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Characteristics of *Klebsiella pneumoniae* Isolates from Stool Samples of Patients with Liver Abscess Caused by Hypervirulent *K. pneumoniae*

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▸ See the editorial "Hypervirulent *Klebsiella pneumoniae*: Liver Abscess Isolates versus Intestinal Flora" in volume 35, number 2, e28.

ABSTRACT

Background: Hypervirulent *Klebsiella pneumoniae* (hvKP) has been the most significant pathogen for liver abscesses in East Asia including the Republic of Korea (ROK). Although gastrointestinal colonization of *K. pneumoniae* may cross the intestinal barrier to invade the liver, characteristics of gastrointestinal carriage *K. pneumoniae* of hvKP liver abscess patients in the ROK are not well known.
Methods: Characteristics of *K. pneumoniae* isolated from stool samples and liver aspirate samples of patients with hvKP liver abscess at a tertiary care hospital in the ROK between 2017 and 2018 were evaluated.
Results: Out of 37 patients with hvKP liver abscess, 11 patients were noted to have *K. pneumoniae* isolated from stool samples and were enrolled for analysis. The median age was 71 years. For hvKP isolates from the liver aspirate samples, the most common serotype was K1 (72.7%) followed by K2 (27.3%). For *K. pneumoniae* isolates from the stool sample, the majority was non-K1/K2 serotype (72.7%). Among non-K1/K2 serotype isolates, high variability of sequence type (ST; ST15, ST307, ST37, ST273, ST2622, and ST42) with high rate of presence of extended-spectrum beta-lactamase (100.0%) was noted. The concordance rate of the *K. pneumoniae* isolates between the liver aspirate samples and the stool samples from the primary hvKP liver abscess was low (27.3%).
Conclusion: This study suggests that significant heterogeneity of *K. pneumoniae* colonizing intestinal tract of the hvKP liver abscess patients. Further studies involving a larger number of hvKP liver abscess patients with continuing surveillance are needed to define the changing epidemiology and the role of gastrointestinal *K. pneumoniae* in the hvKP liver abscess patients in the ROK.

Keywords: *Klebsiella pneumoniae*; Hypervirulent; Liver Abscess; Gastrointestinal Carriage

Author Contributions

Conceptualization: Kim JH, Kim MJ; Data curation: Kim JH, Jeong Y, Lee CK, Kim SB, Yoon YK, Sohn JW, Kim MJ; Formal analysis: Kim JH, Kim MJ; Methodology: Kim JH, Kim MJ; Writing - original draft: Kim JH; Writing - review & editing: Kim JH, Kim MJ.

INTRODUCTION

Pyogenic liver abscess caused by *Klebsiella pneumoniae* has emerged in East Asia including Taiwan, China, and the Republic of Korea (ROK).[1-3] This condition is frequently complicated by an extrahepatic metastatic infection such as septic endophthalmitis and is primarily caused by strains of hypervirulent *K. pneumoniae* (hvKP).[4] For hvKP liver abscess, *K. pneumoniae* serotype K1 is the most prevalent serotype and sequence type (ST) 23 is the predominant type identified from multilocus sequence typing (MLST).[4] In addition, hvKP is known to have virulence genes related to the expression of the hypermucoviscous phenotype manifested as a positive string test, which would facilitate further invasion by enhancing resistance to phagocytosis.[5] Also, virulence genes related to iron uptake for promoting bacterial replication and survival are well characterized in hvKP.[6] These virulence factors are thought to contribute to the pathogenesis of hvKP liver abscess, even in healthy individuals. Previous epidemiological studies suggested that higher rate of *K. pneumoniae* including hvKP intestinal colonization seen in individuals in Asian countries such as Taiwan and ROK[7-9] may have resulted in transient bacteremia followed by dissemination and invasion of the liver. This possible association was further supported by a study from Taiwan,[10] which showed that liver aspirate *K. pneumoniae* isolates had a pulsed-field gel electrophoresis profile identical or closely related to those of fecal or saliva samples from the same patient with hvKP liver abscess. However, the intestinal colonization rate of *K. pneumoniae* including hvKP was found to be different in ROK when compared to that of Taiwan,[7,8] which might have an impact on the characteristics of *K. pneumoniae* of the hvKP liver abscess patients in the ROK. Furthermore, little is known about the characteristics of *K. pneumoniae* colonizing the intestinal tract of the hvKP liver abscess patients in the ROK.

