5.04	53.9	SAMD00046725	-	food	2006	gulf	<i>Kluyvera cryocrescens</i>	<i>Kluyvera cryocrescens</i>
22	NCTC104 83	4.82	54.0	SAMEA103924398	Japan: Tokyo	-	-	-	<i>Kluyvera cryocrescens</i>	<i>Kluyvera cryocrescens</i>
23	NCTC129 93	5.29	53.8	SAMEA2580319	-	-	-	-	<i>Kluyvera cryocrescens</i>	<i>Kluyvera cryocrescens</i>
24	SCW13	5.44	53.4	SAMN12268149	China:Sichu an	environ ment	2019	hospital sewage	<i>Kluyvera cryocrescens</i>	<i>Kluyvera cryocrescens</i>

25	14751	5.00	55.1	SAMN16845442	USA: Louisville, KY	Homo sapiens	2002	sepsis	<i>Kluyvera georgiana</i>	<i>Kluyvera georgiana</i>
26	ATCC 51603	5.07	54.5	SAMN04875430	-	Homo sapiens	-	sputum	<i>Kluyvera georgiana</i>	<i>Kluyvera georgiana</i>
27	DRR01559 1	5.07	54.5	SAMD00012204	-	-	-	-	<i>Kluyvera georgiana</i>	<i>Kluyvera georgiana</i>
28	WCH1410	4.99	54.8	SAMN04482606	China: Chengdu	environ ment	2015	hospital sewage	<i>Kluyvera ascorbata</i>	<i>Kluyvera georgiana</i>
29	DRR01558 7	4.73	52.6	SAMD00004643	-	-	-	-	<i>Kluyvera intermedia</i>	<i>Kluyvera intermedia</i>
30	DRR01571 2	4.66	52.6	SAMD00011660	-	-	-	-	<i>Kluyvera intermedia</i>	<i>Kluyvera intermedia</i>
31	HR2	4.94	52.3	SAMN13135960	China:Gua ngzhou	environ ment	2017	flower	<i>Kluyvera intermedia</i>	<i>Kluyvera intermedia</i>
32	N2-1	4.94	52.3	SAMN13135738	China:Gua ngzhou	environ ment	2017	flower shop	<i>Kluyvera intermedia</i>	<i>Kluyvera intermedia</i>
33	NBRC 102594	4.64	52.6	SAMD00046734	France	environ ment	2006	Surface water	<i>Kluyvera intermedia</i>	<i>Kluyvera intermedia</i>
34	NCTC121 25	4.74	52.6	SAMEA2612496	-	-	-	-	<i>Kluyvera intermedia</i>	<i>Kluyvera intermedia</i>
35	VRES0055 -sc- 2201594	5.15	52.0	SAMEA3181512	-	-	-	-	<i>Kluyvera intermedia</i>	<i>Kluyvera intermedia</i>
36	090646	5.48	54.5	SAMN14563383	China: Chengdu, Sichuan	environ ment	2019	hospital sewage	<i>Kluyvera sichuanensis</i>	<i>Kluyvera sichuanensis</i>
37	13608	5.03	54.9	SAMN16845436	USA: Lexington, KY	Homo sapiens	2003	sepsis	<i>Kluyvera sichuanensis</i>	<i>Kluyvera sichuanensis</i>
38	CHPC 1.251*	4.85	54.9	-	China: Beijing	Homo sapiens	2007	faeces	-	<i>Kluyvera excreta</i>

