Genomic Characterization of *Klebsiella quasipneumoniae* from Clinical Specimens in Singapore

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ABSTRACT A total of 1,281 specimens from 1,024 patients were screened. Phylogenetic analysis classified 44 of these isolates as *Klebsiella quasipneumoniae* subsp. *similipneumoniae* (44/1,281 [3.4%]) and the remaining three as *K. quasipneumoniae* subsp. *quasipneumoniae*. The most common specimen source was urine (21/47 [44.7%]) followed by blood (14/47 [29.8%]). *K. quasipneumoniae* isolates were nonclonal. Carbapenemase-encoding genes (bla_{NDM} and $bla_{OXA-181}$) were detected in only two isolates (2/47 [4.3%]). *K. quasipneumoniae* appears to cause a spectrum of infections similar to those of *K. pneumoniae*, although higher rates of susceptibility to many commonly tested antimicrobials and low prevalence of virulence genes were demonstrated.

KEYWORDS antimicrobial resistance, clinical microbiology, *Enterobacteriaceae*, whole genome

*K*lebsiella pneumoniae is a pathogenic Gram-negative organism capable of causing serious infections in humans and can acquire significant antibiotic resistance. The spectrum of infection is wide, including urinary tract infections and respiratory tract infections. Invasive infections such as liver abscess, endophthalmitis, and meningitis have been attributed to hypervirulent *Klebsiella pneumoniae* (1, 2). Hypervirulence has been associated with a mucoid phenotype in strains with particular capsular serotypes (K1, K2, and K5) and virulence genes, such as siderophore systems yersiniabactin, aerobactin, and salmochelin (*ybt*, *iuc*, and *iro*), genotoxin colibactin (*clb*), and the regulators of mucoid phenotype, *rmpA/rmpA2* (3).

Genomic analysis has distinguished *Klebsiella pneumoniae* into at least seven phylogroups: *K. pneumoniae sensu stricto* (Kp1), *K. quasipneumoniae* subsp. *quasipneumoniae* (Kp2), *K. variicola* (Kp3), *K. quasipneumoniae* subsp. *similipneumoniae* (Kp4), *K. variicola* subsp. *tropicalensis* (Kp5), *K. quasivariicola* (Kp6), and *K. africanensis* (Kp7) (4–8).

There is increasing recognition that both *K. quasipneumoniae* subspecies cause infections in humans (5, 9–11). Of particular concern were reports of *K. quasipneumoniae* subsp. *similipneumoniae* identified as the causative microorganism of neonate septicemia in China (12) and also the cause of a neonatal bacteremia outbreak at a tertiary hospital in Nigeria (13). The *K. quasipneumoniae* subspecies have also been observed to acquire plasmid-mediated carbapenem resistance (14–16), which may complicate treatment regimens. The true prevalence of *K. quasipneumoniae* remains unknown, as it is indistinguishable from *K. pneumoniae* (Kp1) in clinical laboratories which utilize matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) for routine microbial identification (17).

In this study, we investigated the distribution of *K. quasipneumoniae* within the *K. pneumoniae* complex and performed whole-genome sequencing to describe their genomic characteristics with a focus on acquired antimicrobial resistance and virulence genes.

Blood culture isolates in 2019 and all isolates between February and August 2020 identified as *K. pneumoniae* by MALDI-TOF (Bruker MALDI Biotyper; Bruker, Billerica, MA,

