Molecular epidemiology and clinical impact of *Klebsiella* spp. causing bloodstream infections in Hong Kong

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Summary

Background The epidemiological features of the *Klebsiella pneumoniae* causing bloodstream infections in Hong Kong and their potential threats to human health remained unknown.

Methods *K. pneumoniae* strains collected from four hospitals in Hong Kong during the period of 2009–2018 were subjected to molecular typing, string test, antimicrobial susceptibility testing, whole genome sequencing and analysis. Clinical data of patients from whom these strains were isolated were analyzed retrospectively using univariate and multivariate logistic regression approaches.

Findings The 240 *Klebsiella* spp. strains belonged to 123 different STs and 63 different capsule loci (KLs), with KL1 and KL2 being the major type. 86 out of 212 BSI-KP (40.6%) carried at least one of the virulence genes *iuc*, *iro*, *rmpA* or *rmpA2*. Virulence plasmid correlated well with the string test positive result, yet 8 strains without *rmp* genes were also hypermucoviscous, which was due to *wzc* mutation. The mortality rate of bloodstream infection patients was 43.0%. Univariant analysis showed that factors including renal replacement therapy (FDR adjusted $p = 0.0007$), mechanical ventilation (FDR adjusted $p < 0.0001$) and respiratory sepsis (FDR adjusted $p < 0.0001$) were found to pose the highest risk of death upon infection by *Klebsiella* spp.

Interpretation This study revealed the high mortality rate and risk factors associated with bloodstream infections caused by *K. pneumoniae* in Hong Kong, which warrants immediate action to develop effective solution to tackle this problem.

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Keywords: *Klebsiella pneumoniae*; Bloodstream infections; Epidemiology; Risk factors; Mortality rate

Introduction

Klebsiella pneumoniae is a Gram-negative bacillus from the genus *Klebsiella* and family *Enterobacteriaceae*, which could cause a range of severe infections in humans.[1] Over the past decades, *K. pneumoniae* has become an important pathogen due to the increasing prevalence of hospital-associated infections caused by multidrug-resistant *K. pneumoniae* (MDR-KP) and hypervirulent *K. pneumoniae* (HvKP).[2] *K. pneumoniae* is well known for its ability to acquire genetic elements through horizontal gene transfer, resulting in the above two *K. pneumoniae* groups, HvKP and MDR-KP.[3] The HvKP carries integrative conjugative elements (ICEs) and plasmids encoding virulence factor including siderophores (*iro*, *iuc* and *ybt*), the colibactin toxin (*clb*) and regulators of the mucoid phenotype (*rmpA*/*rmpA2*), and are able to cause community-acquired infections in healthy individuals.[4] The MDR-KP produce extended-spectrum β-lactamases

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Research in context

Evidence before this study

We searched, with the following terms and no language restrictions, the PubMed database of NCBI for reports that were published during the period of Jan 1, 2000 to Nov 03, 2023: "*Klebsiella pneumoniae* blood stream infections", "*Klebsiella pneumoniae* blood stream infections mortality" and "blood stream infections *Klebsiella pneumoniae* epidemiology". We found 183 reports under this topic, most of which were published recently, suggesting that *Klebsiella pneumoniae* blood stream infections have been recognized as a great concern of global public health. Although epidemiology and clinical outcomes of *Klebsiella pneumoniae* blood stream infections were described as grave in some reports, there is a lack of comprehensive clinical epidemiology and surveillance data.

Added value of this study

Our findings show that the *Klebsiella* spp. causing blood stream infections in Hong Kong is genetically diverse, comprised of *K. pneumoniae*, *K. quasipneumoniae* subsp. similipneumoniae, *K. quasipneumoniae* subsp. quasipneumoniae and *K. variicola* subsp. variicola strains. KL1 and KL2 *K. pneumonia* strains are major strains causing BSI in Hong Kong. 40.6% of the BSI-KP carried at least one of the virulence genes *iuc*, *iro*, *rmpA* or *rmpA2* in various forms of virulence determinants and virulence plasmid correlated well with the string test positive result, yet 8 strains without *rmpADC* or *rmpA2* genes were also hypermucoviscous, which was identified due to wzc mutation. Most of these strains were susceptible to the last sort antibiotics carbapenem, polymyxin B and tigecycline. The mortality rate of bloodstream infection patients was 43.0%. Factors including renal replacement therapy (FDR adjusted $p = 0.0007$), mechanical ventilation (FDR adjusted $p < 0.0001$) and respiratory sepsis (FDR adjusted $p < 0.0001$) were found to pose the highest risk of death upon infection by *Klebsiella* spp.

Implications of all the available evidence

This study interpreted the epidemiology of *Klebsiella* spp. strains causing bloodstream infections in Hong Kong. Our data revealed the high mortality rate and risk factors associated with bloodstream infections caused by *Klebsiella* spp., which warrants immediate action to develop effective solution to tackle this problem.

(ESBLs) and even carbapenemases, leading to failure of antibiotics therapy against different infections.[5] Both groups are associated with specific sequence types (STs), but recently convergence between these two groups has been observed.[6] Besides, recently, whole genome sequencing (WGS) and relative analysis has verified that some isolates identified as *K. pneumoniae* by biochemical or proteomics assays actually belong to closely related species, such as *K. quasipneumoniae* subsp. quasipneumoniae, *K. quasipneumoniae* subsp. similipneumoniae, *K. variicola* subsp. variicola, *K. variicola* subsp. tropica, *K. quasivariicola* and *K. Africana*.[7] These six species together with *K. pneumoniae* are then designated as *Klebsiella pneumoniae* species complex (KpSC).[7] Of the KpSC members, *K. pneumoniae* is still responsible for the majority of human infections.