39	CHPC 1.254*	5.07	54.8	-	China: Beijing	Homo sapiens	2007	faeces	-	<i>Kluyvera sichuanensis</i>
40	CHPC 1.2972*	4.92	55.0	-	China: Beijing	environ ment	2016	egg surface	-	<i>Kluyvera chilikensis</i>
41	Colony413	2.37	55.1	SAMN17914377	Thailand	-	-	food	<i>Kluyvera ascorbata</i>	<i>Kluyvera</i> sp.
42	L2	4.74	53.4	SAMN03008042	Malaysia	Food	2013	lettuce	<i>Kluyvera cryocrescens</i>	<i>Kluyvera</i> genomosp. 1
43	CHPC 1.982*	4.96	55.5		China: Beijing	Homo sapiens	2008	faeces	-	<i>Kluyvera</i> genomosp. 2
44	KA1	5.28	55.2	SAMN14640326	Spain	Homo sapiens	2014	Human gut	<i>Kluyvera ascorbata</i>	<i>Kluyvera</i> genomosp. 2
45	KA2	5.26	55.2	SAMN08578632	Spain	Homo sapiens	2014	Rectal colonization	<i>Kluyvera ascorbata</i>	<i>Kluyvera</i> genomosp. 2
46	KA5	5.30	55.2	SAMN14640327	Spain	Homo sapiens	2014	Human gut	<i>Kluyvera ascorbata</i>	<i>Kluyvera</i> genomosp. 2
47	MGYG- HGUT- 02491	5.26	55.2	SAMEA5851996	Spain	Homo sapiens	-	human gut	<i>Kluyvera ascorbata</i>	<i>Kluyvera</i> genomosp. 2
48	R10	5.26	55.2	SAMN14640329	Spain	Homo sapiens	2014	Human gut	<i>Kluyvera ascorbata</i>	<i>Kluyvera</i> genomosp. 2
49	WP4-W19- ESBL-05	5.16	55.1	SAMD00194492	Japan:Tokyo	environ ment	2019	wastewater	<i>Kluyvera georgiana</i>	<i>Kluyvera</i> genomosp. 2
50	WP4-W19- ESBL-10	5.44	54.8	SAMD00194497	Japan:Tokyo	environ ment	2019	wastewater	<i>Kluyvera georgiana</i>	<i>Kluyvera</i> genomosp. 2
51	WP8-W19- ESBL-13	4.95	55.1	SAMD00194654	Japan:Tokyo	environ ment	2019	wastewater	<i>Kluyvera georgiana</i>	<i>Kluyvera</i> genomosp. 2
52	WP8-W19- ESBL-15	4.94	55.3	SAMD00194656	Japan:Tokyo	environ ment	2019	wastewater	<i>Kluyvera georgiana</i>	<i>Kluyvera</i> genomosp. 2
53	CRP	5.16	54.8	SAMN21013960	Hong Kong: Ocean Park	environ ment	2018	Ailurus fulgens faeces	<i>Kluyvera</i> sp.	<i>Kluyvera</i> genomosp. 3
54	ERR37129 46	5.08	54.7	SAMEA6368553	Netherlands	-	-	-	<i>Kluyvera georgiana</i>	<i>Kluyvera</i> genomosp. 3

55	PO2S7	5.00	54.9	SAMN13951572	India:Mani pur	environ ment	2018	Oryza sativa	<i>Kluyvera genomosp. 3</i>	<i>Kluyvera</i> genomosp. 3
56	YDC799	5.00	55.0	SAMN07267279	USA: Pittsburgh, PA	Homo sapiens	2017	-	<i>Kluyvera georgiana</i>	<i>Kluyvera</i> genomosp. 3
57	D51-sc- 1712206	4.80	52.9	SAMEA2393042	-	-	-	-	<i>Kluyvera cryocrescens</i>	<i>Kluyvera</i> genomosp. 4
58	e02842a0- 063f-11e6- bb80- 3c4a9275d 6c8	4.76	52.8	SAMEA104428612	-	Homo sapiens	-	gut	<i>Kluyvera cryocrescens</i>	<i>Kluyvera</i> genomosp. 4
59	169	5.20	53.3	SAMN16845438	Argentina: Buenos Aires	Homo sapiens	2003	Human, lower urinary tract infection	<i>Kluyvera sp.</i>	<i>Kluyvera</i> genomosp. 5
60	RHBSTW- 00307	5.23	53.4	SAMN16088452	United Kingdom	environ ment	2017	wastewater influent	<i>Kluyvera cryocrescens</i>	<i>Kluyvera</i> genomosp. 5
Note: Strains labeled in dark blue are misidentified.										

Table S2 Primer sequence information used in this study

Strain	Primer	Enzyme digestion sites	Sequence (5'-3')	Target sequence (bp)
CHPC 1.251	KLUS _{CHPC 1.251} -F	<i>XbaI</i>	GCTCTAGAATGATGACCCTGGGCGTTCG	873
	KLUS _{CHPC 1.251} -R	<i>HindIII</i>	CCCAAGCTTCTAAAGCCCTTCGGTAACGATTTTGC	
CHPC 1.2972	KLUS _{CHPC 1.2972} -F	<i>XbaI</i>	GCTCTAGAATGATGACCCTGGGCGTTCG	873
	KLUS _{CHPC 1.2972} -R	<i>HindIII</i>	CCCAAGCTTCTAAAGCCCTTCGGTAACGATTTTGGC	
CHPC 1.254	KLUS _{CHPC 1.254} -F	<i>XbaI</i>	GCTCTAGAATGATGACCCTGGGCGTTCG	873
	KLUS _{CHPC 1.254} -R	<i>HindIII</i>	CCCAAGCTTCTAAAGCCCTTGGGTAACGATTTTGC	

