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Stool metagenome analysis of patients with *Klebsiella pneumoniae* liver abscess and their domestic partners

James S. Molton[a,b,*], I. Russel Lee[c], Denis Bertrand[d], Ying Ding[e,f], Shirin Kalimuddin[g], David C. Lye[b,c,e,f], Niranjan Nagarajan[b,d], Yunn-Hwen Gan[h], Sophia Archuleta[a,b]

[a] Division of Infectious Diseases, National University Hospital, National University Health System, Singapore 119228, Singapore
[b] Department of Medicine, Yong Loo Lin School of Medicine, National University of Singapore, Singapore 117597, Singapore
[c] National Centre for Infectious Diseases, Singapore 308442, Singapore
[d] Genome Institute of Singapore, A*STAR, Singapore 138672, Singapore
[e] Department of Infectious Diseases, Tan Tock Seng Hospital, Singapore 308433, Singapore
[f] Lee Kong Chian School of Medicine, Nanyang Technological University, Singapore 636921, Singapore
[g] Department of Infectious Diseases, Singapore General Hospital, Singapore 169608, Singapore
[h] Department of Biochemistry, Yong Loo Lin School of Medicine, National University of Singapore, Singapore 117596, Singapore

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ABSTRACT

Objectives: Hypermucoviscous *Klebsiella pneumoniae* is an emerging cause of community-acquired liver abscess. The aim of this study was to investigate whether hypermucoviscous strains could be shared among households.
Methods: The clinical *K. pneumoniae* isolates from a cohort of 24 patients with Klebsiella liver abscess were genotyped, and the stool metagenomes of the index patients and their cohabiting domestic partners were analyzed.
Results: K. pneumoniae was identified in 33% of index patient stools, and one index patient's clinical isolate was identified in their domestic partner's stool.
Conclusions: This could represent a transmission event or could represent exposure to a common environmental source.

Introduction

Hypermucoviscous *Klebsiella pneumoniae* is an emerging cause of community-acquired liver abscess in Asian populations worldwide, affecting predominantly diabetic individuals with a normal biliary system and occasionally resulting in metastatic spread of infection (Siu et al., 2012). Virulence is partly due to the hypermucoviscous phenotype, which is mediated by the K1 and K2 capsule serotypes. In Singapore, Klebsiella liver abscess (KLA) has become the most common cause of liver abscess (Chan et al., 2013). The carriage rate of K1/K2 capsule types in the general population in several Asian countries, including Singapore, is 9.8% (Lin et al., 2012).

While the source of the hypermucoviscous *K. pneumoniae* causing liver abscess is unknown, the gastrointestinal tract appears to be the dominant site of colonization. A study in Taiwan showed that *K. pneumoniae* isolated from patient liver aspirates had an identical pulsed-field gel electrophoresis (PFGE) profile to that of the stool isolate from the same patient, suggesting that the KLA infections originated from the intestinal flora (Fung et al., 2012). Several groups of these liver aspirate *K. pneumoniae* isolates exhibited identical PFGE patterns and possessed similar virulence-associated genes as stool *K. pneumoniae* isolates from healthy individuals, suggesting that healthy people may carry virulent *K. pneumoniae* in their gastrointestinal tract (Fung et al., 2012). In animal models, the strains causing KLA were found to cross the intestinal barrier and pass via the portal vein to the liver (Tu et al., 2009). Gut colonization may result from fecal–oral transmission or environmental exposure (Siu et al., 2012). Clustering of *K. pneumoniae* sequence type (ST) 23 has been identified among three members of a family, resulting in liver abscess in two (Harada et al., 2011). A number of research groups have identified similar hypermucoviscous *K. pneumoniae* strains to those causing liver abscess in the stool of KLA patients and healthy donors (Chung et al., 2012; Fung et al., 2012; Lin et al., 2014; Siu et al., 2011).

* Corresponding author at: Division of Infectious Diseases, University Medicine Cluster, National University Health System, 1E Kent Ridge Road, NUHS Tower Block, Level 10, Singapore 119228, Singapore.
E-mail address: james.molton@wh.org.au (J.S. Molton).

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However, a prospective comparison of *K. pneumoniae* strains in individuals with liver abscess to those colonizing their domestic partners has not yet been performed.

The aim of this study was to genotype the clinical *K. pneumoniae* isolates in a cohort of patients with KLA and to analyze the stool metagenomes of these patients and their corresponding domestic partners living in the same household. The purpose was to identify whether the KLA-causing strains were present in the stools of the patients and/or their domestic partners, which may provide evidence that virulent strains could be shared within a household.

