Molecular Typing and Virulence Analysis of Serotype K1 *Klebsiella pneumoniae* Strains Isolated from Liver Abscess Patients and Stool Samples from Noninfectious Subjects in Hong Kong, Singapore, and Taiwan[▽]

L. Kristopher Siu,^{1,2} Chang-Phone Fung,³ Feng-Yee Chang,⁴ Nelson Lee,⁵ Kuo-Ming Yeh,⁴ Tse Hsien Koh,^{6*} and Margaret Ip^{7*}

Division of Infectious Diseases, National Health Research Institutes,¹ and Graduate Institute of Basic Medical Science, China Medical University,² Taichung, Taiwan; Section of Infectious Diseases, Department of Medicine, Taipei Veterans General Hospital and National Yang-Ming University, Taipei, Taiwan³; Division of Infectious Diseases and Tropical Medicine, Department of Internal Medicine, Tri-Service General Hospital, Taipei, Taiwan⁴; Department of Pathology, Singapore General Hospital, Singapore, Republic of Singapore⁵; and Department of Medicine and Therapeutics⁵ and Department of Microbiology,⁷ Faculty of Medicine, Chinese University of Hong Kong, Hong Kong SAR, People's Republic of China

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Serotype K1 *Klebsiella pneumoniae* with multilocus sequence type 23 (ST23) has been strongly associated with liver abscess in Taiwan. Few data regarding the strain types and virulence of this serotype from other Asian countries are available. Serotype K1 *K. pneumoniae* strains isolated from liver abscess and stool samples from subjects hospitalized in Hong Kong, Singapore, and Taiwan hospitals were examined. Forty-seven serotype K1 isolates were identified: 26 from liver abscess samples and 21 from stool samples. MLST revealed 7 sequence types: 85.1% (40 of 47 isolates) belonged to ST23, 1 isolate belonged to ST163 (a single-locus variant of ST23), and 2 isolates were ST249 (a 3-locus variant of ST23). New STs, namely, ST367, ST425, and ST426, were allocated to 3 of 4 isolates from stool samples. The virulence of these strains was determined by neutrophil phagocytosis and mouse infection models. Except for two ST23 isolates, all *Klebsiella pneumoniae* isolates were resistant to phagocytosis. Resistance to serum killing varied in isolates of ST23, while all non-ST23 strains were susceptible to serum killing except one with ST249 from a liver abscess. All hypervirulent isolates with a 50% lethal dose of $<10^2$ CFU were from ST23, were resistant to phagocytosis and serum killing, and also carried both virulence-associated genes, *rmpA* and aerobactin. Multilocus sequence typing genotype 23 was the most prevalent sequence type among serotype K1 *K. pneumoniae* isolates from both liver abscess and stool samples in the Asia Pacific region. Serotype K1 *K. pneumoniae* isolates with capsule expression leading to phagocytic resistance and with the aerobactin gene were associated with hypervirulence.

Klebsiella pneumoniae is an opportunistic nosocomial pathogen frequently encountered in urinary tract infections, pneumonia, and septicemia (18). However, community-acquired *K. pneumoniae* liver abscess (KP-LA) has frequently been observed in Taiwan in the past 2 decades and appears to be endemic in this locality (7). Recent epidemiologic surveys showed that *K. pneumoniae* as a single agent rather than a polymicrobial infection is the most common causative agent of liver abscess in Singapore and South Korea (5, 23). Sporadic cases have also been reported from the United States, Japan, Canada, and Thailand (11, 12, 22).

K. pneumoniae serotype K1 has been identified to be the major cause of KP-LA and bacteremia. Complications of endophthalmitis and meningitis are often seen in patients with

KP-LA, especially patients with diabetes mellitus (DM) (7). DM is prevalent in our localities, while alcoholism as a predisposing factor has been reported only in South Korea (5). KP-LA isolates have been associated with capsule hypermucoviscosity and the presence of virulence genes *magA* and *rmpA*. However, it is now clear that *magA* is a capsular polymerase, *wzy*_{KPK1}, specific for *K. pneumoniae* serotype K1 capsule formation (24).

