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Clinical and Molecular Characteristics of *Klebsiella pneumoniae* Isolates Causing Bloodstream Infections in Japan: Occurrence of Hypervirulent Infections in Health Care

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ABSTRACT Although hypervirulent *Klebsiella pneumoniae* (hvKp) has been associated with severe community-acquired infections that occur among relatively healthy individuals, information about hvKp infections in health care settings remains limited. Here, we systematically analyzed the clinical and molecular characteristics of *K. pneumoniae* isolates causing bloodstream infections in a cross-sectional study. Clinical characteristics of *K. pneumoniae* bloodstream infections from hospitals across Japan were analyzed by a review of the medical records. Whole-genome sequencing of the causative isolates was performed. Bacterial species were confirmed and hvKp were identified using whole-genome sequencing data. Clinical characteristics of hvKp infections were compared with those of non-hvKp infections by bivariate analyses. Of 140 cases of *K. pneumoniae* bloodstream infections, 26 cases (18.6%) were caused by various clones of hvKp defined by the carriage of cardinal virulence genes. Molecular identification revealed that 24 (17.1%) and 14 (10%) cases were caused by *Klebsiella variicola* and *Klebsiella quasipneumoniae*, respectively. Patients

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with hvKp infections had higher proportions of diabetes mellitus (risk ratio [RR], 1.75; 95% confidence interval [CI], 1.05 to 2.94), and their infections had significantly higher propensity to involve pneumonia (RR, 5.85; 95% CI, 1.39 to 24.6), liver abscess (RR, 5.85; 95% CI, 1.39 to 24.6), and disseminated infections (RR, 6.58; 95% CI, 1.16 to 37.4) than infections by other isolates. More than one-half of hvKp infections were health care associated or hospital acquired, and a probable event of health care-associated transmission of hvKp was documented. hvKp isolates, which are significantly associated with severe and disseminated infections, are frequently involved in health care-associated and hospital-acquired infections in Japan.

KEYWORDS bloodstream infection, disseminated infection, hypervirulent *Klebsiella pneumoniae*, whole-genome sequencing

Klebsiella pneumoniae causes infections mainly in hospitalized patients or patients with underlying medical conditions, but occurrence of severe infections by this organism in healthy community-dwelling persons has been well documented, especially in East and Southeast Asia. The typical presentation of this "invasive syndrome" is liver abscess occasionally accompanied by endophthalmitis or meningitis (1). Detailed analysis of the isolates causing community-acquired liver abscess in Taiwan has led to the first recognition of the presence of hypervirulent *K. pneumoniae* (hvKp) isolates. Molecular analysis revealed that most of these isolates produced K1 capsular polysaccharides and belong to sequence type (ST) 23 (2, 3).

Severe community-acquired infections caused by *K. pneumoniae* isolates other than capsular genotype K1 have been reported from a number of countries and have gained attention in recent years (4, 5). While most of these isolates have capsule types classically recognized as high risk for invasive infections (K1, K2, K5, K20, K54, and K57), some of them have capsules of other types (6). Furthermore, the genetic backgrounds are diverse, even among isolates producing the same capsule. For example, hvKp isolates of capsular genotype K2 belong to different STs (e.g., ST65, ST86, and ST380) (4, 7). In addition to liver abscess, clinical presentations of severe infections caused by hvKp include necrotizing fasciitis, osteomyelitis, and meningitis and are sometimes accompanied by disseminated infections (5, 8, 9). hvKp has also been associated with community-acquired pneumonia with bacteremia (10, 11).

A common feature of various clones of hvKp is carriage of virulence genes such as *rmpA*, *rmpA2*, and those encoding siderophore biosynthesis. Carriage of *rmpA* and *rmpA2* has been associated with hypermucoviscosity of bacterial colonies on agar plates, a frequent feature of hvKp that originally triggered attention to the presence of K1-ST23 isolates in Taiwan (12, 13). Siderophores are small molecules with a high affinity for iron and are common virulence factors of pathogenic bacteria. Among the various siderophores *K. pneumoniae* produces, the production of aerobactin and salmochelin appears to be specific to hvKp (14). These cardinal virulence genes were found on the prototypical hvKp plasmid pLVPK, carried by the *K. pneumoniae* CG43 strain (15). Despite the diversity of genetic backgrounds among hvKp isolates, they commonly carry plasmids resembling pLVPK (16). Virulence genes, such as *rmpA-rmpA2*, genes for aerobactin biosynthesis, and genes for salmochelin biosynthesis, have been found to be sensitive and specific biomarkers to identify hvKp compared with the string test, which has been commonly employed to identify isolates with hypermucoviscosity (6).