Methods

Population and samples

The study recruited eligible adult participants (age ≥21 years) who were divided into two groups. The first group, KLA index patients (IPs), were identified from the inpatient wards of three hospitals in Singapore. Inclusion criteria were abdominal imaging suggestive of liver abscess, plus *K. pneumoniae* isolated from abscess fluid or blood collected within the preceding 14 days. Patients were excluded if additional organisms were isolated (unless deemed to be contaminants). The second group were cohabiting domestic partners (DPs) of KLA index patients. Enrolled IPs were requested to approach one DP who was invited to join the study. The National Healthcare Group Domain Specific Review Board approved the study, and written informed consent was obtained from all participants.

The IPs underwent a first study visit within 14 days of positive culture for *K. pneumoniae*. The first study visit for DPs was conducted within 14 days of their linked IP's first visit. Clinical and demographic data were collected in both groups, and a stool sample was collected within 7 days of the study visit. In the IP group, existing isolates from blood and/or abscess fluid cultures were sent by the respective clinical microbiology laboratories to the research laboratory. The IP group underwent a second study visit on day 28 (±7 days) involving additional clinical data and stool sample collection.

The sample size of 24 participants per group was chosen based on pragmatic considerations of feasibility (recruitment and work of analysis) and available funding, and was considered adequate to fulfill the descriptive objectives of the study.

Sample DNA extraction and sequencing

DNA was extracted directly from stool samples. The majority of the stool samples (except those from the first few participants recruited) were diluted in phosphate buffered saline (PBS) and plated onto HiChrome *Klebsiella* selective agar (Sigma-Aldrich) before DNA extraction. However, some of these *Klebsiella* selective agar plates did not grow colonies, presumably because the antibiotics consumed by some of these patients had eradicated the gut *Klebsiella*. For total microbial DNA extraction directly from stool samples, the PowerSoil DNA isolation kit (MoBio Laboratories) was used according to the manufacturer's instructions, with minor modifications (see **Supplementary Material)**. To extract DNA from enriched *Enterobacteriaceae* (*Klebsiella*) colonies on the HiChrome *Klebsiella* selective agar, the GenElute bacterial genomic DNA kit (Sigma-Aldrich) was used according to the manufacturer's instructions. Illumina sequencing libraries were prepared using standard protocols (see **Supplementary Material), following which** the enriched libraries were pooled in equimolar ratios and sequenced on an Illumina HiSeq sequencing instrument at the Genome Institute of Singapore (2 × 101 bp reads, 41 × 10^6 on average per library). Library preparation and sequencing of *K. pneumoniae* clinical isolates was performed following the same protocol as above, with DNA extracted using the GenElute bacterial genomic DNA kit (Sigma-Aldrich) according to the manufacturer's instructions.

Isolate genome assembly and annotation

Isolate genomes were assembled using the Velvet assembler (version 1.2.10) with parameters optimized by Velvet Optimiser (Zerbino and Birney, 2008), scaffolded with Opera (version 1.4.1) (Gao et al., 2016), and gap-filled with FinIS (version 0.3) (Gao et al., 2012). The strain genome annotation was performed by mapping the virulence factor gene from the PATRIC database (v3.5.43) (Wattam et al., 2017) against the assemblies (diamond blastx v0.9.24, default parameters) and using the software Kleborate (v0.3.0) (Lam et al., 2018; Wyres et al., 2016).

Taxonomic analysis and identification of isolate strains in the gut metagenome

Taxonomic abundance profiles were generated from shotgun metagenomic data using metaphlan2 v2.6.0 (Truong et al., 2015); species with abundance <0.1% were filtered out. To identify the isolate in gut metagenomes, it was required that the majority of the isolate genome sequence was present in the metagenome (95% of 1-kbp windows; see **Supplementary Material) and sequences had to show** a high level of similarity relative to other isolates in this study (see **Supplementary Material**).