K. pneumoniae can cause life-threatening bloodstream infections (BSIs) with significant prevalence and high mortality worldwide.[8] While previous studies have focused on the epidemiology and molecular characterization of MDR-KP and HvKP, only a few studies have assessed the significance of these phenotypes on clinical outcomes. The reported mortality rate of BSI due to *K. pneumoniae* in most studies varies from 32.6% to 56.1%.[9–11] Particularly, carbapenem-resistant *K. pneumoniae* (CRKP) contributed to even higher mortality rates (up to 79%).[9,12] This is multi-factor driven, however, delayed effective therapy definitely played a critical role.[9] On the other hand, the mortality rate of BSIs caused by hypermucoviscous *K. pneumoniae* (HMKP) is much lower than that by CRKP, perhaps due to non-overlapping with MDR of this group.[10] There is limited information regarding the epidemiology of this species in Hong Kong. Herein, we conducted an integrated epidemiological and genomic analysis of *K. pneumoniae* isolates which caused BSIs in Hong Kong to investigate the resistomes, virulence determinants, and the phylogenetic relationship between BSI-KP. Risk factors on clinical outcomes have also been analyzed. Understanding the risk factors associated with these strains in healthcare facilities is important for targeting interventions and reducing hospital transmission.

Methods

Study design and materials

The workflow of the current study is outlined in Fig. 1. *K. pneumoniae* isolates from patients with BSI from four hospitals located in Hong Kong were recruited in this study. CMC (Caritas Medical Centre) is a district general hospital in Kowloon, contains 1100 beds and serves mainly Northwest Kowloon. KWH (Kwong Wah Hospital) is a charitable district general hospital in Kowloon, contains 1100 beds and serves mainly West Kowloon. EH (Pamela Youde Nethersole Eastern Hospital) is an acute district general hospital in Hong Kong Island, contains 1800 beds and serves mainly East Hong Kong Island. QMH (Queen Mary Hospital) is a district general hospital in Hong Kong Island, contains 1700 beds and serves mainly Western and Southern districts.

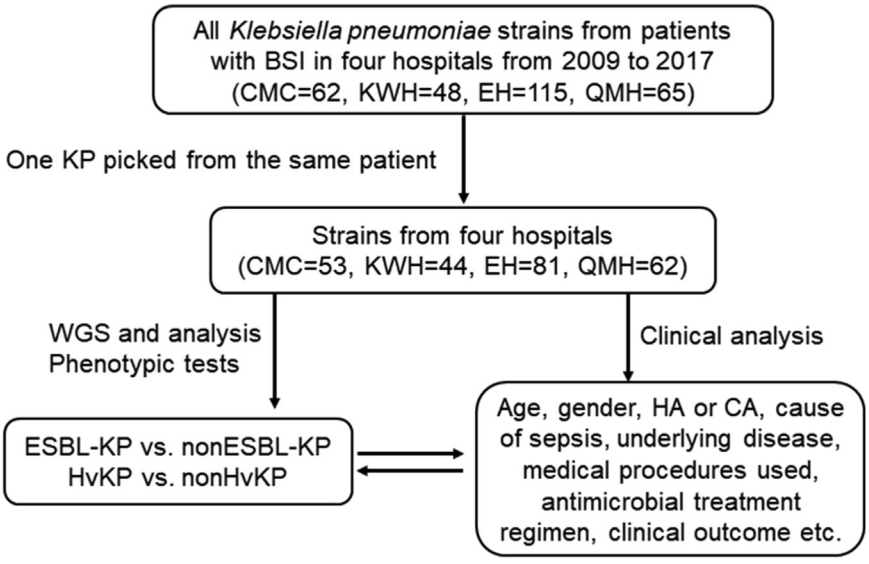

Fig. 1: **Flowchart depicting key procedures of the study.** BSI, bloodstream infections; CMC, Caritas Medical Centre; KWH, Kwong Wah Hospital; EH, Pamela Youde Nethersole Eastern Hospital; QMH, Queen Mary Hospital.

K. pneumoniae strains were collected from blood samples of patients with BSI during their hospitalization and stored at −80 °C. In this study, the *K. pneumoniae* strains in the participating hospitals were re-cultured and purified for further analysis. In brief, *K. pneumoniae* strains collected from CMC during the period of 2013–2018, from KWH during the period of 2009–2017, from EH during the period of 2015–2017, and from QMH during the period of 2016–2017 were included. Only one *K. pneumoniae* strain collected from each of the patients was selected for this study. Finally, 240 strains were selected and subjected to whole genome sequencing to investigate the molecular epidemiological features of these strains. Secondly, we performed a retrospective study to assess the risk factors of infection and the rate of mortality among patients. Retrospective analysis of clinical data of patients was performed. These include hospitals, year, age, gender, cause of sepsis, underlying disease, medical procedures used, antimicrobial treatment regimen, and clinical outcome. A total of 166 patients with full records were included. Comparative analysis of clinical data from patients with clinical outcome of dead or survival was performed to identify risk factors that contributed to clinical morality of BSI.

Procedures

All *K. pneumoniae* strains were cultured on Columbia blood agar containing 5% sheep blood (Becton Dickinson, USA). Strain identity was confirmed by the matrix-assisted laser desorption/ionization-time of flight mass spectrometry apparatus (MALDI-TOF MS) (Bruker, Germany). A string-test was performed on blood agar as previously described.[13] Antimicrobial susceptibility testing was performed by the agar dilution method. *Escherichia coli* strain ATCC 25922 served as quality control strain for susceptibility testing. Antimicrobial agents included ampicillin, ceftazidime, cefotaxime, meropenem, gentamycin, kanamycin, amikacin, chloramphenicol, ciprofloxacin, azithromycin, tetracycline, tigecycline and polymyxin B (Sigma–Aldrich, USA). The susceptibility was interpreted according to both Performance Standards for Antimicrobial Susceptibility Testing by the Clinical and Laboratory Standards Institute (CLSI) of 2023[14] and Clinical breakpoints and guidance by European Committee on Antimicrobial Susceptibility Testing (EUCAST) of 2023.[15] Genomic DNA was extracted using the Genomic purification kit for bacteria (Invitrogen, USA) and sequenced via the 150-bp paired-end Illumina NextSeq 500 platform (Illumina, San Diego, CA). Data has been deposited in the GenBank database under BioProject no. PRJNA1003408. Detailed procedure for WGS data analysis is described in Supplementary Materials.