Therefore, this study aimed to investigate the distribution and characteristics of *K. pneumoniae* colonizing intestinal tract of the hvKP liver abscess patients in the ROK. Also, we compared the intestinal colonization isolates of *K. pneumoniae* with the liver abscess strains of hvKP to evaluate the roles of the intestinal colonization of *K. pneumoniae* in the development of hvKP liver abscess.

METHODS

Study population and bacterial strains

K. pneumoniae isolates from liver aspirate samples and stool samples from hospitalized adult patients diagnosed with hvKP liver abscess at the Korea University Anam Hospital, Seoul, ROK were prospectively collected from 2017 to 2018. Patients were required to meet the inclusion criteria: 1) age ≥ 19 years, 2) confirmation of the primary hvKP liver abscess diagnosis defined with the combination of hvKP isolation from the liver aspirate sample culture and the presence of typical clinical manifestations including fever, right upper abdominal pain, and imaging evidence of liver abscess seen from the computed tomography scan, 3) positive isolation of *K. pneumoniae* from the stool sample culture. Patients with the following criteria were excluded from this study: 1) possible evidence of a secondary liver abscess such as polymicrobial bacteremia or presence of other intra-abdominal sources of infection (e.g., appendicitis), 2) isolation of bacteria other than *K. pneumoniae* from the stool sample culture.

The liver aspirate sample from liver abscess was inoculated into one aerobic and one anaerobic blood culture bottle and was placed into a BacT/ALERT® 3D Microbial Detection System

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(bioMerieux, Inc., Durham, NC, USA). Identification of *K. pneumoniae* was carried out using matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS; Bruker Daltonics, Bremen, Germany). Antibiotic susceptibility testing was performed using an automated antimicrobial susceptibility testing system (Vitek® 2; bioMérieux Vitek, Hazelwood, MO, USA). Results of antibiotic susceptibility were interpreted according to the standards of the Clinical and Laboratory Standards Institute.[11] The string test was performed on identified *K. pneumoniae* isolates from the liver abscess aspirate sample. HvKP was confirmed if there was a positive string test defined as the formation of viscous string > 5 mm in length.[12] The stool sample from the hvKP liver abscess patient was inoculated into the MacConkey media. Identification and antibiotic susceptibility testing of *K. pneumoniae* from the stool sample were carried out using MALDI-TOF MS and Vitek® 2, respectively. The string test was also performed on the identified *K. pneumoniae* isolate from the stool sample.

Clinical variables including hvKP liver abscess patients' demographics, comorbid chronic illnesses, clinical manifestations, outcomes, and microbiological data were collected.

Microbiological characterization

The serotype of *K. pneumoniae* isolates was determined by polymerase chain reaction (PCR) using primers specific for *wzy* and *wzx* alleles for detection of serotype K1, K2, K5, K20, K54, and K57 as described previously.[13] For detection of other serotypes, sequencing polymorphisms of the *wzi* gene by PCR was performed as *wzi* sequencing can offer a reliable determination of the K serotypes of both hvKP and classical *K. pneumoniae* strains.[14] MLST was performed on *K. pneumoniae* isolates by PCR using known sequence primers for seven housekeeping genes (*rpoB*, *gapA*, *mdh*, *pgi*, *phoE*, *infB*, *tonB*).[15] ST was determined by application of the sequenced data of seven housekeeping genes into the *K. pneumoniae* MLST database (http://www.pasteur.fr/mlst/). The phylogeny scheme was generated by assembly and editing of nucleotide sequences obtained from the MLST by the software ClustalX2 v2.1 (http://www.clustal.org/clustal) and BioEdit v7.0.5 (http://www.mbio.ncsu.edu/bioedit/bioedit.html). The phylogenetic tree was displayed with iTOL (http://itol.embl.ed).[16] Genes encoding major virulence factors (*rmpA*, *magA*, *iutA*) were determined by PCR using known primers as described previously.[17,18]

Statistical analysis

Descriptive statistics were used to analyze the data. The Fisher's exact test was used for dichotomous variables and the Mann-Whitney U test was used for continuous variables where applicable. SPSS version 18.0 for Windows (SPSS Inc., Chicago, IL, USA) was used for statistical analyses.

Ethics statement

This study was approved by the Institutional Review Board of the Korea University Anam Hospital (2017AN0378), which exempted the informed consent requirements.