Results

Twenty-four IPs with KLA and 24 DPs were recruited between August 22, 2016 and October 24, 2017. IPs had a median age of 65 years (range 44–93 years); 14/24 (58.3%) were male and the ethnic distribution was 22/24 (91.7%) Chinese and 2/24 (8.3%) Malay. Eight (33.3%) had *K. pneumoniae* isolated from blood, 10/24 (41.7%) had *K. pneumoniae* isolated from abscess fluid, and six had *K. pneumoniae* isolated from both blood and abscess fluid. All clinical isolates were sensitive to ceftriaxone and ciprofloxacin. IPs were exposed to a median of three different antibiotics (range 1–7 antibiotics) in the 30 days prior to recruitment (see **Supplementary Material**Table). DPs had a median age of 56 years (range 41–87 years); 18/24 (75%) were female and the ethnic distribution was 21/24 (87.5%) Chinese, 1/24 (4.2%) Malay, 1/24 (4.2%) Filipino, and 1/24 (4.2%) Eurasian. No DPs had received antibiotics in the preceding 30 days prior to recruitment.

Clinical isolate genome analysis

Of the 24 clinical isolates collected, 22 were analyzed (see Table 1). The majority (68.2%, 15/22) belonged to capsule type K1, a genetically homogeneous group comprising 14 ST23 isolates and one ST2044 isolate. The non-K1 group was genetically heterogeneous, comprising three K2 isolates (two ST2039 and one ST86), two K5 (both ST60), one K20 (ST268), and one K57 (ST161). Virulence and resistance scores were derived from Kleborate (Kleborate). Among the K1 isolates, 12 were assigned a maximum virulence score of 5 (carried yersiniabactin, colibactin, aerobactin, and/or salmochelin) and three had a score of 3 (carried aerobactin and/or salmochelin only without yersiniabactin or colibactin). Among the non-K1, four isolates (two K2, one K20, and one K57) were assigned a virulence score of 5, one K2 isolate had a score of 4 (carried aerobactin and/or salmochelin with yersiniabactin without colibactin), and both K5 isolates had a score of 1 (carried yersiniabactin only). Two isolates (one K20 and one K57) were assigned an antimicrobial resistance score of 1 (extended-spectrum beta-lactamase (ESBL), no carbapenemase regardless

Table 1
Klebsiella species, sequence type, and presence of virulence factors in the index patient's clinical isolates.

Age, years	Sex	Ethnicity	Species	ST	Virulence score	Resistance score	Yersiniabactin	Colibactin	Aerobactin	Salmochelin	*rmpA*	*rmpA2*	wzi	K locus	O locus
70	M	Chinese	*K. pneumoniae*	ST86	4	0	ybt 9; ICEKp3	–	iuc 1	–	–	–	wzi2	KL2	O1v1
51	M	Chinese	*K. pneumoniae*	ST23	5	0	ybt 1; ICEKp10	clb 2	iuc 1	iro 1	–	–	wzi1	KL1	O2v2
65	F	Chinese	*K. pneumoniae*	ST23	5	0	ybt 1; ICEKp10	clb 2	iuc 1	iro 1	rmpA_2*(KpVP-1)	–	wzi1	KL1	O1/O2v2
65	M	Chinese	*K. pneumoniae*	ST23	5	0	ybt 1; ICEKp10	clb 2	iuc 1	iro 1	rmpA_1(KpVP-1)	–	wzi1	KL1	O1v2
70	F	Chinese	*K. pneumoniae*	ST23	5	0	ybt 1; ICEKp10	clb 2	iuc 1	iro 1	rmpA_1*(KpVP-1)	rmpA2_7*	wzi1	KL1	O1/O2v2
47	M	Chinese	*K. pneumoniae*	ST23	5	0	ybt 1; ICEKp10	clb 2	iuc 1	iro 1	rmpA_2(KpVP-1)	rmpA2_5*	wzi1	KL1	O1v2
86	F	Chinese	*K. pneumoniae*	ST23	5	0	ybt 1; ICEKp10	clb 2	iuc 1	iro 1	rmpA_2(KpVP-1)	rmpA2_7	wzi1	KL1	O1v2
44	M	Chinese	*K. pneumoniae*	ST23	5	0	ybt 1; ICEKp10	clb 2	iuc 1	iro 1	rmpA_2(KpVP-1)	rmpA2_3	wzi1	KL1	O1v2
66	M	Chinese	*K. pneumoniae*	ST2039	5	0	ybt 12; ICEKp10	clb 1	iuc 2	iro 2	rmpA_3*(KpVP-2)	–	–	KL2	O1v1
69	M	Chinese	*K. pneumoniae*	ST23	3	0	–	–	iuc 1	iro 1	rmpA_2(KpVP-1)	rmpA2_3*	wzi1	KL1	O1v2
63	F	Malay	*K. pneumoniae*	ST23	5	0	–	clb 2	iuc 1	iro 1	rmpA_1(KpVP-1)	–	wzi1	KL1	O1/O2v2
77	M	Chinese	*K. pneumoniae*	ST23	3	0	–	–	iuc 1	iro 1	rmpA_1(KpVP-1)	rmpA2_5	wzi1	KL1	O1/O2v2
53	M	Malay	*K. pneumoniae*	ST23	5	0	ybt 1; ICEKp10	clb 2	iuc 1	iro 1	–	rmpA2_5*	wzi1	KL1	O1v2
44	M	Chinese	*K. pneumoniae*	ST161-1LV	5	1	ybt unknown	clb unknown	iuc 2A	iro 2	rmpA_7*(VP)	–	–	KL57	O2v2
58	F	Chinese	*K. pneumoniae*	ST23	5	0	ybt 1; ICEKp10	clb 2	iuc 1	iro 1	–	–	wzi1	KL1	O1v2
73	F	Chinese	*K. pneumoniae*	ST268	5	1	ybt 9; ICEKp3	clb 3	iuc 1	iro 1	rmpA2_1*(KpVP-1)	rmpA2_2*	wzi95	KL20	O2v1
47	M	Chinese	*K. pneumoniae*	ST23	3	0	–	–	iuc 1	iro 1	rmpA_2(KpVP-1)	rmpA2_3*	wzi1	KL1	O1v2
67	F	Chinese	*K. pneumoniae*	ST2039	5	0	ybt 12; ICEKp10	clb 1	iuc 2	iro 2	rmpA_3*(KpVP-2)	–	wzi257	KL2	O1v1
48	M	Chinese	*K. pneumoniae*	ST60	1	0	ybt 2; ICEKp1	–	–	iro 3	rmpA_11(ICEKp1)	–	wzi5	KL5	O1v1
71	F	Chinese	*K. variicola*	ST454-2LV	1	0	ybt unknown	–	–	iro unknown	–	–	–	KL11	O3/O3a
59	M	Chinese	*K. pneumoniae*	ST60	1	0	ybt 2; ICEKp1	–	–	iro 3	rmpA_11(ICEKp1)	–	wzi5	KL5	O1v1
58	M	Chinese	*K. pneumoniae*	ST23	5	0	ybt 1; ICEKp10	clb 3	iuc 1	iro 1	rmpA_2(KpVP-1)	rmpA2_6	wzi1	KL1	O1v2