Molecular typing of isolates from KP-LAs in Taiwan has shown that a portion of isolates was clustered by pulsed-field gel electrophoresis (PFGE) in one study, while nonclonal isolates were also observed in another study (2). Turton et al. have identified the genetic similarity of KP-LA isolates from three continents by multilocus sequence typing (MLST) (21). To our knowledge, no data are available on the molecular types and characteristics of serotype K1 isolates from the stools of healthy carriers in the general population, nor have isolates from the stools of healthy carriers been compared to isolates from liver abscesses. In the present study, we have performed MLST for serotype K1 *Klebsiella pneumoniae* isolates from liver abscesses and from carriers without a history of KP-LA and assessed the virulence of isolates with different MLST types from Hong Kong, Singapore, and Taiwan.

* Corresponding author. Mailing address for M. Ip: Department of Microbiology, Faculty of Medicine, Chinese University of Hong Kong, Hong Kong SAR, People's Republic of China. Phone: 852-26321265. Fax: 852-26473227. E-mail: margaretip@cuhk.edu.hk. Mailing address for T. H. Koh: Department of Pathology, Singapore General Hospital, Singapore, Republic of Singapore. Phone: 65-63214942. Fax: 65-62226826. E-mail: koh.tse.hsien@sgh.com.sg.

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TABLE 1. Specific primers used for amplification of target genes of *K. pneumoniae* in this study

Serotype (target gene)	Primer	Size of PCR product (bp)	Reference
K1 (<i>wzy</i> _{KPK1})	5'-GGTGCTCTTACATCATTGC-3' 5'-GCAATGGCCATTTCGTTAG-3'	1,283	20
K2 (<i>wzy</i> _{KPK2})	5'-GACCCGATATTCATACTTGACA GAG-3' 5'-CCTGAAGTAAAATCGTAAATA GATGGC-3'	641	20
K5 (<i>wzy</i> _{KPK5})	5'-TGGTAGTGTGCTCGCGA-3' 5'-CCTGAACCCACCCCAATC-3'	280	20
<i>mmpA</i>	5'-ACTGGGCTACCTCTGCTTCA-3' 5'-CTTGCAATGAGCCATCTTTCA-3'	536	25
Aerobactin	5'-GCATAGGCGGATACGAACAT-3' 5'-CACAGGGCAATTGCTTACCT-3'	556	25
Aerobactin	5'-CTGTCGGCATCGGTTTATT-3' 5'-TGGCGTGTGCGATTATTACCA-3'	531	25

MATERIALS AND METHODS

Bacterial strains. *K. pneumoniae* strains that were isolated from liver abscesses and stool samples from healthy subjects, hospitalized patients without a history of liver abscess, or patients admitted with noninfectious diseases were collected at Prince of Wales hospital in Hong Kong, Singapore General Hospital, National University Hospital in Singapore, and Tri-Service General Hospital in Taiwan from 2002 to 2009. The diagnosis of liver abscess was confirmed by abdominal ultrasonography and/or computerized tomography. Identification of the isolates was according to standard clinical microbiologic methods.

Serotyping and *mmpA* and aerobactin gene detection by PCR. Isolates were serotyped using the capsule swelling reaction with antisera obtained from the Health Protection Agency in the United Kingdom and by PCR as previously described (7). PCRs to determine the presence of the genes specific for serotypes K1, K2, and K5 and the *mmpA* and aerobactin genes were performed using the primers listed in Table 1 (20, 25). A bacterial colony from an overnight culture was added to 300 μ l water and boiled for 15 min to release the DNA template. The reaction mixture was kept at 95°C for 5 min, followed by 40 temperature cycles of 95°C for 1 min, 50°C for 1 min, and 72°C for 2 min and then 72°C for 7 min. The expected PCR products were 1,283 bp in length for *wzy*_{KPK1}, 535 bp for *mmpA*, and either 556 or 531 bp for aerobactin (Table 1).