For analysis of risk factors of infection, we procured electronic medical records on patient demographics, underlying medical conditions, previous treatment prior 30 days before hospitalization, antibiotic treatments and clinical outcome within 30 days after identification of *Klebsiella* spp. strains. Clinical data were collected and analyzed using the R software (R version 4.2.2). The hospital-acquired (HA), health care-associated (HCA) and community-acquired (CA) infections were defined according to previous criteria.[16] Briefly, an HA infection was defined as an infection that occurred 48 h after the patient's admission to hospital. An HCA infection was

defined as an infection occurred within 48 h of the patient's admission to hospital with the following conditions: 1) a history of intravenous therapy or hemodialysis in the 30 days before the bloodstream infection; 2) a history of hospitalization for 2 or more days in the three months before the bloodstream infection; 3) residence in a nursing home or long-term care facility. A CA infection was defined as an infection occurred within 48 h of the patient's admission to hospital and did not fulfill the definitions of HCA infections.

Ethics

All clinical data were collected according to the ORION Checklist.[17] Ethical approval has been obtained for this study with approval number of HKECREC-2017-056 for EH, KC/KE-17-0190/ER-3 for KWH, and KW/EX-17-156118-07 for CMC. The clinical data presented in this study have been extracted directly from the Clinical Management System (CMS) and the Philips IntelliSpace Critical Care and Anaesthesia System (ICCA).

Statistics

Univariate logistic regression was used to determine the level of association between clinical mortality of KP-BSI and different risk factors. All risk factors (except patient age and APACHE IV Score) are treated as 'factor' type, and age and APACHE IV score were treated as numeric. Benjamini-Hochberg (BH) was used to adjust the p value. Significant variables with p value of <0.05 were then selected for multivariate analysis to evaluate risk factors of morality of BSI.

Role of funders

The sponsor of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Prevalence and genetic diversity of BSI-KP in Hong Kong

A total of 290 isolates collected from patients diagnosed as BSIs in four hospitals in Hong Kong during a period from 2009 to 2018 and identified as *K. pneumoniae* using MALDI-TOF MS were recovered in this study. One *K. pneumoniae* strain collected from each of the patients was randomly selected for further analysis. The remains excluded were the isolates from the same patients. Finally, 240 strains were included and subjected to whole genome sequencing (Table 1). These included 53 isolates from CMC during 2013–2018, 44 isolates from KWH during 2009–2017, 81 isolates from EH during 2015–2017 and 62 isolates from QMH during 2016–2017. The sequenced genomes were *de novo* assembled into an average of 102 contigs (2–464) with a mean genome length of 5,529,223 bp (5,136,887–6,124,626 bp) and an average GC content of 57.20% (56.13–57.98%) (Table S1). Among them, 212 isolates were identified as *K. pneumonia* using Kleborate based on the draft genome sequences. While the rest 28 strains were identified as other species of KpSC: 21 *K. quasipneumoniae* subsp. similipneumoniae, 2 *K. quasipneumoniae* subsp. quasipneumoniae and 5 *K. variicola* subsp. variicola (Fig. 2).

The 212 *K. pneumoniae* strains belonged to 106 different STs, of which 67 were only represented by one single isolate (Table S1). The most common STs were ST23 (n = 18), ST25 (n = 12), ST11 (n = 9), ST86 (n = 7), ST29 (n = 5) and ST65 (n = 5). Among all *K. pneumonia* isolates, 55 different capsule loci (KL) were identified, of which 25 were only represented by one single isolate (Table S1). Though no KL was assigned to 16 isolates. The most common KLs were KL2 (n = 36), KL1 (n = 21), KL20 (n = 8), KL27 (n = 8) and KL122 (n = 8). These results indicated that KL1 and KL2 *K. pneumonia* strains were the major strains causing KP-BSIs in Hong Kong. The core-genome of the 212 *K. pneumonia* strains contains 4093 genes, and the pan-genome contains 16,888 genes. Then a core-genome tree was built using the core gene alignment. Phylogenetic analysis showed that the test strains were clustered according to ST types and KL serotypes (Fig. 3, Figure S1). The 21 *K. quasipneumoniae* subsp. similipneumoniae isolates belonged to 17 different STs, of which 15 were only represented by one single isolate (Table S1). Among them, four belonged to ST334 and 2 belonged to ST334-1LV. And the 2 *K. quasipneumoniae* subsp. quasipneumoniae strains and 5 *K. variicola* subsp. variicola strains all belonged to different STs. Yet the STs of these minor KpSC members were non-overlapping with the *K. pneumonia* strains, indicating that the *Klesiella* spp. causing BSIs in Hong Kong is genetically diverse.

Virulence determinants of KpSC strains

Among the 212 *K. pneumonia* strains, 86 (40.6%) carried at least one of the virulence genes *iuc*, *iro*, *rmpA* or *rmpA2* in various forms of virulence determinants. In details, 62 strains carried the pLVPK-like KpVP-1 type virulence plasmid with different coverages (24–98%) (Figure S2). The genome of strains PM43 and PM44 covered 24% of the virulence plasmid pLVPK, harbouring virulence genes *iro* and *rmpADC*. The genome of strain EH91 covered 56% of the virulence plasmid pLVPK. While the genome of other strains covered 70%–98% of plasmid pLVPK (Figure S2, Table S1). These results indicated that the typical virulence plasmid pLVPK has been under evolving among the *K. pneumonia* strains. Four strains all belonging to KL2 type but different ST types from 3 hospitals carried the KpVP-2 type virulence plasmid (Figure S3, Table S1). This kind of pPM27_Vir-like KpVP-2 type virulence plasmid has been proven to be conjugative in our

Hospital	Year	*K. pneumoniae*	*K. quasipneumoniae* subsp. similipneumoniae	*K. quasipneumoniae* subsp. quasipneumoniae	*K. variicola* subsp. variicola
CMC	2013	1	0	0	0
	2014	5	0	0	0
	2015	16	1	0	1
	2016	13	1	0	0
	2017	11	0	0	0
	2018	4	0	0	0
	Total	50	2	0	1
KWH	2009	10	1	0	0
	2010	4	0	0	1
	2011	1	0	0	0
	2012	5	0	0	0
	2013	2	0	0	1
	2014	2	0	0	0
	2015	7	2	0	0
	2016	4	0	0	1
	2017	3	0	0	0
	Total	38	3	0	3
EH	2015	26	3	0	0
	2016	25	4	0	1
	2017	21	1	0	0
	Total	72	8	0	1
QMH	2016	39	7	2	0
	2017	13	1	0	0
	Total	52	8	2	0