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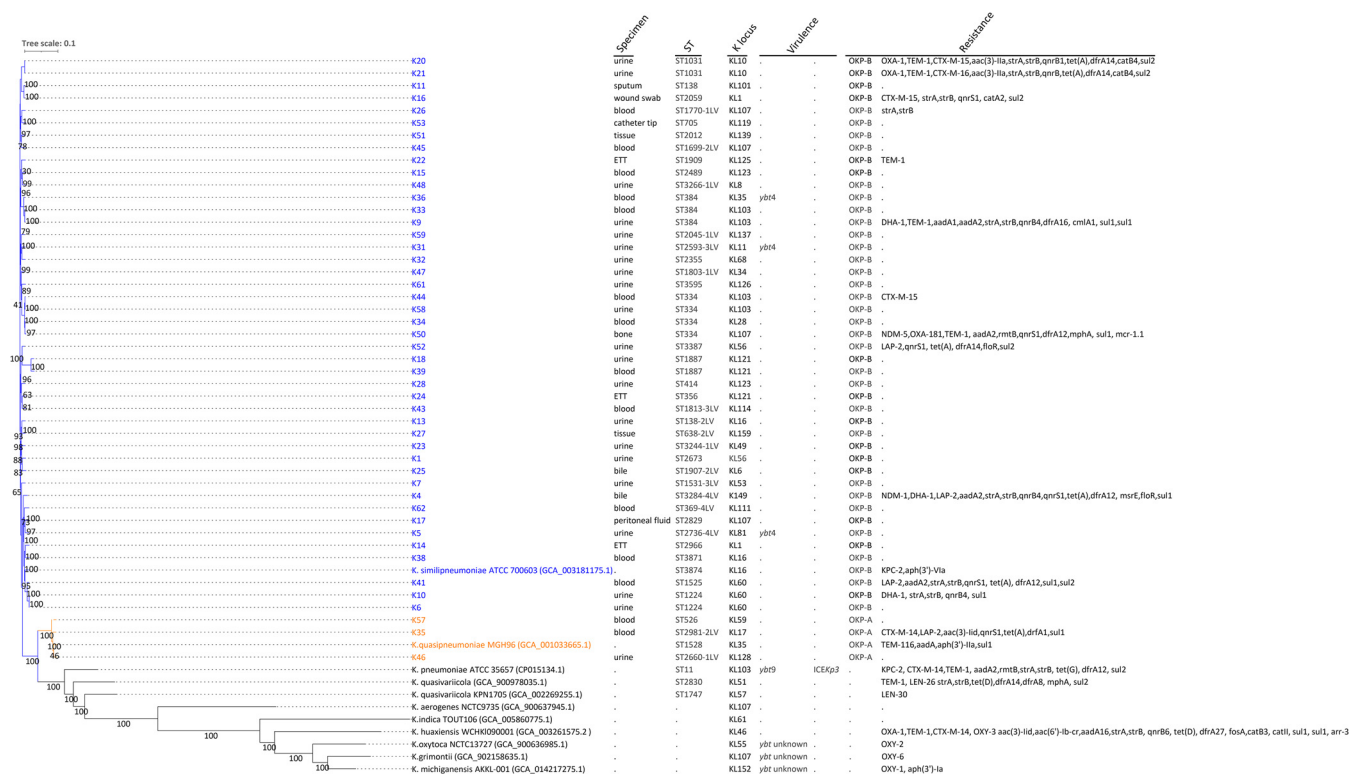
USA) were included. Further identification of these isolates was performed using PCR for detection of OKP β-lactamase (bla_{OKP}), a known chromosomal species-specific marker for *K. quasipneumoniae* (16). The primers okpBF, 5′-GCCAGCCCTCAGCCGCTTGAG-3′, and okpBR, 5′-ATAGATCACCACGATACGCTCCGC-3′, were used for PCR amplification, producing 714-bp amplicons, which then underwent Sanger sequencing. Routine susceptibility testing was performed using Vitek 2 (bioMérieux, Marcy-l'Étoile, France), and antimicrobial susceptibility was interpreted based on European Committee on Antimicrobial Susceptibility Testing (EUCAST) susceptibility break points (version 11.0).