F, female; M, male; ST, sequence type.

of colistin resistance) from a maximum score of 3 (carbapenemase with colistin resistance regardless of ESBL), while the remaining 20 isolates had a score of 0 (no ESBL or carbapenemase regardless of colistin resistance).

Stool metagenome K. pneumoniae *strain analysis*

KLA index patient group

All 24 IPs provided a stool specimen on day 1, while 23 provided a stool specimen on day 28. Stool microbiome analysis with shotgun metagenomics revealed that *K. pneumoniae* was present in 8/24 (33.3%) samples on day 1 and 10/23 (43.5%) samples on day 28 (median abundance 0.3% and 0.15%). As expected, post antibiotic treatment, no complete genome (>95% coverage) of a corresponding clinical strain could be identified in any patient at either time point based on genomic analysis of the *K. pneumoniae* strains in the stool. Importantly, the 5–15% of the clinical strain's genome that could not be detected included regions encoding virulence factors such as salmochelin, aerobactin, and yersiniabactin. This suggests that the strains in the stool lacked the same virulence potential as the strains in the liver abscess/blood.

Domestic partner group

All 24 DPs provided a stool specimen. We identified reads mapping to >95% of genomic windows (1-kbp) corresponding to the IP's clinical strain in the gut metagenomes of six DPs, and identified a complete genome of a corresponding IP's clinical strain in one DP's gut metagenome (99% of the isolate genome detected, with a low average number of single nucleotide variants (SNVs) (23 SNVs), average nucleotide identity (ANI) >99.999, $P < 0.2 \times 10^{-8}$, Wilcoxon rank sum test). Analysis of the genome coverage of this IP's clinical strain in their DP's stool microbiome indicated that multiple *K. pneumoniae* strains were present and that the IP's clinical strain represented only 5% of the total *K. pneumoniae* reads present (see Figure 1). While this strain was identified in the IP's clinical sample and the stool sample of their DP, it was not isolated in the IP's own stool.