Table 1: **Number of KpSC strains causing BSI in four different hospitals in Hong Kong.**

previous study.[18] Besides, the chromosomal integrative conjugative element ICE*Kp1* carrying *iro3* and *rmp3* was identified in 11 strains from 4 hospitals, belonging to KL1, KL2, KL5, KL35, KL63 and KL108 (Table S1). The aerobactin encoding gene cluster *iuc3* has been identified in 13 strains from four hospitals (Table S1). Strain EH74 as a representative strain was subjected to Nanopore sequencing. The *iuc3* was located on the 235,277 bp $IncFIB_K/IncFII_K$ type plasmid pEH74_iuc3, and the other 12 strains carried similar backbone of plasmid pEH74_iuc3 (Figure S4). Lastly, the conjugative IncFIA/IncFII plasmid pEH13_2 carried the *iuc5* locus together with several resistance genes.[19] While only one *K. quasipneumoniae* subsp. similipneumoniae strain was

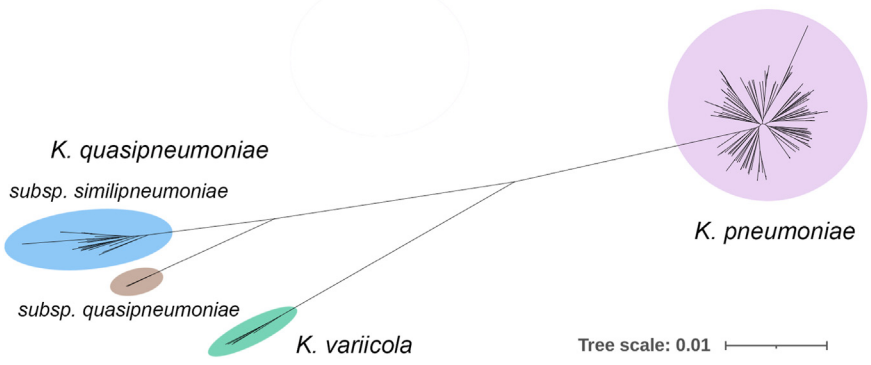

Fig. 2: **The phylogroups of the 240 *K. pneumoniae* isolates.** Among them, 212 isolates were identified as *K. pneumoniae*. While 21 were identified as *K. quasipneumoniae* subsp. similipneumoniae, 2 were identified as *K. quasipneumoniae* subsp. quasipneumoniae, 5 isolates were identified as *K. variicola* subsp. variicola.

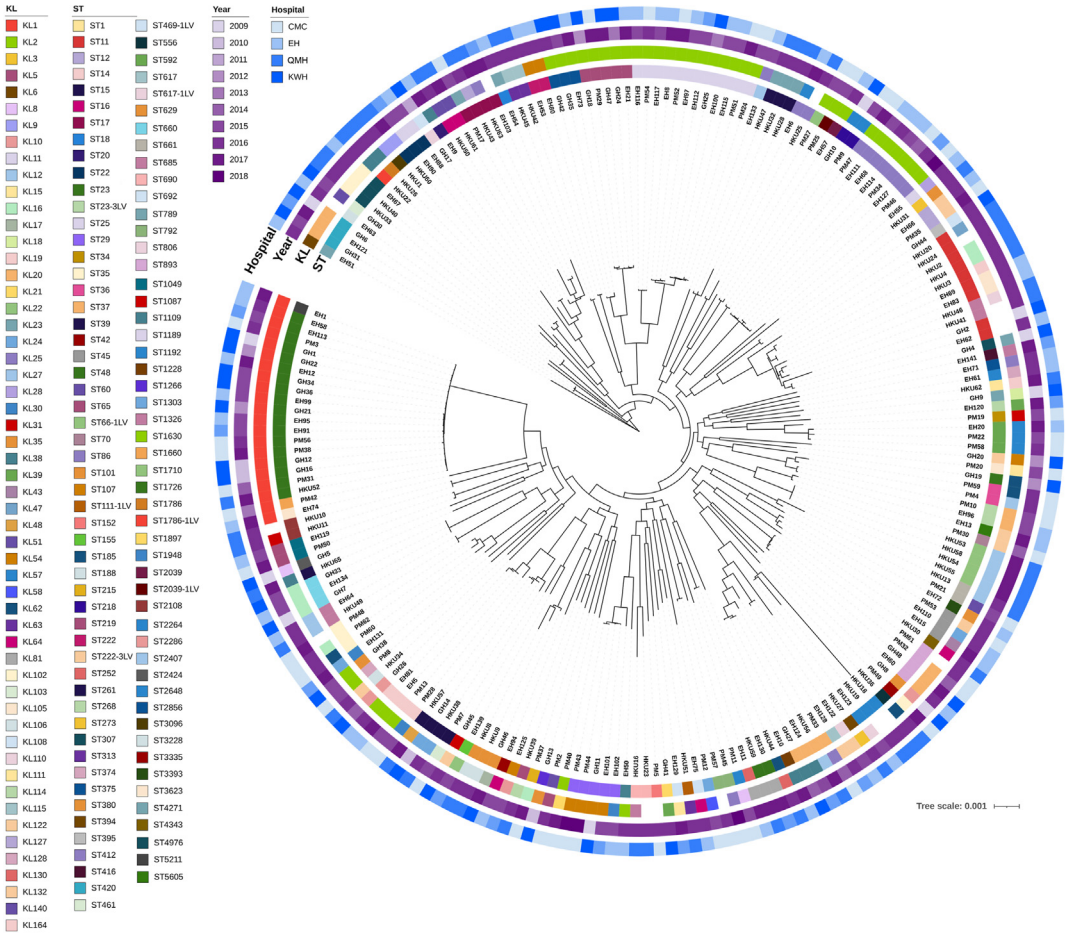

***Fig. 3*: The phylogroups of the 212 *K. pneumoniae* isolates.** The tree was annotated with STs, KLs, isolation years and hospitals from inside to outside.

found to carry *iuc3*, the rest *K. quasipneumoniae* and *K. variicola* strains all did not carry any of the virulence plasmid associated virulence genes (Table S1). The prevalence of these virulence plasmid associated genes among the isolates was 50.9%, 50.0%, 40.7% and 8.1% for hospitals CMC, KWH, EH and QMH, respectively (Table S1).