RESULTS

Clinical characteristics

There was a total of 37 patients diagnosed with the primary hvKP liver abscess during the study period. These primary hvKP liver abscesses were caused by the extended-spectrum beta-lactamase (ESBL) negative and ciprofloxacin-susceptible hvKP isolates except for

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one patient whose liver abscess was caused by the both ESBL-negative and ciprofloxacin-susceptible hvKP isolate and *K. pneumoniae* isolate with positive ESBL and sensitivity to ciprofloxacin. Out of 37 patients with the primary hvKP liver abscess, 11 patients were noted to have *K. pneumoniae* isolated from the stool samples and were enrolled for analyses. There were 8 men (72.7%) and 3 women (27.3%). The median age was 71 years (interquartile range [IQR], 50–75 years). The majority of the patients (72.7%) were without significant underlying comorbidities. Diabetes mellitus (DM) was noted in 1 patient (9.1%). The median Pitt bacteremia score at the hospital admission was 0 (IQR, 0–1). Presence of metastatic infection was noted in 2 patients (18.2%; one patient with endophthalmitis and another patient with endophthalmitis and abscess in the prostate and bilateral psoas muscle) who did not have underlying comorbidities. Third generation cephalosporin (ceftriaxone or cefotaxime) was the most commonly used in-hospital antibiotic treatment (9/11; 81.8%). There was no 30-day mortality among these patients (**Table 1**).

Microbiological characterization

For hvKP isolates from the liver aspirate samples of 11 patients, the most common serotype was K1 (8/11; 72.7%) followed by K2 (3/11; 27.3%). The string test was positive in all hvKP isolates. All K1 serotype isolates were ST23 (8/11; 72.7%). There were ST25 (1/11; 9.1%) and ST86 (2/11; 18.2%) for K2 serotypes. Presence of the major virulence factors (*rmpA*, *magA*, and *iutA*) were noted in all K1/ST23 isolates. However, the absence of *magA* was seen in all K2 serotype isolates (two ST86 isolates and one ST25 isolate). All hvKP isolates were susceptible to ciprofloxacin without the presence of ESBL. For *K. pneumoniae* isolates from the stool samples of 11 patients, the majority was non-K1/K2 serotype (8/11; 72.7%). There were two K1/ST23 (18.2%) and one K2/ST25 (9.1%) isolates. The median time between the initiation of antibiotic therapy for hvKP liver abscess and collection of stool *K. pneumoniae* isolates was 14 days. The string test was positive only in K1/K2 serotype isolates. Among non-K1/K2 serotype isolates, high variability of ST was noted; ST15, ST307, ST37, ST273, ST2622, and ST42 were observed. With regard to antibiotic susceptibility, K1/K2 serotype isolates were susceptible to ciprofloxacin without ESBL. However, a high rate of ciprofloxacin resistance (6/8; 75.0%) and presence of ESBL (8/8; 100.0%) were noted among non-K1/K2 serotype isolates. The major virulence factors (*rmpA*, *magA*, and *iutA*) were absent in all non-K1/K2 serotype isolates. For K1/K2 serotype isolates, these virulence factors were present except for *magA*, which was absent in the K2 serotype isolate (**Table 2**). The phylogenetic tree analysis of *K. pneumoniae* isolates from the liver aspirate sample, and the stool sample by MLST showed a considerable heterogeneity of *K. pneumoniae* isolates from the stool sample (**Fig. 1**).

Table 1. Clinical characteristics of patients with liver abscess caused by hypervirulent *Klebsiella pneumoniae*

Patient number	Age, yr	Gender	Pitt bacteremia score	Comorbidities	Metastatic infection	In-hospital antibiotic treatment	30-day mortality
Patient 1	50	Men	5	None	None	CTX	No
Patient 2	31	Women	0	None	None	Cipro	No
Patient 3	74	Men	0	CKD, CBD cancer	None	CTX, Cipro	No
Patient 4	70	Men	0	None	None	CTX	No
Patient 5	75	Men	0	None	Prostate, psoas muscle, eye	CEF, Cipro	No
Patient 6	58	Men	0	None	None	CTX	No
Patient 7	91	Women	2	None	None	CTX, Cipro	No
Patient 8	71	Women	1	None	None	CEF	No
Patient 9	82	Men	0	HCC	Eye	CTX	No
Patient 10	47	Men	1	None	None	Cipro, AMP-Sulb	No
Patient 11	75	Men	0	DM	None	CTX	No

CTX = ceftriaxone, Cipro = ciprofloxacin, CKD = chronic kidney disease, CBD = common bile duct, CEF = cefotaxime, AMP-Sulb = ampicillin-sulbactam, DM = diabetes mellitus.