Table S3 Phenotypic characteristics of *K. sichuanensis* 090646^T, *K. excreta* CHPC 1.251^T and *K. chilikensis* CHPC 1.2972^T

Characteristic	<i>K. sichuanensis</i> 090646 ^T	<i>K. excreta</i> CHPC 1.251 ^T	<i>K. chilikensis</i> CHPC 1.2972 ^T
Motility	+	+	+
Indole production*	–	+	+
Voges–Proskauer reaction	–	–	–
Citrate utilization	+	+	+
H ₂ S production	–	–	–
Malonate utilization	+	+	+
NO ₃ ->NO ₂	+	+	+
ONPG test	–	–	–
Oxidase	–	–	–
Catalase	–	–	–
Lipase	–	–	–
Sorbitol fermentation*	+	–	–
β-D-Glucosidase (ESC)	+	+	+
β-galactosidase (PNPG)	+	+	+
Urease	–	–	–
Lysine decarboxylase	+	+	+
Arginine dihydrolase	–	–	–
Ornithine decarboxylase	+	+	+
Gelatinase (GEL)	–	–	–
Acid production from:			
D-glucose	+	+	+
Sucrose*	–	+	+
Dulcitol	–	–	–
D-sorbitol	+	+	+
Amygdalin	+	+	+
Glycerol	–	–	–
D-mannose	–	–	–

D-galactose	+	+	+
Esculin	+	+	+
methyl α -D-glucopyranoside	+	+	+
Assimilation of:			
Glucose	+	+	+
Arabinose (ARA)	+	+	+
D-Mannose (MNE)	+	+	+
D-Mannitol (MAN)	+	+	+
N-Acetyl-D-glucosamine (NAG)	+	+	+
Maltose (MAL)	+	+	+
Gluconate (GNT)	+	+	+
Mannitol	+	+	+
α -Glucosidase*	-	-	+

Note: The biochemical reactions marked with “*” are the differential biochemical indicators of the three species.

Table S4 The results of antibiotic susceptibility testing.

	AMI	AMP	A/S2	AZM	AZT	CTX	FOX	CAZ	CAP	CIP	CT	ETP	IPM	MEM	NA	NIT	S	TET	TGC	SXT	CAZ-NXL
CHP C 1.251	≤4	8	2/1	16	≤0.25	≤0.25	≤2	≤0.25	≤4	≤0.015	0.5	≤0.25	≤0.25	≤0.12	≤4	≤3	8	2	≤0.25	≤0.5/9.5	≤0.25/4
CHP C 1.254	≤4	8	2/1	32	≤0.25	≤0.25	≤2	≤0.25	≤4	≤0.015	0.5	≤0.25	≤0.25	≤0.12	≤4	≤3	8	2	≤0.25	≤0.5/9.5	≤0.25/4
CHP C 1.2972	≤4	16	2/1	32	≤0.25	≤0.25	≤2	≤0.25	≤4	≤0.015	0.5	≤0.25	≤0.25	≤0.12	≤4	≤3	8	2	≤0.25	≤0.5/9.5	≤0.25/4
CHP C 1.982	≤4	≤2	2/1	16	≤0.25	≤0.25	≤2	≤0.25	≤4	≤0.015	0.5	≤0.25	0.5	≤0.12	≤4	≤3	<2	≤1	≤0.25	≤0.5/9.5	≤0.25/4

Note: Amikacin: AMI; Ampicillin: AMP; Ampicillin-Sulbactam: A/S2; Azithromycin: AZM; Aztreonam: AZT; Cefotaxime: CTX; Cefoxitin: FOX; Ceftazidime: CAZ; Chloramphenicol: CAP; Ciprofloxacin: CIP; Colistin: CT; Ertapenem: ETP; Imipenem: IPM; Meropenem: MEM; Nalidixic Acid: NA; Nitrofurantoin: NIT; Streptomycin: S; Tetracycline: TET; Tigecycline: TGC; Trimethoprim-Sulfamethoxazole: SXT; ceftazidime-avibactam: CAZ-NXL