Discussion

The aim of this study was to genotype the clinical *K. pneumoniae* isolates in a cohort of patients with KLA and analyze the stool metagenomes of these patients and their corresponding domestic partners. An IP's clinical strain was identified in their DP's stool, despite that strain not being detected in the IP's own stool (possibly due to antibiotic exposure). The identification of the same strain in an IP and their DP could possibly represent a transmission event between the two individuals (in either direction), or could alternatively represent exposure to a common environmental source. *K. pneumoniae* has been recovered in 21% of raw and ready-to-eat retail food samples screened in Singapore, and 8% of these carried genetic elements (*wcaG* and capsule types K1, K2, and K54) associated with increased virulence (Hartantyo et al., 2020). However, no isolates carried the *rmpA* gene linked to community-acquired KLA, so a dietary source has not yet been proven. Fecal–oral transmission of *K. pneumoniae* was observed in a mouse model (Young et al., 2020). Such transmission may also occur in humans within a household.

K. pneumoniae was identified in only 33% of IP stool samples, and in those, 5–15% of the genome of the clinical strain was not detected. The strains identified in the IPs' stool often lacked the virulence factors seen in the clinical strain. One potential reason for the unexpectedly low proportion of *K. pneumoniae* in IP stool samples could be that all patients were exposed to antibiotics prior to the day 1 stool collection. This was inevitable because all

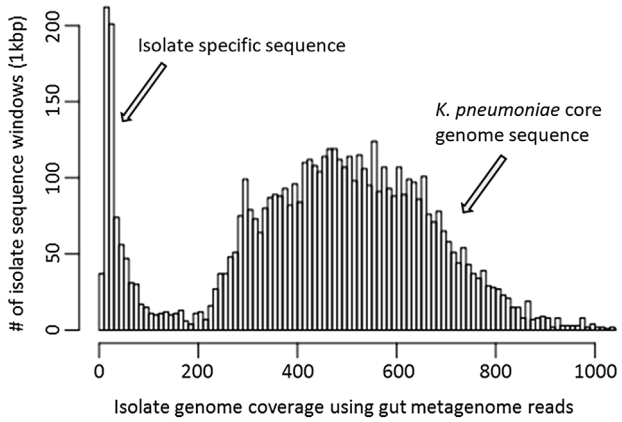

Figure 1. Gut microbiome read coverage of the virulent *Klebsiella pneumoniae* strain. Gut microbiome reads were obtained from the domestic partner of a *K. pneumoniae*-infected patient. The bimodal distribution revealed that multiple *K. pneumoniae* strains were present in the gut microbiome, where shared *K. pneumoniae* genomic regions are represented by the higher coverage mode (~475× coverage) and the lower coverage mode representing the average coverage of the virulent strain (~25× coverage, representing 5% of total coverage and therefore the population).

patients were diagnosed with KLA and appropriately treated with antibiotics.

The clinical isolate detected in the DP stool co-existed with other *Klebsiella* strains. This suggests that there may be no inherent fitness advantage in the gut for hypermucoviscous *K. pneumoniae*. The co-existence of different *K. pneumoniae* strains also opens up the possibility that plasmids encoding virulence or antibiotic resistance could be exchanged between these strains (Chen et al., 2020). It is unclear whether colonization by the clinical isolate in DP is transient, and this can only be resolved with repeated sampling of DP stools. Although attempts to decolonize ESBL have been unsuccessful, the use of antibiotics or fecal microbiota transplants may be worth investigating for the decolonization of this antibiotic-sensitive, community-acquired *K. pneumoniae*, so that it does not become a potential reservoir for transmission.

In summary, the same hypermucoviscous *K. pneumoniae* isolate in the liver abscess of a patient was identified in their domestic partner's stool, suggesting that either both partners were exposed to a common environmental source or a transmission event occurred between the partners. Further work to explore the source, transmissibility, and duration of carriage of hypermucoviscous *K. pneumoniae* is necessary to determine approaches to primary and secondary prevention of the growing global problem of KLA.

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Conflict of interest

All authors report no conflicts of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.ijid.2021.04.012.