Illumina NovaSeq 6000 sequencing (Illumina Inc., San Diego, CA, USA) was used to generate and assemble 150-bp paired-end reads. An average sequencing depth of 250× was achieved for the genomes. Raw reads were trimmed using Trimmomatic v. 0.38 (18) and then assembled with SPAdes version 3.14.0 (19). Genome annotation was carried out using Prokka (20). Kleborate (21) was used to identify the multilocus sequence type, species identity, *K. pneumoniae* integrating conjugative element (ICE*Kp*)-associated and plasmid-associated virulence loci and antimicrobial resistance. Kleborate also assigns a virulence score based on the presence of *ybt*, *clb*, and *iuc* as follows: 0, none present; 1, *ybt* only; 2, *clb* without *iuc* (regardless of *ybt*; however, *ybt* is almost always present when *clb* is); 3, *iuc* only; 4, *iuc* and *ybt* without *clb*; and 5, all three genes present. Abricate using the database PlasmidFinder 2.1 (https://cge.cbs.dtu.dk/services/PlasmidFinder/) was used to determine the presence of plasmid replicons. Single-nucleotide polymorphisms (SNPs) were identified using Snippy (https://github.com/tseemann/snippy). A total of 71,339 SNPs were included for phylogenetic analysis with 1,000 bootstrap replicates using FastTree (22). Tree editing and annotation were performed using interactive Tree Of Life (iTOL) (23). Average nucleotide identity (ANI) values were calculated using the Pyani package (https://github.com/widdowquinn/pyani). The following reference genomes were used for ANI comparisons: *K. quasipneumoniae* subsp. *similipneumoniae* strain ATCC 700603 (GenBank assembly accession GCA_003181175.1), *K. quasipneumoniae* subsp. *quasipneumoniae* MGH96 (GenBank assembly accession GCA_001033665.1), and *K. pneumoniae* ATCC 35657 strain KPN1482 (GenBank CP015134.1).

A total of 1,281 isolates from 1,024 patients were included. The specimen sources were diverse and included respiratory samples (bronchoalveolar lavage fluid and sputum), urine, rectal swabs, wound swabs, tissue, bone, pus, bile, and pleural and peritoneal fluid. Of the 1,281 isolates, 47 were bla_{OKP} positive. bla_{OKP-A} is associated with *K. quasipneumoniae* subsp. *quasipneumoniae* and bla_{OKP-B} with *K. quasipneumoniae* subsp. *similipneumoniae* (Fig. 1). All 47 isolates were whole-genome sequenced, and both phylogenetic analysis and Kleborate classified 44 of these isolates as *K. quasipneumoniae* subsp. *similipneumoniae* (44/1,281 [3.43%]) and the remaining three as *K. quasipneumoniae* subsp. *quasipneumoniae* (3/1,281 [0.23%]) (Fig. 1). The species identification was also supported by ANI values of >99% compared to their species reference genome (data not shown). The most common specimen source was urine (21/47 [44.7%]) followed by blood (14/47 [29.8%]) (Fig. 1). Our results are consistent with previous studies in which *K. quasipneumoniae* typically forms a smaller proportion of the *K. pneumoniae* complex, with *K. pneumoniae sensu stricto* being the dominant species (4, 24). Likewise, Long et al. (16) suggested that approximately 2% of human infections attributed to *K. pneumoniae* were actually caused by *K. quasipneumoniae*.

The *K. quasipneumoniae* isolates were nonclonal, with 39 diverse sequence types (STs) (Fig. 1). The most commonly identified ST was ST334 (4/47 [8.5%]). *K. quasipneumoniae* ST334 has been reported as a potential emerging outbreak-associated antimicrobial-resistant clone (25). In a Cambodian neonatal unit, ST334 was the most prevalent *K. quasipneumoniae* subsp. *similipneumoniae* sequence type, representing 30.5% of all isolates analyzed (26). Additionally, in a Pakistani hospital, the major sequence type observed was ST334, making up 29.2% of all *K. quasipneumoniae* hospital isolates. In our cohort, none of our *K. quasipneumoniae* isolates came from neonates. Of the ST334 isolates, one particular isolate was phenotypically multidrug resistant and was also the only isolate bearing dual carbapenemases (NDM-5 and OXA-181) and plasmid-mediated