While these studies have advanced our understanding of the pathogenesis and epidemiology of hvKp, data regarding health care-associated and hospital-acquired infections caused by hvKp remain limited. To address this knowledge gap, we systematically analyzed the clinical and molecular characteristics of *K. pneumoniae* isolates causing bloodstream infections at hospitals across Japan.

(This study was presented in part at the 55th Interscience Conference on Antimicrobial Agents and Chemotherapy, October 2015, San Diego, CA.)

MATERIALS AND METHODS

Ethics. The protocols were approved by the institutional review boards of all participating hospitals, including the coordinating hospital (Cancer Institute Hospital, Japanese Foundation for Cancer Research; approval number 2013-1063). Informed consent to participate in the study was obtained from the patients or their proxies (if the patient had significant altered mental status or had expired).

Participants. Patients 20 years of age or older at 23 acute care hospitals in Japan from December 2013 through March 2014 whose blood cultures grew *K. pneumoniae* were prospectively and sequentially recruited for this cross-sectional study. These hospitals were located in seven geographic regions across the country (see Table S1 in the supplemental material). *K. pneumoniae* was identified by automated systems at each hospital. For patients with multiple episodes of *K. pneumoniae* bloodstream infections during the study period, only the first episode was included.

Clinical data collection. Infectious disease specialists at each participating hospital collected the following clinical information of the patients from medical records: age; sex; severity of underlying diseases according to the Charlson Comorbidity Index (17); immunocompromised conditions (see Table S2); source of bloodstream infections defined by culture results of relevant clinical samples (definite), specific radiographic or laboratory findings other than cultures (probable), or suggestive clinical signs combined with risk factors for urinary tract or biliary tract infections (possible); presence of disseminated infection defined by multiple organ involvement; presence of severe sepsis or septic shock according to sepsis-2 criteria (18); and 30-day crude mortality. The origins of infections were classified into community acquired, health care associated, or hospital acquired. Hospital-acquired infection was defined as infection documented by a positive blood culture obtained more than 48 h after hospital admission, health care-associated infection was defined as infection documented by a positive blood culture obtained on an outpatient basis or within 48 h of admission from a patient with recent history of medical care (e.g., hospitalization in an acute care hospital for 2 or more days within 90 days, receipt of intravenous chemotherapy within 30 days, or residence in a nursing home or long-term-care facility), and community-acquired infection was defined as infection documented by a positive blood culture obtained on an outpatient basis or within 48 h of admission from a patient who did not fulfill the criteria for a health care-associated infection (19).

Microbiological and molecular analyses. *K. pneumoniae* isolates from each hospital were collected and analyzed at a central research laboratory for the study. The string test was performed with a standard inoculation loop to evaluate the hypermucoviscosity, and the formation of viscous strings >5 mm was regarded as positive (12).

The genomes of all study isolates were sequenced and assembled according to the methods detailed in the supplemental text. Identification of bacterial species and capsular genotyping were performed with KmerFinder and Kaptive web, respectively (supplemental text). Multilocus sequence typing and identification of β-lactamase genes and virulence genes were implemented on the *Klebsiella* locus/sequence definitions database (https://bigsdb.pasteur.fr/klebsiella/). Characteristics of β-lactamase genes were queried on the Comprehensive Antibiotic Research Database (20). Of the *K. pneumoniae* isolates confirmed by KmerFinder, those carrying any of the virulence genes, *rmpA*, *rmpA2*, *iroBCDN* (salmochelin siderophore biosynthesis), *iucABCD* (aerobactin siderophore biosynthesis), and *iutA* (aerobactin transporter), were defined as hvKp and others were defined as classic *K. pneumoniae* (6, 14). Single-nucleotide polymorphism (SNP) identification was performed for isolates of K1-ST23 and K62-ST36 with the methods detailed in the supplemental text.