PFGE. Total DNA was prepared and PFGE was performed as described previously (23). The restriction enzyme XbaI (New England BioLabs, Beverly, MA) was used at the manufacturer's suggested temperature. Restriction fragments were separated by PFGE in a 1% agarose gel (Bio-Rad, Hercules, CA) in 0.5 $\times$ TBE buffer (45 mM Tris, 45 mM boric acid, 1.0 mM EDTA, pH 8.0) for 22 h at 200 V at a temperature of 14°C, with ramped times of 2 to 40 s, using a Bio-Rad CHEF Mapper apparatus (Bio-Rad Laboratories, Richmond, CA). Gels were then stained with ethidium bromide and photographed under UV light. The resulting genomic DNA profiles, or fingerprints, were interpreted according to established guidelines (19).

MLST. MLST was performed according to the method of Turton et al. (21). Sequences of seven housekeeping genes were obtained for isolates from liver abscess patients and carriers. Sequence information was compared with that available from the MLST website (<http://www.pasteur.fr/mlst/>) developed by Keith Jolley (9). Alleles and sequence types (STs) were assigned accordingly. Sequences of any alleles that were not in the database were submitted to the curator and a new allele number was obtained. A difference in two or more alleles was considered to indicate that the sequence types being compared were unrelated.

Fluorescence labeling of bacteria. Labeling was performed as previously described (15). The *K. pneumoniae* isolate and control suspensions were individually incubated overnight at 37°C. The concentration was approximated using photometry (Olympus). The percentage of viable bacteria in an aliquot of each population was determined by quantitative plate counting. Populations were then heat killed for 60 min in a 70°C water bath, and quantitative colony count determination of population viability was performed again. The bacteria were washed with phosphate-buffered saline (PBS) and labeled with fluorescein isothiocyanate (FITC) by incubation with 0.1 μ g/ml FITC (Sigma Chemical Co., St. Louis, MO) in 0.10 M NaHCO₃, pH 9.0, for 60 min at 25°C. Bacteria were washed of unbound fluorochrome with PBS by three cycles of centrifugation

(13,000 rpm, 10 min). The FITC-labeled bacteria were resuspended at a concentration of 2×10^8 cells/ml in PBS, divided into equal volumes, and stored at -70°C. Aliquots were thawed just prior to use.

Phagocytosis assay. Phagocytosis was measured using a standard assay. Normal human serum pooled from healthy volunteers was divided into equal volumes and stored at -70°C. Serum was thawed immediately prior to use and stored on ice until it was added to the phagocytosis assay. Briefly, for the assay, 100 μ l of a neutrophil suspension (representing 1×10^6 cells), 100 μ l of freshly thawed pooled normal human serum (10% [vol/vol] opsonization), and 600 μ l PBS were added to sealable Falcon polypropylene tubes (10 by 75 mm; BD Biosciences, Franklin Lakes, NJ). The suspension was prewarmed with shaking for 5 min at 37°C. Two hundred microliters FITC-labeled bacteria (representing 4×10^7 CFU/ml) was added to 800 μ l to produce a final volume of 1.0 ml. Each tube was capped and incubated in a shaking water bath at 37°C with continuous agitation for 15 min. The result for a sample in an unincubated tube served as the result for the 0-min time point. At each designated time, samples were removed and placed in an ice bath. The cells in each suspension were removed by centrifugation at $250 \times g$ for 6 min, and the cell pellet was resuspended in 1.0 ml of ice-cold PBS and maintained at 4°C. A 600- μ l volume of the suspension was transferred into a new tube, and ethidium bromide was added to a final concentration of 50 mg/liter before measurement. Excess ethidium bromide was used to suppress the extracellular fluorescence. Bacteria that were not localized in neutrophils appeared red upon microscopic examination.