Label	Specimen	ST	K locus	Virulence	OKP	Resistance
K20	urine	ST1031	KL10	.	OKP-B	OXA-1,TEM-1,CTX-M-15,aac(3)-IIa,strA,strB,qnrB1,tet(A),dfrA14,catB4,sul2
K21	urine	ST1031	KL10	.	OKP-B	OXA-1,TEM-1,CTX-M-16,aac(3)-IIa,strA,strB,qnrB,tet(A),dfrA14,catB4,sul2
K11	sputum	ST138	KL101	.	OKP-B	.
K16	wound swab	ST2059	KL1	.	OKP-B	CTX-M-15, strA,strB, qnrS1, catA2, sul2
K26	blood	ST1770-1LV	KL107	.	OKP-B	strA,strB
K53	catheter tip	ST705	KL119	.	OKP-B	.
K51	tissue	ST2012	KL139	.	OKP-B	.
K45	blood	ST1699-2LV	KL107	.	OKP-B	.
K22	ETT	ST1909	KL125	.	OKP-B	TEM-1
K15	blood	ST2489	KL123	.	OKP-B	.
K36	urine	ST3266-1LV	KL8	.	OKP-B	.
K33	blood	ST384	KL35	ybt4	OKP-B	.
K9	blood	ST384	KL103	.	OKP-B	.
K59	urine	ST384	KL103	.	OKP-B	DHA-1,TEM-1,aadA1,aadA2,strA,strB,qnrB4,dfrA16,cmlA1,sul1,sul1
K31	urine	ST2045-1LV	KL137	.	OKP-B	.
K32	urine	ST2593-3LV	KL11	ybt4	OKP-B	.
K47	urine	ST2355	KL68	.	OKP-B	.
K61	urine	ST1803-1LV	KL34	.	OKP-B	.
K58	urine	ST3595	KL126	.	OKP-B	.
K34	blood	ST334	KL103	.	OKP-B	CTX-M-15
K50	blood	ST334	KL103	.	OKP-B	.
K52	blood	ST334	KL28	.	OKP-B	.
K18	bone	ST334	KL107	.	OKP-B	NDM-5,OXA-181,TEM-1, aadA2,rmtB,qnrS1,dfrA12,mphA, sul1, mcr-1.1
K39	urine	ST3387	KL56	.	OKP-B	LAP-2,qnrS1, tet(A), dfrA14,floR,sul2
K28	urine	ST1887	KL121	.	OKP-B	.
K24	blood	ST1887	KL121	.	OKP-B	.
K43	urine	ST414	KL123	.	OKP-B	.
K13	ETT	ST356	KL121	.	OKP-B	.
K27	blood	ST1813-3LV	KL114	.	OKP-B	.
K23	urine	ST138-2LV	KL16	.	OKP-B	.
K1	tissue	ST638-2LV	KL159	.	OKP-B	.
K25	urine	ST3244-1LV	KL49	.	OKP-B	.
K7	urine	ST2673	KL56	.	OKP-B	.
K4	bile	ST1907-2LV	KL6	.	OKP-B	.
K62	urine	ST1531-3LV	KL53	.	OKP-B	.
K17	bile	ST3284-4LV	KL149	.	OKP-B	NDM-1,DHA-1,LAP-2,aadA2,strA,strB,qnrB,qnrS1,tet(A),dfrA12,msrE,floR,sul1
K5	blood	ST369-4LV	KL111	.	OKP-B	.
K14	peritoneal fluid	ST2829	KL107	.	OKP-B	.
K38	urine	ST2736-4LV	KL81	ybt4	OKP-B	.
K. similipneumoniae ATCC 700603 (GCA_003181175.1)	ETT	ST2966	KL1	.	OKP-B	.
K41	blood	ST3871	KL16	.	OKP-B	.
K10		ST3874	KL16	.	OKP-B	KPC-2,aph(3')-VIa
K6	blood	ST1525	KL60	.	OKP-B	LAP-2,aadA2,strA,strB,qnrS1, tet(A), dfrA12,sul1,sul2
K57	urine	ST1224	KL60	.	OKP-B	DHA-1, strA,strB,qnrB4, sul1
K35	blood	ST1224	KL60	.	OKP-B	.
K. quasipneumoniae MGH96 (GCA_001033665.1)	blood	ST526	KL59	.	OKP-A	.
K46	blood	ST2981-2LV	KL17	.	OKP-A	CTX-M-14,LAP-2,aac(3)-IId,qnrS1,tet(A),drfA1,sul1
		ST1528	KL35	.	OKP-A	TEM-116,aadA,aph(3')-IIa,sul1
	urine	ST2660-1LV	KL128	.	OKP-A	.
K. pneumoniae ATCC 35657 (CP015134.1)	.	ST11	KL103	ybt9	ICEKp3	KPC-2, CTX-M-14,TEM-1, aadA2,rmtB,strA,strB,tet(G), dfrA12, sul2
K. quasivariicola (GCA_900978035.1)	.	ST2830	KL51	.	.	TEM-1, LEN-26 strA,strB,tet(D),dfrA14,dfrA8, mphA, sul2
K. quasivariicola KPN1705 (GCA_002269255.1)	.	ST1747	KL57	.	.	LEN-30
K. aerogenes NCTC9735 (GCA_900637945.1)	.	.	KL107	.	.	.
K.indica TOUT106 (GCA_005860775.1)	.	.	KL61	.	.	.
K. huaxiensis WCHKl090001 (GCA_003261575.2)	.	.	KL46	.	.	OXA-1,TEM-1,CTX-M-14, OXY-3 aac(3)-IId,aac(6')-Ib-cr,aadA16,strA,strB, qnrB6, tet(D), dfrA27, fosA,catB3,catII,sul1,sul1, arr-3
K.oxytoca NCTC13727 (GCA_900636985.1)	.	.	KL55	ybt unknown	.	OXY-2
K.grimontii (GCA_902158635.1)	.	.	KL107	ybt unknown	.	OXY-6
K. michiganensis AKKL-001 (GCA_014217275.1)	.	.	KL152	ybt unknown	.	OXY-1, aph(3')-Ia