Statistical analysis. Continuous data are expressed as medians with interquartile ranges. Categorical variables are shown as proportions. Sensitivity, specificity, positive predictive value, and negative predictive value of the string test for identification of hvKp were computed with 95% confidence intervals (CIs). Bivariate analyses of the clinical characteristics of the patients with hvKp infection and those with infection by other isolates were performed, and risk ratios (RRs) and 95% CIs were calculated. EZR (ver. 1.37) was used for the statistical analysis (21).

Accession number(s). All genome sequences have been deposited in the NCBI database under BioProject accession number PRJDB7163.

RESULTS

Of the 206 patients with *K. pneumoniae* bloodstream infections identified during the study period, 144 patients (69.9%) agreed to participate in this study (Fig. 1). The remaining 62 patients (30.1%) declined to participate and were not included in the analysis.

Species identification. Assembled genomes of the *K. pneumoniae* isolates had an average of 75.2 contigs (standard deviation [SD], 66.9) and N_{50} value of 400,608 bp (SD, 301,862 bp). KmerFinder identified 102 *K. pneumoniae*, 24 *Klebsiella variicola*, and 14 *Klebsiella quasipneumoniae* isolates. Three isolates and one isolate were identified as *Raoultella planticola* and *Raoultella ornithinolytica*, respectively, and were excluded from the further analysis. Therefore, 140 cases were included in final analysis (Fig. 1).

Virulence gene profile and hypermucoviscosity. Of the 102 *K. pneumoniae* isolates, 26 isolates (25.5%) were hvKp, and the remaining 76 isolates (74.5%) were classic *K. pneumoniae*. Of the 26 hvKp isolates, all carried *iroBCDN*, 23 (88.5%) carried both

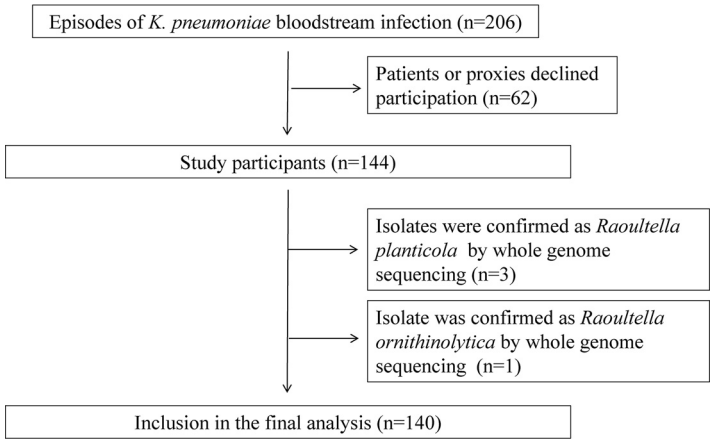

FIG 1 Patient inclusion.

iucABCD and *iutA*, and 22 (84.6%) carried *rmpA* and/or *rmpA2*. In addition, two isolates of *K. variicola* carried *rmpA* and *iroBCDN* (see Table S3 in the supplemental material).

The string test was positive for 30 isolates, including 18 hvKp isolates, 9 classic *K. pneumoniae* isolates, and 3 *K. variicola* isolates (Table S3). Sensitivity, specificity, positive predictive value, and negative predictive value of string tests for identification of hvKp were 69.2% (95% CI, 48.2 to 85.7%), 89.5% (95% CI, 82.3 to 94.4%), 60.0% (95% CI, 40.6 to 77.3%), and 92.7% (95% CI, 86.2 to 96.8%), respectively.

Clinical characteristics. The median age of the 140 patients was 74 years (interquartile range, 67 to 81 years), and 87 patients (62.1%) were male. Fifty-five cases (39.3%) were community acquired, 34 cases (24.3%) were health care associated, and 51 cases (36.4%) were hospital acquired. The sources of bloodstream infections were confirmed in 121 cases (86.4%), with definite diagnosis in 59 cases (42.1%), probable diagnosis in 43 cases (30.7%), and possible diagnosis in 19 cases (13.6%). Patient backgrounds and characteristics of infections with respect to the groups of isolates are presented in Tables 1 and 2, respectively. hvKp infections were community acquired in 10 cases (38.5%), health care associated in 8 cases (30.8%), and hospital acquired in 8 cases (30.8%). Classic *K. pneumoniae* infections were community acquired in 30 cases (39.4%), health care associated in 18 cases (23.7%), and hospital acquired in 28 cases (36.8%). *K. variicola* infections were community acquired in 9 cases (37.5%), health care associated in 3 cases (12.5%), and hospital acquired in 12 cases (50%). *K. quasipneumoniae* infections were community acquired in 6 cases (42.9%), health care associated in 5 cases (35.7%), and hospital acquired in 3 cases (21.4%). Detailed characteristics of hvKp bloodstream infections are presented in Table 3.