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Table 2. Microbiological characteristics of *Klebsiella pneumoniae* isolates from liver abscess aspirates and stool samples from patients with liver abscess caused by hypervirulent *K. pneumoniae*

Patient number	Source	Isolate name	Antimicrobial susceptibility	String test	Serotype	MLST ST	Virulence gene		
							rmpA	*magA*	*iutA*
Patient 1	Liver	KUH-L1	ESBL-negative, Cipro-sensitive	Positive	K1	23	Positive	Positive	Positive
	Stool	KUH-F1	ESBL-negative, Cipro-sensitive	Positive	K1	23	Positive	Positive	Positive
Patient 2	Liver	KUH-L2	ESBL-negative, Cipro-sensitive	Positive	K1	23	Positive	Positive	Positive
	Stool	KUH-F2	ESBL-positive, Cipro-resistant	Negative	K24	15	Negative	Negative	Negative
Patient 3	Liver	KUH-L3	ESBL-negative, Cipro-sensitive	Positive	K1	23	Positive	Positive	Positive
	Stool	KUH-F3	ESBL-positive, Cipro-resistant	Negative	KN2	307	Negative	Negative	Negative
Patient 4	Liver	KUH-L4	ESBL-negative, Cipro-sensitive	Positive	K2	86	Positive	Negative	Positive
	Stool	KUH-F4	ESBL-positive, Cipro-sensitive	Negative	K12	37	Negative	Negative	Negative
Patient 5	Liver	KUH-L5	ESBL-negative, Cipro-sensitive	Positive	K2	86	Positive	Negative	Positive
	Stool	KUH-F5	ESBL-positive, Cipro-resistant	Negative	NT	273	Negative	Negative	Negative
Patient 6	Liver	KUH-L6	ESBL-negative, Cipro-sensitive	Positive	K1	23	Positive	Positive	Positive
	Stool	KUH-F6	ESBL-positive, Cipro-resistant	Negative	K11	2,622	Negative	Negative	Negative
Patient 7	Liver	KUH-L7	ESBL-negative, Cipro-sensitive	Positive	K1	23	Positive	Positive	Positive
	Stool	KUH-F7	ESBL-negative, Cipro-sensitive	Positive	K1	23	Positive	Positive	Positive
Patient 8	Liver	KUH-L8	ESBL-negative, Cipro-sensitive	Positive	K2	25	Positive	Negative	Positive
	Stool	KUH-F8	ESBL-negative, Cipro-sensitive	Positive	K2	25	Positive	Negative	Positive
Patient 9	Liver	KUH-L9	ESBL-negative, Cipro-sensitive	Positive	K1	23	Positive	Positive	Positive
	Stool	KUH-F9	ESBL-positive, Cipro-sensitive	Negative	K64	42	Negative	Negative	Negative
Patient 10	Liver	KUH-L10	ESBL-negative, Cipro-sensitive	Positive	K1	23	Positive	Positive	Positive
	Stool	KUH-F10	ESBL-positive, Cipro-resistant	Negative	KN2	307	Negative	Negative	Negative
Patient 11	Liver	KUH-L11	ESBL-negative, Cipro-sensitive	Positive	K1	23	Positive	Positive	Positive
	Stool	KUH-F11	ESBL-positive, Cipro-resistant	Negative	KN2	307	Negative	Negative	Negative

ESBL= extended-spectrum beta-lactamase, Cipro = ciprofloxacin, MLST = multilocus sequence typing, ST = sequence type, NT = nontypeable.