The carriage of other virulence genes among *K. pneumonia* strains has been presented on the phylogroup tree (Figure S5, Table S2). The type 3 fimbriae (*mrk*) cluster was present in 92% (n = 195) of the isolates. The yersiniabactin locus (*ybtAEPQSTUX-fyuA-irp1-irp2*) was detected in 47% (n = 100) of the isolates. While the colibactin locus (*clbABCDEFGHILMNOPQ*) was found in 15% (n = 31) of the isolates, which was highly associated with ICE*Kp10*. Notably, 20 of the 31 strains carrying *clb* belonged to KL1 and 7 belonged to KL2. Genes encoding allantoinase (*all*) was present in 22% (n = 46) of the isolates. The *kvgAS* genes which were involved in capsule formation were found in 10% (n = 21) of the isolates. The ferric iron uptake genes *kfuABC* were detected in 43% (n = 91) of the isolates. The microcin E492 locus were detected in 10% (n = 21) of the isolates. While no yersiniabactin, colibactin and microcin E492 encoding genes as well as the *kvgAS* genes were identified among the *K. quasipneumoniae* and *K. variicola* strains. Though they all carried the type 3 fimbriae (*mrk*) cluster and the ferric iron uptake genes *kfuABC*.

Resistance determinants of KpSC strains

Among the 212 BSI-KP isolates, a total of 51 different acquired antimicrobial resistance genes (ARGs) against 11 classes of antimicrobials were identified in 114 isolates (53.8%). The identified ARGs included 14 genes encoding resistance to aminoglycosides, 8 genes to β-lactamases, 7 genes to macrolides, 6 genes to trimethoprim, 4 genes to chloramphenicols, 3 genes to fluoroquinolones, 3 genes to sulphonamides, 3 genes to tetracyclines, 1 gene to fosfomycins, 1 gene to rifamycin

and 1 gene to polymyxin (Table S3). Except for the intrinsic genes *oqxAB* (n = 211) and bla_{SHV} (n = 210), the most prevalent ARGs were *sul1* (n = 77), *tet*(A) (n = 52) and bla_{TEM-1} (n = 51). The resistance genes to the last sort antibiotics were rare among these BSI-KP isolates. Only 2 strains belonging to ST11 from QMH carried the carbapenemase encoding gene bla_{KPC-2}. One strain from QMH carried the gene *mcr-8.1*, which resulted in polymyxin resistance.[20] One strain harboured the *tmexC1-tmexD1-toprJ1* cluster, which mediated resistance to tigecycline.[21] The resistance determinants of the *K. quasipneumoniae* and *K. variicola* strains were similar to the *K. pneumoniae* strains, though no carbapenem, polymyxin or tigecycline resistance genes were identified (Table S3). And β-lactamase encoding genes bla_{LAP}, bla_{OXA-1}, $bla_{CTX-M-1}$, $bla_{CTX-M-9}$, bla_{TEM-1} and bla_{DHA-1} were identified from 9 strains. The overall prevalence of ESBL genes among the isolates was 5.7%, 11.4%, 22.2% and 80.6% for hospitals CMC, KWH, EH and QMH, respectively (Table S1).

Phenotypic virulence and resistance profiles

The string test performed on all the 240 strains indicated that 29% (n = 69) of the strains were hypermucoviscous, including 68 *K. pneumoniae* and 1 *K. variicola* strain (Table S4). According to the WGS data, 77 *K. pneumoniae* strains carried the mucoid regulators *rmpADC* and/or *rmpA2*, yet only 61 of the 77 strains were phenotypic hypermucoviscous. This might be due to the truncation or mutation of these genes (Table S1). In addition, 8 strains (7 *K. pneumoniae* strains and 1 *K. variicola* strain) which did not harbor the *rmpADC* or *rmpA2* genes were identified as hypermucoviscous. Analysis of the capsular biosynthesis clusters of these 8 strains indicated that they belonged to 7 KLs and their *wzc* genes were all site-mutated (Table S5). It has been reported that the hypercapsule *wzc* mutants are highly associated with BSIs by MDR *K. pneumoniae*, and may be resulted from antibiotic treatment.[22]

The antimicrobial susceptibility testing (AST) showed that almost all the *K. pneumoniae* strains exhibited resistance to ampicillin (98%), while a quite lower rate resistance to other tested antibiotics was observed (Table 2, Table S4). The resistance rate of *K. pneumoniae* strains to ceftazidime and cefotaxime was 17% and 36% respectively. And the majority of these strains exhibited susceptibility to amikacin (98%), meropenem (97%), polymyxin B (97%) and tigecycline (99%). Expect for the bla_{KPC-2}, the meropenem resistance was also found to be mediated by mutation of Omp35 or Omp36 together with the β-lactamase genes. And expect for the *mcr-8.1* gene, the resistance of polymyxin B was primarily caused by the truncation of the *mgr*(B) gene. All of the 21 *K. quasipneumoniae* subsp. similipneumoniae strains exhibited resistance to ampicillin, while 19% exhibited resistance to ceftazidime and 52% exhibited resistance to cefotaxime. And no resistance to the last sort antibiotics meropenem, polymyxin B and tigecycline was observed. An exception is a *K. quasipneumoniae* subsp. similipneumoniae strain HKU14 with a truncated *mgr*(B) gene which showed resistance to polymyxin B. Similar resistance profiles have been observed among the two *K. quasipneumoniae* subsp. quasipneumoniae strains and the five *K. variicola* subsp. variicola strains.