When comparing patients with hvKp infections and those with infections by other isolates, the hvKp group had a significantly higher rate of diabetes mellitus and had significantly higher propensity to present with pneumonia, liver abscess, and disseminated infections (Table 4). The 30-day crude mortality rates were not significantly different between the two groups.

Two *K. variicola* isolates carrying *rmpA* and *iroBCDN* caused intravenous catheter-associated bloodstream infections with septic shock and bacteremic urinary tract infection, both hospital acquired (Table 5).

***β*-Lactamase genes.** All except one *K. pneumoniae* isolate carried bla_{SHV}, all *K. variicola* isolates carried bla_{LEN}, and all *K. quasipneumoniae* isolates carried bla_{OKP} (Table S3). Twelve *K. pneumoniae* isolates carried broad-spectrum β-lactamase genes, including those encoding SHV-type extended-spectrum β-lactamase (ESBL) ($n = 5$), CTX-M-type ESBL ($n = 4$), CMY-type cephalosporinase ($n = 1$), and both carbapenemase and CTX-M-type ESBL genes ($n = 2$). The carbapenemase genes were bla_{IMP-6} ($n = 1$) and bla_{GES-4} ($n = 1$). While one hvKp isolate carried $bla_{CTX-M-2}$, all other isolates with β-lactamase genes for expanded spectrum of activity were classic *K. pneumoniae*.

TABLE 1 Baseline characteristics of the patients with *K. pneumoniae* bloodstream infections divided into groups by molecular identification

Characteristic	Value			
	Hypervirulent *K. pneumoniae* ($n = 26$)	Classic *K. pneumoniae* ($n = 76$)	*K. variicola* ($n = 24$)	*K. quasipneumoniae* ($n = 14$)
Age (yrs) (median [IQR][a])	75.5 (68.5–83.5)	73 (66.75–80)	78 (68.5–81.5)	72 (64.75–80.75)
Male sex (*n* [%])	14 (53.8)	52 (68.4)	16 (66.7)	5 (35.7)
Isolation setting (*n* [%])				
Community acquired	10 (38.5)	30 (39.4)	9 (37.5)	6 (42.9)
Healthcare associated	8 (30.8)	18 (23.7)	3 (12.5)	5 (35.7)
Hospital acquired	8 (30.8)	28 (36.8)	12 (50)	3 (21.4)
Diabetes mellitus (*n* [%])	12 (46.2)	21 (27.6)	6 (25)	3 (21.4)
Solid tumors (*n* [%])	13 (50)	35 (46.1)	15 (62.5)	5 (35.7)
Hematological malignancy (*n* [%])	1 (3.8)	4 (5.3)	0 (0)	0 (0)
Immunocompromised conditions (*n* [%])				
Neutropenia	1 (3.8)	4 (5.3)	1 (4.2)	0 (0)
Cellular immunodeficiency	1 (3.8)	6 (7.9)	0 (0)	2 (14.3)
Humoral immunodeficiency	0 (0)	1 (1.3)	0 (0)	0 (0)
Charlson score (*n* [%])				
0	3 (11.5)	8 (10.5)	2 (8.3)	2 (14.3)
1–2	7 (26.9)	28 (36.8)	8 (33.3)	6 (42.9)
3–4	6 (23.1)	21 (27.6)	8 (33.3)	3 (21.4)
≥5	10 (38.5)	19 (25)	6 (25)	3 (21.4)

[a]IQR, interquartile range.