References

Chan DS, Archuleta S, Llorin RM, Lye DC, Fisher D. Standardized outpatient management of *Klebsiella pneumoniae* liver abscesses. Int J Infect Dis 2013;17(3):e185–8.
Chen Y, Marimuthu K, Teo J, Venkatachalam I, Cherng BPZ, De Wang L, et al. Acquisition of plasmid with carbapenem-resistance gene blaKPC2 in hypervirulent *Klebsiella pneumoniae*, Singapore. Emerg Infect Dis 2020;26(3):549–59.
Chung DR, Lee H, Park MH, Jung SI, Chang HH, Kim YS, et al. Fecal carriage of serotype K1 *Klebsiella pneumoniae* ST23 strains closely related to liver abscess isolates in Koreans living in Korea. Eur J Clin Microbiol Infect Dis 2012;31(4):481–6.
Fung CP, Lin YT, Lin JC, Chen TL, Yeh KM, Chang FY, et al. *Klebsiella pneumoniae* in gastrointestinal tract and pyogenic liver abscess. Emerg Infect Dis 2012;18(8):1322–5.
Gao S, Bertrand D, Chia BK, Nagarajan N. OPERA-LG: efficient and exact scaffolding of large, repeat-rich eukaryotic genomes with performance guarantees. Genome Biol 2016;17:102.
Gao S, Bertrand D, Nagarajan N. FinIS: improved in silico finishing using an exact quadratic programming formulation. Algorithms in bioinformatics. Springer; 2012. p. 314–25.
Harada S, Tateda K, Mitsui H, Hattori Y, Okubo M, Kimura S, et al. Familial spread of a virulent clone of *Klebsiella pneumoniae* causing primary liver abscess. J Clin Microbiol 2011;49(6):2354–6.
Hartantyo SHP, Chau ML, Koh TH, Yap M, Yi T, Cao DYH, et al. Foodborne *Klebsiella pneumoniae*: virulence potential, antibiotic resistance, and risks to food safety. J Food Prot 2020;83(7):1096–103.
Kleborate. Kleborate; Available from: https://github.com/katholt/Kleborate/wiki/Scores-and-counts. [Accessed 20 March 2021].
Lam MMC, Wick RR, Wyres KL, Gorrie CL, Judd LM, Jenney AWJ, et al. Genetic diversity, mobilisation and spread of the yersiniabactin-encoding mobile element ICEKp in *Klebsiella pneumoniae* populations. Microb Genom 2018;4(9).
Lin JC, Koh TH, Lee N, Fung CP, Chang FY, Tsai YK, et al. Genotypes and virulence in serotype K2 *Klebsiella pneumoniae* from liver abscess and non-infectious carriers in Hong Kong, Singapore and Taiwan. Gut Pathogens 2014;6:21.
Lin YT, Siu LK, Lin JC, Chen TL, Tseng CP, Yeh KM, et al. Seroepidemiology of *Klebsiella pneumoniae* colonizing the intestinal tract of healthy Chinese and overseas Chinese adults in Asian countries. BMC Microbiol 2012;12:13.
Siu LK, Fung CP, Chang FY, Lee N, Yeh KM, Koh TH, et al. Molecular typing and virulence analysis of serotype K1 *Klebsiella pneumoniae* strains isolated from liver abscess patients and stool samples from noninfectious subjects in Hong Kong, Singapore, and Taiwan. J Clin Microbiol 2011;49(11):3761–5.
Siu LK, Yeh KM, Lin JC, Fung CP, Chang FY. *Klebsiella pneumoniae* liver abscess: a new invasive syndrome. Lancet Infect Dis 2012;12(11):881–7.
Truong DT, Franzosa EA, Tickle TL, Scholz M, Weingart G, Pasolli E, et al. MetaPhlAn2 for enhanced metagenomic taxonomic profiling. Nat Methods 2015;12(10):902–3.
Tu YC, Lu MC, Chiang MK, Huang SP, Peng HL, Chang HY, et al. Genetic requirements for *Klebsiella pneumoniae*-induced liver abscess in an oral infection model. Infect Immun 2009;77(7):2657–71.
Wattam AR, Davis JJ, Assaf R, Boisvert S, Brettin T, Bun C, et al. Improvements to PATRIC, the all-bacterial Bioinformatics Database and Analysis Resource Center. Nucleic Acids Res 2017;45(D1):D535–42.
Wyres KL, Wick RR, Gorrie C, Jenney A, Follador R, Thomson NR, et al. Identification of Klebsiella capsule synthesis loci from whole genome data. Microb Genom 2016;2(12)e000102.
Young MT, Bray AS, Nagpal RK, Caudell DL, Yadav H, Zafar MA. An animal model to study *Klebsiella pneumoniae* gastro-intestinal colonization and host-to-host transmission. bioRxiv 2020;.
Zerbino DR, Birney E. Velvet: algorithms for de novo short read assembly using de Bruijn graphs. Genome Res 2008;18(5):821–9.