Phagocytosis assay using flow cytometry. A FACScan apparatus emitting an argon laser beam at 488 nm (Becton Dickinson Immunocytometry Systems, San Jose, CA) was used to detect FITC fluorescence. The sideways scatter (SSC) threshold was 52. The detector was set at E00, 350, and 427 for forward scatter (FSC), SSC, and fluorescence 1 (FL1-H, green), respectively. Fluorescence values were collected after the detector was gated on the FSC and SSC combination. A total of 10,000 cells were processed using Cellquest (version 1.0) software (Becton Dickinson Immunocytometry Systems). Fluorescence distribution data collected using a logarithmic amplifier were displayed as single histograms for FL1-H. By processing unstained and FITC-stained bacterial phagocytosis mixtures, the boundary of positive and negative fluorescence was determined. The percentage of ingested bacteria was assessed after the addition of ethidium bromide.

Susceptibility to serum killing. Serum bactericidal activity was measured using the method of Hughes et al. (8) as modified by Podschun et al. (17). Bacteria grown in nutrient broth were collected during the early logarithmic phase. The viable bacterial concentration was adjusted to 1×10^6 CFU/ml. Twenty-five microliters of bacteria was added to 75 μ l of pooled human sera contained in a Falcon polypropylene tube (10 by 75 mm; BD Biosciences, Franklin Lakes, NJ). Tubes were agitated for 0, 60, 120, or 180 min. To determine the number of viable bacteria after exposure to serum, an aliquot of each bacterial suspension was removed at the designated time point, diluted 10-fold by addition of Mueller-Hinton broth, plated on Mueller-Hinton agar, and assayed as described immediately below.

Results were expressed as a percentage of the inoculum, and responses in terms of viable counts were graded from 1 to 6 as described previously (17). Grade 1 represented viable counts of <10% of the inoculum after 1 and 2 h and <0.1% after 3 h. Grade 2 represented viable counts of 10 to 100% of the inoculum after 1 h and <10% after 3 h. Grade 3 represented viable counts that exceeded those of the inoculum after 1 h but that were <100% after 2 and 3 h. Grade 4 represented viable counts of >100% of the inoculum after both 1 and 2 h but <100% after 3 h. Grade 5 represented viable counts which were >100% of the inoculum at 1, 2, and 3 h but which decreased during the third hour. Finally, grade 6 represented viable counts that exceeded those of the inoculum after 1, 2, and 3 h and that increased throughout this time period. Each strain was tested at least three times. A strain was considered serum resistant or serum sensitive if the grading was the same in all experiments. Each isolate was classified highly sensitive (grade 1 or 2), intermediately sensitive (grade 3 or 4), or resistant (grade 5 or 6).

Mouse lethality test. For determination of the 50% lethal dose (LD₅₀) in mice, six mice were used as a sample population for each bacterial concentration. Bacterial concentration was calculated by determination of the number of CFU, and the concentration for inoculation tested ranged from 10^4 to 10^8 CFU/ml. Intraperitoneal (i.p.) injection was used to assess virulence. Symptoms and signs of infection were observed for 14 days. Survival of the inoculated mice was recorded and the LD₅₀ was calculated using the SigmaPlot (version 7.0) program from SPSS Inc. (Chicago, IL).

TABLE 2. MLST of serotype K1 isolates from liver abscess patients and stool samples from carriers without a history of KP-LA in Hong Kong, Singapore, and Taiwan^a

Serotype (<i>n</i> = 577)	ST (no. of isolates)	No. of isolates						Allelic profile						
		HK		SG		TW		<i>gapA</i>	<i>inf</i>	<i>mdh</i>	<i>pgi</i>	<i>pho</i>	<i>rpoB</i>	<i>tonB</i>
		LA	STC	LA	STC	LA	STC							
K1 (47)	23 (40)	5	5	9	2	10	9	2	1	1	1	9	4	12
	163 (1)			1				2	1	1	1	9	1	12
	249 (2)	1					1	2	1	44	1	16	4	66
	367 ^b (1)		1					18	15	26	16	11	13	106 ^b
	425 ^b (1)		1					42	22	63 ^b	22	86 ^b	38	114
	426 ^b (1)		1					18	15	18	64 ^b	11	13	50
	138 (1)				1			18	22	26	23	31	13	49
Non-K1 (530)	NA	24	92	40	35	18	321							

^a HK, Hong Kong; SG, Singapore; TW, Taiwan; LA, liver abscess; STC, stool carriage; NA, not available.^b New STs or alleles in this study.