Capsular genotype and clonality of the isolates. The isolates were classified into a number of clones defined by capsular genotypes and STs, and isolates of a given ST had a corresponding specific capsular genotype with a few exceptions (Table S3). Of the hvKp clones, K1-ST23 ($n = 7$), K2-ST65 ($n = 2$), K2-ST86 ($n = 4$), K20-ST268 ($n = 2$), K57-ST218 ($n = 3$), and K62-ST36 ($n = 4$) were the common combinations (Table 3). Three sets of distinct capsular genotype and ST combinations were recovered from the patients at the same hospitals: three K62-ST36 isolates from health care-associated infections, two K1-ST23 isolates from community-acquired infections, and two K20-

TABLE 2 Clinical characteristics and outcomes of *K. pneumoniae* bloodstream infections divided into groups defined by molecular analysis

Characteristic	No. (%) patients infected with:			
	Hypervirulent *K. pneumoniae* ($n = 26$)	Classic *K. pneumoniae* ($n = 76$)	*K. variicola* ($n = 24$)	*K. quasipneumoniae* ($n = 14$)
Source of bloodstream infection[a]				
Biliary tract	6 (23.1)	32 (42.1)	8 (33.3)	8 (57.1)
Urinary tract	8 (30.8)	22 (28.9)	6 (25)	4 (28.6)
Intra-abdominal	1 (3.8)	3 (3.9)	2 (8.3)	1 (7.1)
Pneumonia	4 (15.4)	3 (3.9)	0 (0)	0 (0)
Intravenous catheter related	0 (0)	3 (3.9)	2 (8.3)	1 (7.1)
Liver abscess	4 (15.4)	2 (2.6)	1 (4.2)	0 (0)
Endophthalmitis	2 (7.7)	0 (0)	0 (0)	0 (0)
Meningitis	1 (3.8)	0 (0)	0 (0)	0 (0)
Unknown	3 (11.5)	11 (14.5)	5 (20.8)	0 (0)
Disseminated infection	3 (11.5)	2 (2.6)	0 (0)	0 (0)
Severity of infection				
Severe sepsis	17 (65.4)	46 (60.5)	15 (62.5)	13 (92.9)
Septic shock	6 (23.1)	12 (15.8)	3 (12.5)	2 (14.3)
Altered mental status	7 (26.9)	13 (17.1)	5 (20.8)	2 (14.3)
30-day crude mortality	2 (7.7)	4 (5.3)	1 (4.2)	2 (14.3)

[a]If a patient had multiple infected organs (disseminated infection), all were counted separately.

TABLE 3 Microbiological, molecular, and clinical characteristics of hypervirulent *K. pneumoniae* bloodstream infections[a]

Strain no.	Area	Hospital	Age group[b]	Setting[c]	String test	K type[d]	ST[e]	Source of BSI[f]	Septic shock	Disturbed consciousness	30-day death	Charlson score	Malignancy	Diabetes mellitus	Immunosuppression
14133	Kanto	C	80	CA	+	1	23	Liver abscess	+	+	−	1–2	−	+	−
14056	Kanto	D	50	HC	+	1	23	Unknown	−	−	−	1–2	Endometrial cancer	−	−
14005	Chubu	I	60	HA	−	1	23	Biliary tract infection	−	−	−	3–4	Bile duct cancer	+	−
14036	Kinki	M	60	CA	+	1	23	Liver abscess	−	−	−	0	−	−	−
14041	Kinki	M	70	CA	+	1	23	Liver abscess, endophthalmitis	−	−	−	1–2	−	−	−
14030	Chugoku	R	80	CA	+	1	23	Biliary tract infection	−	−	−	≧5	Bile duct cancer	+	−
14089	Shikoku	T	90	HC	−	1	23	Urinary tract infection	−	−	−	≧5	Renal cell carcinoma	+	Neutropenia
14087	Tohoku	A	90	CA	+	2	65	Pneumonia	−	+	−	3–4	Gastric cancer, bladder cancer	+	−
14106	Kinki	L	70	CA	−	2	65	Unknown	+	−	−	3–4	−	−	−
14139	Kanto	C	90	CA	+	2	86	Urinary tract infection	−	−	−	0	−	−	−
14111	Kanto	F	70	CA	+	2	86	Biliary tract infection	−	−	−	≧5	Pancreatic cancer	+	−
14023	Kinki	O	40	HA	+	2	86	Pneumonia	−	+	−	1–2	Intravascular lymphoma	−	−
14090	Kyushu	V	70	HA	+	2	86	Urinary tract infection, endocarditis	+	−	−	3–4	−	−	−
14123	Kyushu	U	80	HA	+	20	268	Urinary tract infection	−	−	−	≧5	−	+	−
14128	Kyushu	U	60	HA	−	20	268	Intra-abdominal infection	+	−	−	≧5	Hepatocellular carcinoma	−	−
14120	Kanto	H	70	CA	−	20	1544	Urinary tract infection	−	+	−	≧5	Colon cancer	+	−
14081	Tohoku	A	80	CA	+	35	1266	Urinary tract infection	−	−	−	1–2	−	+	−
14062	Kinki	N	80	HC	−	54	29	Urinary tract infection	+	−	−	1–2	−	−	−
14059	Kanto	D	80	HA	+	57	218	Pneumonia	−	+	+	≧5	Oropharynx cancer	+	−
14017	Kanto	G	60	HC	+	57	218	Pneumonia	+	+	+	1–2	−	+	−
14098	Kinki	L	70	HC	+	57	218	Biliary tract infection	−	−	−	0	−	−	−
14013	Chubu	I	70	HA	−	57	412	Liver abscess, meningitis, endophthalmitis	−	+	−	3–4	Bile duct cancer	+	Cellular immunodeficiency
14001	Chubu	I	70	HC	+	62	36	Unknown	−	−	−	≧5	Pancreatic cancer	−	−
14002	Chubu	I	70	HC	+	62	36	Biliary tract infection	−	−	−	≧5	Pancreatic cancer	−	−
14009	Chubu	I	60	HC	+	62	36	Biliary tract infection	−	−	−	≧5	Pancreatic cancer	−	−
14126	Kyushu	U	70	HA	−	62	36	Urinary tract infection	−	−	−	3–4	−	−	−