Risk factors of BSI caused by KpSC and clinical outcomes

Complete clinical data were collected from a total of 166 patients from whom these strains were recovered. Then we performed a retrospective study to determine the risk factors associated with infections caused by the KpSC isolates. These 166 patients have been admitted to hospitals for a stay of 0.5–272 days, and all have been admitted to intensive care unit (ICU). Among the 166 patients, discharge destination was home in 48.8% (n = 81), convalescence hospital in 6.0% (n = 10), acute care hospital in 2.4% (n = 4) and in hospital-death in 42.8% (n = 71). 52% of the *Klebsiella* spp. (n = 87) was isolated from patients without health care exposure within 48 h after they were admitted to hospitals, which was defined as community-acquired (CA). And 13% of the *Klebsiella* spp. was healthcare-associated (HCA). While the rest of the patients were infected by *Klebsiella* spp. more than 2 days after they were admitted to hospitals, which was defined as hospital-acquired (HA). These patients were admitted to hospitals for surgeries and their immunocompromised conditions may lead to *Klebsiella* infections during their hospitalization. The 166 *Klebsiella* spp. strains included 149 *K. pneumoniae*, 12 *K. quasipneumoniae* subsp. similipneumoniae and 5 *K. variicola* subsp. variicola, associated with mortality of 43.0%, 33.3% and 60%, respectively, indicating that all these species could cause fatal BSIs. The average age of these 166 patients was 66.4 years old (range: 27–93 years old), and the male patients accounted for 59% (n = 98). The most frequent comorbidities of these patients were diabetes mellitus (n = 61), chronic kidney diseases (n = 23) and solid tumor (n = 21). Further, the cause of sepsis has been identified as mainly hepatobiliary (n = 50), respiratory (n = 38), gastrointestinal (n = 37) and urosepsis (n = 26). 16 out of the 166 BSI patients have received steroids within 30 days before the culture of *Klebsiella* spp. strains and 64 patients have received antibiotics within 30 days before this hospitalization. In this hospitalization, 58 out of the 166 BSI patients received renal replacement, 95 patients received mechanical ventilation and 106 patients have encountered sepsis shock. The average APACHE IV score was 108.6 (range: 31–233) and the average APACHE IV risk of death was 0.49 (range: 0.02–1). Most of the patients (95%) had been treated with antibiotics, with augmentin (30%) and piperacillin/tazobactam (29%) being the most

Antibiotics	Range	*K. pneumoniae*		*K. quasipneumoniae* subsp. similipneumoniae		*K. quasipneumoniae* subsp. quasipneumoniae		*K. variicola* subsp. variicola	
		%S	%R	%S	%R	%S	%R	%S	%R
Ampicillin	≤2–>128	0.9	98	0	100	0	100	0	80
Ceftazidime	≤0.03–>128	75	17	67	19	50	50	60	40
Cefotaxime	≤0.03–>128	64	36	48	52	50	50	60	40
Meropenem	≤0.03–>128	97	3	100	0	100	0	100	0
Gentamycin	≤0.5–>128	77	23	100	0	50	50	80	20
Kanamycin	≤0.5–>128	84	13	90	10	100	0	60	40
Amikacin	≤0.5–>128	98	2	100	0	100	0	100	0
Chloramphenicol	≤0.5–>128	74	25	57	43	50	50	80	20
Ciprofloxacin	≤0.03–>128	60	28	71	10	50	50	60	20
Azithromycin	≤0.5–>128	80	20	90	10	100	0	80	20
Tetracycline	≤0.5–>128	64	32	62	38	50	50	100	0
Tigecycline	≤0.25–16	99	1	100	0	100	0	100	0
Polymyxin B	≤0.25–128	97	3	95	5	100	0	100	0

Table 2: **Antimicrobial susceptibility of clinical KpSC strains.**

commonly prescribed drugs, followed by levofloxacin (8%). The rest of the antibiotics included ceftriaxone, cefotaxime, ceftazidime, cefuroxime, meropenem, ertapenem, tienam, gentamicin and amikacin.

We identified the clinical characteristics affecting the development of BSIs of HvKP and ESBL-KP, by comparing patient demographics, clinical characteristics, causes of sepsis, prior treatments, antibiotic exposures, and outcomes of patients with HvKP and nonHvKP, ESBL-KP and nonESBL-KP, respectively (Tables S6 and S7). The factors determined to be more relevantly related with HvKP-BSI, using the univariable logistic regression analysis, included community-acquired (FDR adjusted p = 0.0020), appropriate empirical antibiotic treatment (FDR adjusted p = 0.0197), isolation year of 2016 (FDR adjusted p = 0.0347), sepsis shock (FDR adjusted p = 0.0461) and hepatobiliary sepsis (FDR adjusted p = 0.0567). And it was found to negatively correlated with gastrointestinal sepsis (FDR adjusted p = 0.0079) and hospital-acquired (FDR adjusted p = 0.0107) (Table S6). Though the underlying reason why HvKP was negatively associated with gastrointestinal sepsis remained unknown. One possible reason was that the majority of the gastrointestinal sepsis-KP in this study was acquired in hospital, where non-hvKP were more prevalent. The factors determined to be more relevantly related with ESBL-KP-BSI, using the univariable logistic regression analysis, included inappropriate empirical antibiotic treatment (FDR adjusted p = 0.0014), hospital-acquired (FDR adjusted p = 0.0506), prior antibiotics use (FDR adjusted p = 0.0506) and respiratory sepsis (FDR adjusted p = 0.0518) (Table S7).