FIG 1 Core SNP phylogenetic tree of 47 *Klebsiella quasipneumoniae*. The metadata include specimen source, sequence type (ST), K-loci, virulence factors, presence of bla_{OKP}, and other acquired resistance determinants. LV, locus variant; ETT, endotracheal aspirate; *ybt*, yersiniabactin; ICE*Kp*, *Klebsiella* integrative conjugative element. The blue branch labels belong to *K. quasipneumoniae* subsp. *similipneumoniae*, and the orange labels belong to *K. quasipneumoniae* subsp. *quasipneumoniae*. The bootstrap values are indicated on the nodes, where black asterisks represent a bootstrap value of 100.

mcr-1.1 (Fig. 1). ST2727 *K. quasipneumoniae* subsp. *similipneumoniae* has been reported in an outbreak event in a neonatal intensive care unit (NICU) (12). However, none of the isolates in our study belonged to this sequence type. Typically, the number of SNP differences for isolates with the same sequence type was observed to be <500, while nonrelated sequence types had SNP differences of >1,100 (see Fig. S1 in the supplemental material).

Acquired antimicrobial resistance genes were observed in 13 isolates (13/47 [27.6%]). Carbapenemase-encoding genes (bla_{NDM} and $bla_{OXA-181}$) were detected in two isolates (2/47 [4.3%]). Other observed resistance genes included those encoding extended-spectrum β-lactamases (bla_{CTX-M}), oxacillinases (bla_{OXA-1}), penicillinases (bla_{TEM}), aminoglycoside-modifying enzymes (*aacA*, *aadA*, *strA*, and *strB*), fluoroquinolone resistance proteins (*qnrB4* and *qnrS*), dihydrofolate reductase (*dfr*), and dihydropteroate synthase (*sul1* and *sul2*) conferring trimethoprim-sulfamethoxazole resistance as well as plasmid-mediated colistin resistance (*mcr-1.1*) (Fig. 1).

We reviewed the phenotypic drug susceptibility data for *K. quasipneumoniae*; in addition, antibiograms from *K. pneumoniae* identified during the study period were used as a comparator. Overall, there was a trend toward higher rates of susceptibility to the tested antibiotics (with the exceptions of those for meropenem and amikacin, which were similar) for *K. quasipneumoniae* than for *K. pneumoniae* (Table 1).