[a]Isolates were aligned according to capsular genotype and sequence type.
[b]Ages of the patients were described as groups within a 10-year range (e.g., age group 80 represents ages from 80 through 89).
[c]CA, community acquired; HC, health care associated; HA, hospital acquired.
[d]K type, capsular genotype.
[e]ST, sequence type.
[f]BSI, bloodstream infection.

TABLE 4 Comparison of clinical characteristics of hypervirulent *K. pneumoniae* infections and those caused by other isolates

Clinical characteristic	No. (%) of patients infected with:		Risk ratio (95% confidence interval)[a]
	Hypervirulent *K. pneumoniae* ($n = 26$)	Other isolates ($n = 114$)	
Diabetes mellitus as an underlying condition	12 (46.2)	30 (26.3)	1.75 (1.05–2.94)
Pneumonia	4 (15.4)	3 (2.6)	5.85 (1.39–24.6)
Liver abscess	4 (15.4)	3 (2.6)	5.85 (1.39–24.6)
Disseminated infections	3 (11.5)	2 (1.8)	6.58 (1.16–37.4)
Septic shock	6 (23.1)	17 (14.9)	1.55 (0.68–3.54)
Altered mental status	7 (26.9)	20 (17.5)	1.54 (0.73–3.24)
30-day crude mortality	2 (7.7)	7 (6.1)	1.25 (0.28–5.69)

[a]Risk ratio of hypervirulent *K. pneumoniae* infections to have each characteristic compared with those caused by other isolates.

ST268 isolates from hospital-acquired infections. The K62-ST36 isolates and K1-ST23 shared identical profiles of β-lactamase genes, whereas the two K20-ST268 isolates had different profiles. Notably, a pair of K62-ST36 isolates (strain numbers 14001 and 14002) were genetically almost identical with only one core genome SNP, while other pairs had greater core genome SNPs ranging from 93 to 108. The core genome SNP numbers of seven K1-ST23 isolates, including those recovered in the same hospital, ranged between 165 and 399.

Classic *K. pneumoniae*, *K. variicola*, and *K. quasipneumoniae* had diverse capsular genotypes and STs (Table S3). Of interest, the two *K. variicola* isolates carrying *rmpA* and *iroBCDN* both belonged to ST919 and had capsular genotype K2.

DISCUSSION

In this study, we analyzed the clinical and molecular characteristics of *K. pneumoniae* bloodstream infections in Japan. Twenty-six of the 140 *K. pneumoniae* bloodstream infections were caused by hvKp identified by several molecular biomarkers. hvKp isolates consisting of various clones were distributed across the country.