We then attempted to identify risk factors that are potentially related to clinical mortality of BSI-KP infections using univariate logistic regression analysis. Factors including renal replacement therapy (FDR adjusted p = 0.0007), mechanical ventilation (FDR adjusted p < 0.0001) and respiratory sepsis (FDR adjusted p < 0.0001) were found to pose the highest risk of death upon infection by *Klebsiella* spp. (Table 3). Other factors associated with death included hospital-acquired infections (FDR adjusted p = 0.0141) and chronic kidney diseases (FDR adjusted p = 0.0765). While factors such as community-acquired infections (FDR adjusted p = 0.0034), hepatobiliary sepsis (FDR adjusted p = 0.0101), diabetes mellitus (FDR adjusted p = 0.0159), urosepsis (FDR adjusted p = 0.0436) and age (55–80) (FDR adjusted p = 0.0436) were found to negatively related with clinical mortality of BSI-KP infections. This meant that the presence of these factors was associated with a lower risk of death from BSI-KP infections. The risk factors with an FDR adjusted p < 0.05 from the univariate analysis were retrieved and subjected to the multivariable logistic regression analysis. Only APACHE IV risk of death (OR 406.19, 95% CI 1.40–157083.4, p = 0.0401) and respiratory sepsis (OR 4.47, 95% CI 1.11–20.54, p = 0.0418) remained adversely and independently associated with mortality, whereas a negative association was observed for hepatobiliary sepsis (OR 0.20, 95% CI 0.04–0.89, p = 0.0413) (Table 4).

Discussion

In this study, we investigated molecular epidemiology and clinical outcome of BSIs caused by *K. pneumoniae*, a life-threatening pathogen in Hong Kong during 2009–2018. A total of 240 *Klebsiella* spp. strains collected from blood samples of patients with BSI in four hospitals were included in this study. The 240 strains belonged to 130 different STs, indicating that the *Klesiella* spp. causing BSIs in Hong Kong is genetically diverse. Our

Factors	Death n = 71 (%)	Recovered n = 81 (%)	*p* value	FDR adjusted *p*
Species				
K. pneumoniae	64 (90.1)	71 (87.7)	0.8885	0.9605
K. quasipneumoniae subsp. similipneumoniae	4 (5.6)	8 (9.9)	0.4954	0.6392
K. variicola subsp. variicola	3 (4.2)	2 (2.5)	0.4381	0.6109
Hospitals				
CMC	24 (33.8)	24 (29.6)	0.2964	0.5389
EH	31 (43.7)	40 (49.4)	0.4582	0.6109
KWH	16 (22.5)	17 (21.0)	0.8012	0.8902
Year				
2009–2014	12 (16.9)	19 (23.5)	0.1125	0.2368
2015	29 (40.8)	24 (29.6)	0.0344	0.0984
2016	15 (21.1)	32 (39.5)	0.0778	0.1729
2017	15 (21.1)	20 (24.5)	0.9908	0.9908
Age (years)				
<55	18 (25.4)	13 (16.0)	0.2333	0.4443
55–80	37 (52.1)	59 (72.8)	0.0117	0.0436
>80	16 (26.2)	9 (11.1)	0.0390	0.1041
Sex (male)	42 (59.2)	50 (61.7)	0.9785	0.9908
Infectious background				
Hospital-acquired	34 (47.9)	16 (19.8)	0.0028	0.0141
Health care-associated	11 (15.5)	10 (12.3)	0.3435	0.5496
Community-acquired	26 (36.6)	59 (72.8)	0.0005	0.0034
Comorbidities				
Diabetes mellitus	17 (15.5)	34 (42.0)	0.0036	0.0159
Cirrhosis	4 (5.6)	3 (3.7)	0.4384	0.6109
Congestive heart failure	4 (5.6)	3 (3.7)	0.4384	0.6109
Kidney disease	15 (21.1)	6 (7.4)	0.0230	0.0765
Haematological Malignancy	6 (8.4)	1 (1.2)	0.0479	0.1197
Solid tumor	10 (14.1)	9 (11.1)	0.6314	0.7428
Previous treatments				
Steroids	5 (7.0)	7 (8.6)	0.3318	0.5496
Antibiotics	32 (45.1)	26 (32.1)	0.1370	0.2740
Severity of illness				
Renal replacement therapy	37 (52.1)	17 (20.1)	0.0001	0.0007
Mechanical ventilation	61 (85.9)	24 (29.6)	<0.0001	<0.0001
Septic shock	52 (72.3)	45 (55.5)	0.0309	0.0949
APACHE IV Score (mean ± SD)	107 ± 45	106 ± 42	<0.0001	<0.0001
APACHE IV risk of death (mean ± SD)	0.46 ± 0.32	0.49 ± 0.30	<0.0001	<0.0001
Causes of sepsis				
Gastrointestinal	17 (23.9)	18 (22.2)	0.6581	0.7521
Hepatobiliary	12 (16.9)	35 (43.2)	0.0018	0.0101
Respiratory	30 (42.2)	5 (6.17)	<0.0001	<0.0001
Urosepsis	5 (7.0)	19 (23.4)	0.0120	0.0436
Others/unknown	7 (9.8)	4 (4.9)	/	/
Antibiotic treatment				
Fail	7 (9.8)	10 (12.3)	0.4557	0.6109
Success	53 (74.6)	71 (87.7)	0.0596	0.1402
No antibiotics given	11 (15.5)	0 (0)	0.9879	0.9908
Type of strains				
Virulence plasmid	29 (40.8)	38 (46.9)	0.3324	0.5496
String test	24 (33.8)	30 (37.0)	0.5873	0.7119
ESBL	12 (16.9)	10 (12.3)	0.5670	0.7088

Table 3: **Analysis of risk factors that significantly contribute to clinical mortality of BSI.**

Factors	Odds ratio	95% CI	p value
Age (55–80)	0.50	0.17–1.44	0.2009
Hospital-acquired	1.59	0.32–8.41	0.5755
Community-acquired	1.39	0.28–7.05	0.6840
Diabetes mellitus	0.46	0.15–1.36	0.1689
Renal replacement therapy	0.48	0.12–1.70	0.2720
Mechanical ventilation	2.02	0.62–6.84	0.2458
APACHE IV Score	1.04	0.97–1.06	0.7320
APACHE IV risk of death	406.19	1.40–157083.4	**0.0401**
Hepatobiliary sepsis	0.20	0.04–0.89	**0.0413**
Respiratory sepsis	4.47	1.11–20.54	**0.0418**
Urosepsis	0.43	0.07–2.20	0.3204

The bold indicated p value < 0.05.