RESULTS

MLST profiles of isolates from Hong Kong, Singapore, and Taiwan and detection of virulence-associated *rmpA* and aerobactin genes. A total of 47 isolates from 577 *K. pneumoniae* isolates collected from three sites were confirmed to be serotype K1 by serotyping and PCR and selected for this study. Twenty-six and 21 *K. pneumoniae* isolates were isolated from liver abscess patients and stool of uninfected subjects, respectively (Table 2). These represented a prevalence rate of 24% of serotype K1 for LA patients and a carriage rate of 4.5% for stool samples from carriers. Forty of 47 (85.1%) serotype K1 isolates belonged to ST23. One liver abscess isolate was ST163. ST23 and ST163 are clonally related, the latter being a single-locus variant (SLV) with an allelic difference in the *rpoB* gene. Two isolates were ST249, including one isolated from a liver abscess and another isolated from a stool sample from a carrier. There was one isolate each belonging to STs 367, 425, 426, and 138, and all these isolates were from stool samples from carriers. STs 367, 425, and 426 were new STs found in this study. Except for ST23 and ST163, all other STs were non-clonally related (Table 2). PCR results revealed that all isolates carried *rmpA*. In addition, all isolates of ST23 and the ST163 SLV of ST23 carried both *rmpA* and aerobactin. All the remaining isolates except one strain of ST367 were negative for aerobactin.

Neutrophil phagocytosis and serum resistance of *K. pneumoniae* isolates with different MLST types. Overall, there was no statistically significant difference in resistance to neutrophil phagocytosis among strains of different MLST types in this study. Two ST23 isolates, one from a liver abscess and the other from a stool sample from a carrier, were relatively susceptible to neutrophil phagocytosis, and these two isolates were both from Hong Kong. The remainder of the isolates demonstrated resistance to neutrophil phagocytosis, compared to the capsule-deficient control strains, which were highly susceptible to phagocytosis (Fig. 1).

Except for two ST249 isolates, all isolates with STs other than ST23 were susceptible to serum complement killing. The majority of ST23 isolates, 10 of 16 from stool samples from carriers and 17 of 24 from liver abscesses, demonstrated intermediate resistance or resistance (of grade 4 or 5) to serum

complement killing. Serum killing of susceptible strains with ST23 was found in all three geographical regions, and these strains were isolated from liver abscess patients and stool samples from uninfected carriers. Although ST163 is clonally related to ST23, the isolate of ST163 was susceptible to serum complement killing (Table 3).

PFGE for selected serotype K1 isolates with different MLSTs and susceptibility to neutrophil phagocytosis and serum killing. Eight isolates in the ST23 group were further typed by PFGE, according to their phagocytic and serum susceptibilities: (i) susceptible to both phagocytosis and serum complement killing, (ii) susceptible to phagocytosis but resistant to serum complement killing, and (iii) resistant to both

FIG. 1. Effects of all isolates on phagocytosis according to their STs and body sites and regions of isolation. The phagocytosis rate is calculated as the percentage of neutrophils ingesting FITC-labeled bacteria at 15 min. Strains from liver abscess patients and stool samples from carriers are labeled with open and filled symbols, respectively, according to their region of isolation. The isolates from Hong Kong (HK), Singapore (SG), and Taiwan (TW) are identified by circles, triangles, and squares, respectively. Two ST23 laboratory capsule-deficient strains (Δ K1) from a previous study (24) were included as a control for highly phagocytic susceptible strains. Except for two ST23 isolates with a phagocytic rate of over 50% at 15 min, all isolates were highly resistant to phagocytosis by neutrophils.