Of the hvKp isolates identified, K1-ST23 isolates were most common, which was consistent with previous studies in Japan and other Asian countries (22, 23). Nevertheless, various other clones were also observed, including established hvKp clones such as K2-ST86 and previously unrecognized clones such as K62-ST36. Minor differences in the patterns of carriage of virulence genes could be explained by probable variations in the pLVPK-like virulence plasmid due to insertions and deletions, which are known to occur (16, 24). Higher risk of hvKp infection in patients with diabetes and its propensity to cause pneumonia, liver abscess, and disseminated infections have been reported in infections caused by K1 isolates, hypermucoviscous isolates, and isolates carrying *rmpA* (10, 25, 26). These clinical characteristics were preserved in our hvKp isolates that were defined by a more comprehensive set of biomarkers. This may suggest that identification of hvKp among blood isolates using biomarkers may be able to guide comprehensive screening for disseminated infection and implementation of antibiotic therapy with good tissue and central nervous system penetration. While the string test is a popular method to identify hvKp due to its simplicity to perform in the clinical microbiology laboratory, data from this report and others demonstrate that its sensitivity and specificity are not optimal, and the test would not be adequate for standalone use in the identification of hvKp (6).

Healthcare-associated and hospital-acquired hvKp infections were common in our study. Previous studies have presented severe community-acquired infections in relatively healthy persons as the typical feature of hvKp infections (10, 11). Nevertheless, it appears conceivable that hvKp isolates would also pose a high risk for severe infections among hospitalized patients or patients with comorbidities. It is also possible that hvKp infections occur as health care-associated and hospital-acquired infections commonly in countries with demographic and public health characteristics similar to those of Japan.

K62-ST36 was newly recognized as an hvKp clone in this study. A pair of K62-ST36

TABLE 5 Microbiological, molecular, and clinical characteristics of *K. variicola* bloodstream infections[a]

Strain no.	Area	Hospital	Age group[b]	Setting[c]	String test	K type[d]	ST[e]	Source of BSI[f]	Septic shock	Disturbed consciousness	30-day death	Charlson score	Malignancy	Diabetes mellitus	Immunosuppression
14028	Chugoku	R	80	HA	−	2	919	Intravenous catheter-related infection	+	−	+	3–4	−	−	−
14085	Tohoku	A	70	HA	+	2	919	Urinary tract infection	−	−	−	≥5	Colon cancer	−	−
14037	Kinki	M	80	CA	+	10	3954	Biliary tract infection	−	−	−	12	−	−	−
14011	Chubu	I	80	HA	−	16	3950	Intravenous catheter-related infection	−	−	−	3–4	Oral cavity cancer	−	−
14012	Chubu	I	80	CA	−	16	3950	Biliary tract infection	+	+	−	3–4	Pancreatic cancer	−	−
14006	Chubu	I	50	HC	−	18	1903	Biliary tract infection	−	−	−	1–2	Pancreatic cancer	−	−
14007	Chubu	I	60	HA	−	18	1903	Intra-abdominal infection	−	−	−	1 − 2	Gastric cancer	−	−
14078	Tohoku	B	60	HA	−	19	3956	Unknown	−	+	−	3 − 4	Lung cancer	−	Neutropenia
14134	Kanto	C	80	HC	−	24	3961	Urinary tract infection	−	+	−	≥5	−	−	−
14035	Kinki	M	80	CA	−	30	3953	Biliary tract infection	−	−	−	1 − 2	−	−	−
14025	Kinki	O	70	HA	−	34	363	Urinary tract infection	−	−	−	≥5	Colon cancer	+	−
14136	Kanto	C	70	HA	−	47	3962	Urinary tract infection	−	−	−	1 − 2	Lung cancer	−	−
14077	Tohoku	B	70	HA	−	57	3955	Biliary tract infection	−	−	−	3 − 4	Colon cancer	−	−
14010	Chubu	I	60	HA	−	60	697	Unknown	−	−	−	1–2	Bile duct cancer	−	−
14021	Kyushu	W	80	CA	−	64	3951	Urinary tract infection	−	+	+	0	−	−	−
14109	Kanto	F	70	CA	−	64	3959	Intra-abdominal infection	−	−	−	≥5	Colon cancer	−	−
14103	Kinki	L	70	HC	−	105	1142	Urinary tract infection	−	−	−	≥5	−	+	−
14033	Kinki	M	30	CA	−	114	3952	Biliary tract infection	−	−	−	1 − 2	−	−	−
14096	Kinki	L	70	CA	−	125	355	Liver abscess	−	−	−	3 − 4	Thyroid cancer	+	−
14079	Tohoku	B	70	HA	−	125	3957	Unknown	−	−	−	3 − 4	Lung cancer, oral cavity cancer	+	−
14115	Chubu	J	80	CA	+	125	3960	Biliary tract infection	−	−	−	≥5	−	+	−
14058	Kanto	D	60	HA	−	127	3239	Unknown	+	+	−	3 − 4	Gastric cancer	+	−
14086	Tohoku	A	70	HA	−	UD	3958	Unknown	−	−	−	1 − 2	Gastric cancer	−	−
14095	Kinki	L	60	CA	−	UD	919	Biliary tract infection	−	−	−	0	−	−	−