Table 4: **Results of multivariate logistic regression analysis showing factors associated with clinical mortality of BSI.**

data show that KL1 and KL2 *K. pneumonia* strains are major strains causing KP-BSI in Hong Kong, while ST11 and ST307 *Kp* isolates were highly associated with BSIs in mainland China.[23] The prevalence of CRKP and carbapenemases among carbapenem-nonsusceptible *K. pneumoniae* remained low in Hong Kong.[24,25] Thus, the popular STs associated with carbapenem resistance have not become predominant in Hong Kong. Consistently, these *Klebsiella* spp. strains exhibited lower rate resistance to most antibiotics, especially, most of these strains were susceptible to the last sort antibiotics carbapenem, polymyxin B and tigecycline. Similarly, a previous study reported that the carbapenem resistance among Enterobacterales was rare in Hong Kong.[26] Though *K. pneumoniae* strains carrying the carbapenemase gene bla_{KPC-2}, the polymyxin resistance gene *mcr-8.1* and tigecycline resistance gene *tmexC1-tmexD1-toprJ1* have been isolated. It is worth mentioning that 76 strains (32.7%) carried the ESBL encoding genes and most of them were collected from QMH hospital during 2016–2017. The ESBL-encoding *K. pneumoniae* is increasingly reported recently,[27,28] and the dissemination of AMR will result in inappropriate empirical therapies, which is a concerning.

On the contrary, the prevalence of HvKP among the BSI-KP was high, with 40.6% of the *K. pneumoniae* carried at least one of the virulence genes *iuc*, *iro*, *rmpA* or *rmpA2*. While only one *K. quasipneumoniae* subsp. similipneumoniae strain carried *iuc3*, and the rest *K. quasipneumoniae* and *K. variicola* strains all did not carry any of the virulence plasmid associated virulence genes. Except for the typical pLVPK-like KpVP-1 type virulence plasmid, we have identified a conjugative KpVP-2 type virulence plasmid carrying *rmpADC*, *iro* and *iuc*,[18] a conjugative plasmid carrying *iuc5* together with several AMR genes[19] and a conjugative plasmid carrying *iuc3* with AMR genes. Besides, the chromosomal ICE*Kp1* carrying *iro* and *rmpADC* was identified in strains from all four hospitals, belonging to diverse KL types. These conjugative plasmids and mobile elements will promote the transmission of virulence phenotypes and particularly the co-transfer of these phenotypes with AMR among *K. pneumoniae*. The hypermucoviscous phenotype corelated well with the mucoid regulators *rmpADC* and/or *rmpA2*, while 8 strains (7 *K. pneumoniae* and 1 *K. variicola*) without *rmpADC* or *rmpA2D2* genes were also hypermucoviscous. This was contributed by the *wzc* mutations of these strains.[29] It has been reported that the hypercapsule *wzc* mutants are highly associated with BSIs by MDR *K. pneumoniae* and may be resulted from antibiotic treatment.[22] However, 3 out of the 8 strains were susceptible. The underlying mechanism of this phenomenon needs further study. Yet the hypercapsule by *wzc* mutants contributes to phagocytosis resistance and dissemination, resulting in higher mortality *in vivo*.[22] Generally, this is the first report of the hypermucoviscous *K. variicola* strain mediated by *wzc* mutation. Our data calls for more surveillance of *wzc* mutations in clinical strains and its impacts on hypermucoviscous phenotype and virulence.

Another objective of this study is to identify the outcome and risk factors that are associated with BSIs caused by *K. pneumoniae*. We identified a mortality rate of 43.0% of patients with BSIs, which was highly associated with mechanical ventilation, respiratory sepsis and renal replacement therapy. ESBL-producing strains caused a higher mortality rate than nonESBL-producing strains (FDR adjusted $p = 0.8685$), which might be due to inappropriate empirical antibiotic treatment (FDR adjusted $p = 0.0014$).[30] And factors determined to be more relevantly related to ESBL-producing strains also included hospital-acquired (FDR adjusted $p = 0.0506$) and respiratory sepsis (FDR adjusted $p = 0.0518$), which all associated with higher mortality rate. On the other hand, HvKP, which strongly associated with community-acquired (FDR adjusted $p = 0.0020$), appropriate empirical antibiotic treatment (FDR adjusted $p = 0.0197$) and hepatobiliary sepsis (FDR adjusted $p = 0.0567$), caused a lower mortality rate than non-HvKP (FDR adjusted $p = 0.5114$). That HMKP caused BSIs with lower mortality rate than that by CRKP has been reported.[10]

There are several limitations in this study that should be mentioned. Firstly, it was a regional analysis, and the results may not be universal and representative. Secondly, the time span was large while the sample size was relatively small, which leads to little information regarding the timeline changes. Lastly, the number of the ESBL-producing strains was less than that of the nonESBL-producing strains, which may result in some missing information. The situation of KP-BSI in Hong Kong will be continuously monitored to provide sufficient information of this life-threatening pathogen.

In conclusion, our data have identified the high prevalence of HvKP among the BSI-KP population and the increasing of ESBL-KP especially from 2016 in

Hong Kong. The conjugative plasmids and mobile elements carrying virulence determinants will promote the transmission of virulence phenotypes and particularly the co-transfer of these phenotypes with AMR among *K. pneumoniae*. The ESBL-KP has been increasingly identified in Hong Kong and was highly associated with in-hospital mortality. Therefore, adoption of new infection prevention and control (IPC) to arrest transmission of MDR-KP and MDR-HvKP in the hospital environment is urgently required.

Contributors

XMY performed the experiments and data analysis and drafted the manuscript. MYM collected the clinical strains and provided patient information. HH contributed to the data analysis. BKWC helped with strain characterization. QH helped with AST experiments. EWC edited the manuscript. SC and HPS designed the study and edited the manuscript. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Data sharing statement

The assembled genome sequences of the strains used in this study have been deposited in the GenBank database under BioProject number **PRJNA1003408**. Because of patient privacy constraints, raw data is under controlled access and available upon request to corresponding authors. Additional data related to this paper is available from the corresponding authors upon request.

Declaration of interests

We declare no competing interests.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ebiom.2024.104998.

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