TABLE 3. Serotype K1 isolates from stool and liver abscess samples with different STs by serum complement killing

Source	ST (no. of isolates)	No. of isolates with the following results by serum complement killing ^a :					
		Intermediately sensitive or resistant			Sensitive		
		HK	SG	TW	HK	SG	TW
Liver abscess	23 (24)	2	7	8	3	2	2
	163 ^b (1)					1	
	249 (1)	1					
Stool carriage	23 (16)	4	2	4	1		5
	138 (1)					1	
	249 (1)			1			
	367 (1)				1		
	425 (1)				1		
	426 (1)				1		

^a HK, Hong Kong; SG, Singapore; TW, Taiwan.^b Clonal ST23-like related ST.

phagocytosis and serum complement killing. In addition, two isolates with ST249 were also included. Except for two isolates from the first group with indistinguishable pulsed-field patterns, all the other isolates had PFGE profiles that were unrelated.

Mouse lethality by intraperitoneal injection of serotype K1 isolates with different MLSTs, susceptibilities to neutrophil phagocytosis and serum killing, and virulence-associated aerobactin genes. Eight isolates belonging to ST23 and all non-ST23 isolates were selected for mouse lethality studies. Isolates had LD₅₀s that varied from <10² to 3.0 × 10⁵ CFU (Table 4). All hypervirulent isolates (LD₅₀, <10² CFU) were of ST23 and were represented by both stool carriage and liver abscess isolates. All these isolates were resistant to phagocytosis and serum killing and also possessed both the *ompA* and aerobactin virulence-associated genes. Isolates susceptible to both neutrophil phagocytosis and serum killing were relatively less virulent (LD₅₀, ~10⁴ CFU). Although one ST23 isolate showed susceptibility to serum killing, it was as virulent as an ST23 isolate with resistance to both phagocytosis and serum killing (Table 4). Strains that were negative for the aerobactin gene showed relatively low virulence (LD₅₀, ~10⁵ CFU). In a comparison of isolates with similar resistance to phagocytosis and serum killing and with or without the aerobactin gene, ST23 and ST249 isolates showed different mouse mortalities, which implicates aerobactin as a potential independent virulence factor and which will require further elucidation with experimental work using isogenic strains. *In vitro* parameters of neutrophil phagocytosis, serum resistance, and the presence of the aerobactin gene for virulence assessment were correlated to *in vivo* mouse lethality.

DISCUSSION

Turton et al. were the first group to identify a high prevalence of ST23 in liver abscesses in Taiwan and revealed the clonal relationship of strains in their study using PFGE (21). In our study, we demonstrated that ST23 was also predominant in serotype K1 *K. pneumoniae* isolates causing liver abscess and

TABLE 4. Virulence analysis by combining results obtained from phagocytosis, serum complement killing, and mouse lethality assays

Source ^a	Type by MLST	Aerobactin carriage	Susceptibility ^b		LD ₅₀ (CFU)
			Phagocytosis	Serum	
Liver	ST23	+	S	S	~1.2 × 10 ⁴
Carriage	ST23	+	S	S	~2.7 × 10 ⁴
Liver	ST23	+	R	S	~2.6 × 10 ³
Carriage	ST23	+	R	S	<10 ²
Carriage	ST23	+	R	R	<10 ²
Liver	ST23	+	R	R	<10 ²
Liver	ST23	+	R	R	<10 ²
Liver	ST23	+	R	R	<10 ²
Liver	ST163 ^c	+	R	S	~3.5 × 10 ³
Liver	ST249	—	R	R	~3.7 × 10 ⁵
Carriage	ST249	—	R	R	~3.0 × 10 ⁵
Carriage	ST138	—	R	S	~2.4 × 10 ⁵
Carriage	ST367	+	R	S	~1.0 × 10 ³
Carriage	ST425	—	R	S	~1.6 × 10 ⁵
Carriage	ST426	—	R	S	~3.0 × 10 ⁵

^a All isolates carried *ompA*.^b S, susceptible; R, resistant.^c Clonal ST23-like related ST for which partial data have been previously published (1).