[a]Isolates were aligned according to capsular genotype and sequence type.
[b]Ages of the patients were described as groups within a 10-year range (e.g., age group 80 represents ages from 80 through 89).
[c]CA, community acquired; HC, health care associated; HA, hospital acquired.
[d]K type, capsular genotype; UD, undetermined with Kaptive database.
[e]ST, sequence type.
[f]BSI, bloodstream infection.

isolates, which were isolated from the same hospital as health care-associated infections, had only single SNPs in their core genomes. While the route of transmission was unclear, transmission during hospitalization was a distinct possibility, since both patients had pancreatic cancer and were cared for at the same hospital. Considering only clinical isolates from blood were studied in our study, this possible in-hospital transmission might suggest ongoing transmission of hvKp in health care settings that are currently unrecognized. Since transmission and subsequent infection caused by hvKp could pose significant risk of adverse outcome in debilitated inpatients, aggressive prevention measures, such as implementation of contact precaution for patients colonized with hvKp, may be considered.

Although spread of multidrug-resistant hvKp is an emerging problem globally, only one hvKp isolate carried an ESBL gene, and none carried a carbapenemase gene in this study (24, 27). Nevertheless, carbapenemase-producing hvKp was recently reported in Japan, and the future trend of antimicrobial resistance among hvKp isolates requires close monitoring (28).

Of 140 isolates identified as *K. pneumoniae* by automated methods, 24 and 14 were molecularly identified as *K. variicola* and *K. quasipneumoniae*, respectively. These species are difficult to differentiate from *K. pneumoniae* by conventional biochemical methods (29). Carriage of bla_{LEN} by *K. variicola* isolates and bla_{OKP} by *K. quasipneumoniae* isolates was in accordance with previous reports and supports the results of KmerFinder (29). Clinical significance of the carriage of virulence genes, *rmpA* and *iroBCDN*, found in two *K. variicola* ST919 isolates is unknown thus far. A single-hospital study showed a higher mortality rate of bloodstream infections caused by *K. variicola* than those caused by *K. pneumoniae*, and together with our findings might suggest the clinical significance of *K. variicola* as a distinct virulent species within the genus *Klebsiella* (30).

Our study has several limitations. First, this study was conducted in a single country. hvKp infections in other countries may have different clinical characteristics, especially if hvKp clones with distinct characteristics were involved. Nevertheless, the consistency of clinical characteristics of hvKp infections in our study with those reported in previous studies suggests the generalizability of the findings. Second, the limited number of hvKp isolates may have resulted in missed characteristics. Furthermore, clinical characteristics of *K. variicola* and *K. quasipneumoniae* infections could not be adequately assessed in our analysis due to the limited number of cases. Third, the use of whole-genome sequencing to identify hvKp applied in this study is difficult to employ in general clinical settings.

In conclusion, hvKp bloodstream infections, which were identified by molecular biomarkers, showed distinct clinical characteristics. Notably, health care-associated and hospital-acquired infections accounted for the majority of hvKp infections. The predominance of health care-associated and hospital-acquired hvKp infections observed in this study challenges the current paradigm of hvKp focused on community-acquired infections and suggests the need for further studies on diagnosis, treatment, and infection prevention of hvKp infections in health care settings.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/JCM.01206-19.

SUPPLEMENTAL FILE 1, PDF file, 0.1 MB.
SUPPLEMENTAL FILE 2, XLSX file, 0.1 MB.
SUPPLEMENTAL FILE 3, XLSX file, 0.1 MB.
SUPPLEMENTAL FILE 4, XLSX file, 0.1 MB.

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