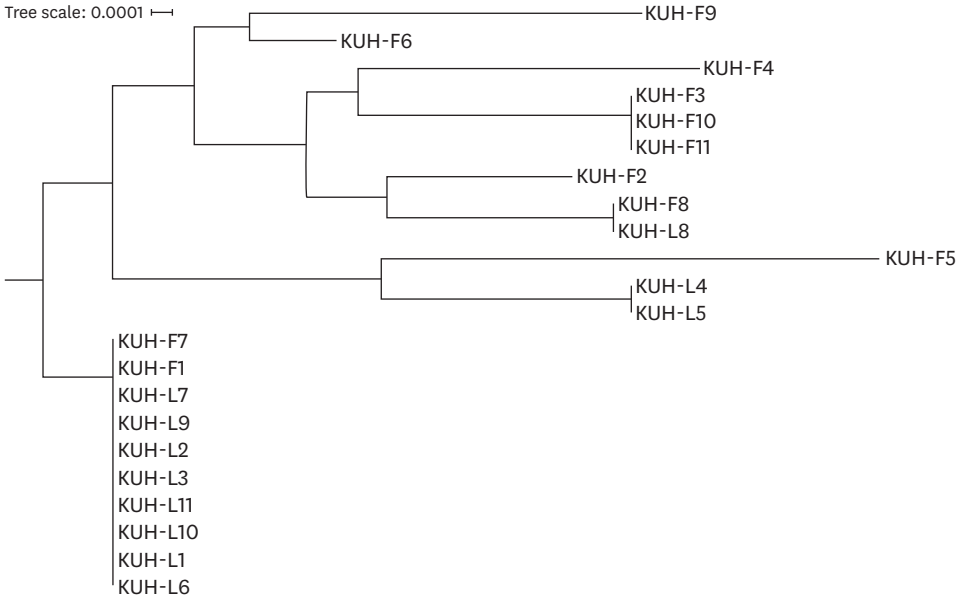

Fig. 1. Phylogenetic tree of *Klebsiella pneumoniae* isolates of the liver aspirates and the stool samples from hypervirulent *K. pneumoniae* liver abscess patients.

Comparison of characteristics between the concordant and discordant group

The concordance rate of the *K. pneumoniae* isolates between the liver aspirate sample and the stool sample from the primary hvKP liver abscess was low (27.3%). Between the concordant and discordant group of *K. pneumoniae* strains, there was no significant clinical difference in terms of age distribution, gender, and presence of co-morbidities and metastatic infection. However, higher Pitt bacteremia score was noted in the concordant group than in the discordant

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Table 3. Comparison of characteristics between the concordant and discordant group of *Klebsiella pneumoniae* strains

Characteristics	Concordant group	Discordant group	*P* value
No. of patients	3	8	
Age, yr	71	64	0.682
Gender			0.152
Men	1 (33.3)	7 (87.5)	
Women	2 (66.7)	1 (12.5)	
Pitt bacteremia score	2.7	0.1	0.006
Presence of comorbidities	0 (0.0)	3 (37.5)	0.491
Presence of metastatic infection	0 (0.0)	2 (25.0)	1.000
Liver *K. pneumoniae* isolate			
Serotype K1	2 (66.7)	6 (75.0)	1.000
Serotype K2	1 (33.3)	2 (25.0)	1.000
Presence of virulence factors			
rmpA	3 (100.0)	8 (100.0)	NA
magA	2 (66.7)	6 (75.0)	1.000
iutA	3 (100.0)	8 (100.0)	NA
ESBL-positive	0 (0.0)	0 (0.0)	NA
Stool *K. pneumoniae* isolate			
Serotype K1/K2	3 (100.0)	0 (0.0)	0.006
Serotype non K1/K2	0 (0.0)	8 (100.0)	
Presence of virulence factors			
rmpA	3 (100.0)	0 (0.0)	0.006
magA	2 (66.7)	0 (0.0)	0.055
iutA	3 (100.0)	0 (0.0)	0.006
ESBL-positive	0 (0.0)	8 (100.0)	0.006

Data are presented as mean or number (%).
NA = not available, ESBL = extended-spectrum beta-lactamase.

group (mean, 2.7 vs. 0.1; $P = 0.006$). Although there was no difference of microbiological characteristics regarding *K. pneumoniae* isolates from the liver aspirate sample, the difference in the distribution of serotypes, the presence of virulence factors, and presence of ESBL was noted in the *K. pneumoniae* isolates from the stool sample. This difference was mainly due to a higher prevalence of non-K1/K2 serotype *K. pneumoniae* isolates in the discordant group than in the concordant group (100.0% vs. 0.0%; $P = 0.006$), which showed characteristics of the high rate of ESBL positivity without specific features of hvKP (**Table 3**).

DISCUSSION

Despite the limitations due to a small number of hvKP liver abscess patients, our results clearly showed that K1 and ST23 were the most common serotype and ST type from the liver aspirate sample of the hvKP liver abscess patients. These are consistent with a previous study conducted in the ROK,[3] which showed the predominance of K1/ST23 in the *K. pneumoniae* liver abscess. Also, in accordance with an earlier study[19] which showed more diversity of ST types in K2 serotype compared with K1 serotype, our K2 isolates had 2 ST types while all K1 isolates had 1 ST type (ST23). Additionally, distribution of the major virulence factors (*rmpA*, *magA*, and *iutA*) in the *K. pneumoniae* isolates from the liver aspirate sample in the present study were consistent with the previous studies.[1-3] However, unlike the previous study[3] which reported the mean age of 60 years and the substantial prevalence rate of DM (39.9%) for patients with *K. pneumoniae* liver abscess, our hvKP liver abscess patients were older (median, 71 years) with lower prevalence rate of comorbidities including DM (9.1%). Although our analysis was limited by the small numbers of patients, our results suggest that hvKP liver abscess can occur in healthy older individuals. Furthermore, the elderly are known to be more susceptible to *K.*