carried in stools of uninfected subjects in Hong Kong, Singapore and Taiwan. In contrast to the findings of Turton et al. (21), our ST23 isolates from LA patients and stool samples from carriers were not related by PFGE, indicating that KP-LA may not be caused by a specific clone. The diversity in our isolates might be explained by the fact that they were isolated from two patient sources (liver abscess and stool) and from three different regions in Asia. In general, non-ST23 isolates were mostly isolated from stool samples from carriers and appeared to be relatively less virulent, although one case of KP-LA was due to ST249. This might reflect that non-ST23 isolates are relatively uncommon in stool carriers in the Asia Pacific region or that other host factors might also play a role in KP-LA. Further studies may elucidate the background prevalence of serotype K1 *K. pneumoniae* isolates in the Asia Pacific region and the significance of non-ST23 isolates in KP-LA.

In this study, no significant difference in virulence was identified between ST23 isolates recovered from LA and from the stools of carriers ($P = 0.733$, Fisher's exact test). The virulence of these strains was demonstrated by the presence of the aerobactin gene and the low LD₅₀ in the mouse lethality model. Previous studies had specifically mentioned hypermucoviscosity as an *in vitro* parameter for the virulence phenotype (6, 10, 14). However, the results obtained from this study showed that all serotype K1 isolates were hypermucoviscous but varied in resistance to phagocytosis and serum and in mouse lethality (Table 4). This result was in line with the observation from Turton et al. (21). Nonhypermucoviscous strains could be as virulent as hypermucoviscous strains. Since all K1 isolates in this study carried *ompA*, we are unable to assess the contribution of this gene in virulence. A previous study confirmed that *ompA* is a regulator of capsule formation (3). Loss of this regulator will downregulate capsule synthesis, leading to the loss of phagocytic resistance and the mucoid phenotype. The LD₅₀ of a strain with this type of deficiency is ≥10⁷ CFU,

implicating the importance of virulence in the presence of a capsule (15, 23). In our study, the LD₅₀s of selected isolates fell between <10² and 10⁵ CFU, indicating that a normally expressed K1 capsule is not the sole factor for hypervirulence. Our study observed that phagocytic resistance and carriage of the aerobactin gene were two independent determinants contributing to mouse lethality. A lack of any one of these factors would reduce at least 100-fold the bacterial concentration for the LD₅₀. All hypervirulent strains (LD₅₀s, <10² CFU) were resistant to phagocytosis and possessed the aerobactin gene. One ST367 isolate that was resistant to phagocytosis and that carried the aerobactin gene had an LD₅₀ of approximately 10³ CFU, indicating that some other factor(s) may also play a role in hypervirulence. Previous studies have also documented other virulence factors, including *kfu* (an iron uptake system) and *allS* (a gene associated with allantoin metabolism), that may contribute to virulence in KP-LA (4, 16). Unfortunately, the previous study also indicated that all serotype K1 isolates contained these two determinants (25). Furthermore, the virulence of non-K1 isolates containing these two virulence determinants could be as low as an LD₅₀ of >10⁷ CFU. Thus, future studies comparing these two strain types, ST367 and ST23, may shed further light on another determinant(s) that may contribute to virulence.

Our present data demonstrate the prevalence of *K. pneumoniae* serotype K1 ST23 in KP-LA in the Asia region. Outside Asia, KP-LA has been reported more frequently in patients of Asian descent than non-Asians (12, 13). Most of these cases had no travel history before disease occurred. Whether Asians are genetically more susceptible to LA or there are other factors that contribute to the increased prevalence in Asians is yet unknown. Our present data could not answer this question but have given an indication that hypervirulence may not be the sole factor in serotype K1 leading to disease in the Asia Pacific region. In conclusion, MLST genotype 23 in serotype K1 *K. pneumoniae* was strongly associated with its carriage prevalence and liver abscess in the Asia Pacific region. A few virulence determinants are demonstrated, but other genetic factors of these strains and host factors may play a role in the pathogenesis of LA and remain to be elucidated.

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