Our observations paralleled the study by Lam et al. (27), in which no ICE*Kp* was detected, and *ybt4* (plasmid encoded and not mobilized by ICE*Kp*) was the only virulence factor detected in three *K. quasipneumoniae* subsp. *similipneumoniae* isolates (Fig. 1). It has been noted that ICE*Kp*, an integrative conjugative element which mobilizes the *ybt* locus, was rare or absent from *K. pneumoniae*'s closest relatives, *K. variicola* and *K. quasipneumoniae* (27). *iuc*, *clb*, *iro*, and *rmpA/rmpA2* genes were not detected in any isolate.

Of the three *ybt*⁺ isolates, one was from blood and the other two were from urine. The overall virulence score as predicted by the Kleborate tool (21) was 0 (no virulence factors

TABLE 1 Susceptibility rates of *K. quasipneumoniae* and *K. pneumoniae* isolated during the 6-month study period from February to August 2020

Species	Antibiotic susceptibility (% [no. of susceptible isolates/total no. of isolates])							
	Amoxicillin/ clavulanic acid	Ceftriaxone	Piperacillin- tazobactam	Meropenem	Gentamicin	Amikacin	Ciprofloxacin	Sulfamethoxazole- trimethoprim
K. quasipneumoniae	80.9 (38/47)	72. (34/47)	87.2 (41/47)	97.9 (46/47)	91.5 (43/47)	97.9 (46/47)	80.9 (38/47)	83.0 (39/47)
K. pneumoniae	70.8 (862/1,218)	69.6 (848/1,218)	75.8 (920/1,214)	98.1 (1,194/1,218)	87.4 (1,065/1,218)	97.8 (1,191/1,218)	64.4 (784/1,218)	71.2 (867/1,218)

detected) or 1 (only *ybt* detected) (21). In contrast, it was common to observe scores of >2 in the more pathogenic *K. pneumoniae* isolates (21). The *ybt*$^+$ isolates also did not have either K1, K2, or K5 capsular serotypes. In addition, a capsule synthesis locus (K-locus) was detected for every isolate (Fig. 1). Thirty-nine distinct K-loci were identified, reflecting the immense diversity of the capsular serotype. The K-loci did not appear to associate with phylogenetic clusters (Fig. 1). Two of our isolates had the K1 serotype and were recovered from a wound swab and an endotracheal tube specimen (Fig. 1), which are not sample sites typically associated with hypervirulent *K. pneumoniae* infections. Genomes from the *Klebsiella* liver abscess syndrome study (28) were also investigated to identify potential *K. quasipneumoniae* isolates that were misidentified as *K. pneumoniae*. We performed the same bioinformatic analysis described in that study on the 70 genomes (NCBI BioProject PRJNA351910) and found that one of the genomes was *bla*$_{OKP-B}$-positive *K. quasipneumoniae* subsp. *similipneumoniae*. Interestingly, this clinical *Klebsiella* liver abscess isolate (TTSH04) harbored a novel multilocus sequence type (MLST; ST2037) and capsule serotype (28) and had a virulence score of 0. *K. quasipneumoniae* isolates with the K1 capsule as well as the complement of virulence factors (*ybt*, *iro*, and *rmpA*) have also been reported as the causative agent of liver abscess (29, 30).

In our study, the proportion of *K. quasipneumoniae* isolates identified within *K. pneumoniae* was small (3.67%). Consistent with other reports, *K. quasipneumoniae* isolates bearing virulence genes were the minority (three *ybt*$^+$ isolates) (31), which may indicate that the virulence potential of *K. quasipneumoniae* is limited. Despite this, the sample source distribution of *K. quasipneumoniae* isolates suggests that the spectrum of infections is similar to that of *K. pneumoniae sensu stricto* and cannot be dismissed as nonpathogenic. Also, *K. quasipneumoniae* has been sporadically reported in the literature as the causative agent of liver abscesses exhibiting a hypervirulent phenotype. *K. quasipneumoniae* may still represent significant pathogen potential despite higher overall susceptibility rates than for *K. pneumoniae* isolates.

Data availability. The raw reads of the isolates sequenced in this study have been deposited under BioProject number PRJNA704495.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.
SUPPLEMENTAL FILE 1, PDF file, 0.7 MB.

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