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pneumoniae infection due to aging-related changes in immune responses,[20] and a recent increase of the detection of hvKP among *K. pneumoniae* isolates in the elderly has been reported in China recently.[21] Given the similar epidemiologic features of hvKP liver abscess between ROK and China,[1-3] continued epidemiologic surveillance of hvKP liver abscess in the elderly may need to be considered to define the possibly changing epidemiology of hvKP liver abscess in the ROK.

The majority of stool *K. pneumoniae* isolates from hvKP liver abscess patients in our study were non-K1/K2 serotypes. Moreover, we observed that the concordance rate between the liver aspirate *K. pneumoniae* isolates and stool *K. pneumoniae* isolates was low (27.3%). Additionally, discordant stool *K. pneumoniae* isolates did have significant heterogeneity in terms of ST types with ESBL and antibiotic resistance. These are in contrast to a previous study conducted in Taiwan[10] which reported a high degree of identicalness between liver aspirate *K. pneumoniae* isolates and stool *K. pneumoniae* isolates from *K. pneumoniae* liver abscess patients. There are several explanations. As shown in the previous study,[10] gastrointestinal colonization of hvKP is one of the predisposing factors for hvKP liver abscess. However, different rates of stool colonization of *K. pneumoniae* including hvKP strains among individuals were noted in the previous studies conducted in Taiwan, ROK, and China.[8,9,22] Moreover, an increasing trend of fecal carriage of ESBL-positive Enterobacteriaceae including *K. pneumoniae* on the global level has been reported recently.[23] Extrapolating from these, it can be inferred that there might be greater heterogeneity of intestinal colonization of *K. pneumoniae* including hvKP strains and other *K. pneumoniae* strains with ESBL in the hvKP liver abscess patients. The low concordance rate between the liver aspirate *K. pneumoniae* isolates and stool *K. pneumoniae* isolates in our study supports this inference. Also, our result of the low growth rate of *K. pneumoniae* from the stool sample (11/37; 29.7%) of overall hvKP liver abscess patients along with the low concordance rate suggest that intestinally colonized hvKP may be able to outcompete other intestinally colonized *K. pneumoniae* strains with ESBL or other Enterobacteriaceae such as *Escherichia coli* for crossing the intestinal barrier to invade the liver. Furthermore, there was one patient whose liver aspirate culture revealed positive growth of the both ESBL-negative and ciprofloxacin-susceptible hvKP isolate and *K. pneumoniae* isolate with positive ESBL and sensitivity to ciprofloxacin. Although this patient was not included in the main analysis due to lack of isolation of *K. pneumoniae* from the stool sample, this case illustrates possible basis for the emergence of ESBL-positive hvKP liver abscess infection following co-colonization of hvKP and ESBL positive *K. pneumoniae*. Recent reports of increasing trends in the prevalence rates of ESBL-positive hvKP isolates in China[21,24] along with our case suggest possible dissemination of the ESBL plasmid into hvKP isolates, which should be monitored closely. Of note, there was a higher Pitt bacteremia score in the concordant group in our study. Whether this was representing an increased disease severity due to enhanced translocation of hvKP from a higher burden of intestinally colonized hvKP or an incidental observation is difficult to conclude but this warrants further investigation.

This study has limitations. First, this was a single-center, observational study with a small number of hvKP liver abscess patients. Thus, sampling bias from a small number of patients and *K. pneumoniae* isolates might have existed to affect the analysis. Second, selection bias regarding the stool *K. pneumoniae* isolates might have led to an underestimation of the concordance rate as the stool samples were collected after hvKP liver abscess patients had already been treated with antibiotic therapy, which might have affected the yield of the stool culture.

In conclusion, despite the aforementioned limitations, the current study suggests the significant heterogeneity of *K. pneumoniae* colonizing the intestinal tract of the hvKP liver

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abscess patients in the ROK. In the era of increasing elderly population and intestinal colonization with ESBL-positive Enterobacteriacea including *K. pneumoniae*, further studies involving a larger number of hvKP liver abscess patients with continuing surveillance are needed to define the changing epidemiology and the role of gastrointestinal *K. pneumoniae* in the hvKP liver abscess patients in the ROK.

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