MAJOR ARTICLE

Klebsiella pneumoniae Genotype K1: An Emerging Pathogen That Causes Septic Ocular or Central Nervous System Complications from Pyogenic Liver Abscess

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Background. Since 1986, researchers have noted a syndrome of *Klebsiella pneumoniae* pyogenic liver abscess that is complicated by endophthalmitis or central nervous system infections. There are limited data regarding the role of bacterial genotype in the pathogenesis of this syndrome.

Methods. We conducted a retrospective cohort study involving 177 cases of *K. pneumoniae* pyogenic liver abscess treated during 1997–2005 at a tertiary university hospital in Taiwan. We performed bacterial *cps* genotyping by polymerase chain reaction detection of serotype-specific alleles at *wzy* and *wzx* loci and used an in vitro serum assay to evaluate the virulence of bacterial strains.

Results. Septic ocular or central nervous system complications developed in 23 patients (13%). Logistic regression analysis showed that genotype K1 was the only significant risk factor (adjusted odds ratio, 4.8; 95% confidence interval, 1.5–15.7, $P = .009$). The serum resistance assay indicated that, on average, K1 strains ($n = 100$) were significantly more virulent than were strains of K2 ($n = 36$), K20/K5/K54 ($n = 21$), or other genotypes ($n = 20$) ($P < .001$ for each comparison). In addition to the serotype-specific *cps* region, the genomic background of K1 strains also differed significantly from that of non-K1 strains (20-kb *kfu*/PTS region, 97/100 vs. 13/77; $P < .001$). Of the 19 cases in which genotype K1 strains caused complications, 8 patients (42%) did not have identifiable underlying medical diseases.

Conclusions. *K. pneumoniae* genotype K1 is an emerging pathogen capable of causing catastrophic septic ocular or central nervous system complications from pyogenic liver abscess independent of underlying diseases in the host.

Klebsiella pneumoniae usually causes urinary tract infections, pneumonia, and other infections in hospitalized persons whose immunity is compromised by underlying diseases, such as diabetes mellitus [1]. Since 1986, researchers in Taiwan and several other areas have noted a distinctive syndrome of community-acquired pyogenic liver abscess that is complicated by metastatic endophthalmitis or CNS infections [2–8]. Despite aggressive therapy, the outcomes frequently involve catastrophic disability [2–9].

There are controversies about the pathogenesis of this syndrome [10–40]. Fung et al. [10] reported 134 patients with *K. pneumoniae* liver abscess, of whom 14 had septic endophthalmitis. For all patients and for the subset who had septic endophthalmitis, there was a high prevalence of diabetes mellitus (105 [78.4%] of 134 patients and 13 [92.9%] of 14 patients, respectively) and capsular serotype K1/K2 strains (85/19 [63.4%/14.2%] of 134 patients and 12/2 [85.7%/14.3%] of 14 patients, respectively) [10]. The same group reported that K1/K2 isolates were more resistant to phagocytosis than were non-K1/K2 isolates [11] and that poor glycemic control selectively impairs the neutrophil phagocytosis of K1/K2 strains in diabetic patients [12]. They therefore proposed that bacterial capsular serotype

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K1/K2 is a major virulence determinant and that poor glycemic control is a risk factor for susceptibility to K1/K2 *K. pneumoniae* liver abscess and complicated endophthalmitis [10–13]. However, other studies from Taiwan have consistently reported a lower prevalence of diabetes mellitus, whether in cases of pyogenic liver abscess (64.4%–67.5%) [14–17] or in those with septic ocular complications (7 [58%] of 12 patients) [3] and 7 [64%] of 11 patients [4]). The prevalence of diabetes mellitus in cases of *K. pneumoniae* liver abscess is even lower in Korea (39.9%) [18]. In addition, other researchers have found some encapsulated serotype K2 strains to be avirulent [19–21]. Experiments using capsule-switch recombinants also showed that K2 capsule per se confers only marginal virulence to recipients of avirulent K21a strain, despite in vitro acquisition of resistance to phagocytosis [22]. Thus, the roles of diabetes mellitus and K2 capsule in the pathogenesis of this syndrome remain unsettled.

Another potential pathogenic factor is serotype K1 capsule. In the 1980s, animal studies showed some *K. pneumoniae* capsular serotype K1 strains to be highly virulent [20, 23, 24], yet little was known about the clinical aspects of this pathogen [25]. Seroepidemiological surveys suggest that serotype K1 is infrequent among *K. pneumoniae* isolates from North America, Europe, and Australia [26–29], but is the most common serotype (21.7%) in northern Taiwan [30]. It should be noted that *K. pneumoniae* pyogenic liver abscess is a heterogeneous group of diseases, not a single entity [31]. Intra-abdominal local host factors (e.g., biliary tract diseases and malignancy) can also be involved in the occurrence of *K. pneumoniae* pyogenic liver abscesses [32–37]. To minimize confounding from such local factors, we have focused on strains isolated from cases of primary liver abscess in our previous bacteriologic studies [38–40]. Using transposon mutagenesis, we identified a previously unknown virulence gene, *magA,* and the surrounding 33-kb genomic region [38], which we subsequently proved to be the genetic determinants of capsular serotype K1 [39]. In addition, 2 large chromosomal regions harboring additional virulence genes are selectively present on the serotype K1 genome (with a few exceptions) [40]. Genotype K1 strains carrying all 3 genomic regions are strongly associated with primary liver abscess and metastatic infections [40].

The relative contributions of the above-mentioned factors in the pathogenesis of this distinctive syndrome remain unclear. An important limitation of previous studies is that the clinical significance of a bacterial or host characteristic was either extrapolated from in vitro data or assumed from its prevalence among cases, instead of being determined by its effect on the incidence of cases among a cohort. Furthermore, most studies did not distinguish risk factors for septic ocular or CNS complications from those for pyogenic liver abscess.

This is a retrospective cohort study that focuses on the predictors for the development of septic ocular or CNS complications in patients with *K. pneumoniae* pyogenic liver abscess. We developed a *cps* genotyping method that is based on PCR detection of *magA* (*wzy*_K1) and its alleles. We compared genotype K1 and non-K1 groups for the risk of septic ocular or CNS complications, host underlying diseases, bacterial resistance to human serum, and bacterial genomic background.

PATIENTS, MATERIALS, AND METHODS

Location and study sample. This study was conducted at National Taiwan University Hospital (Taipei), a university-affiliated medical center with a 2200-bed capacity that provides primary and tertiary referral care. Culture, identification, and susceptibility testing of isolates were performed according to standard microbiological methods [41, 42]. We collected clinical *K. pneumoniae* strains from isolates obtained by culture of blood and/or liver abscess from hospitalized patients and stored all strains at −80°C before use. We reviewed the medical records of patients from whom the collected strains were isolated during 1997–2005. Cases of pyogenic liver abscess that met the following criteria were included: (1) presence of pyogenic liver abscess confirmed by percutaneous aspiration or surgical drainage, and (2) *K. pneumoniae* isolated from either liver abscess culture or blood culture sampled when the pyogenic liver abscess was initially diagnosed. Information on patient characteristics and outcomes was systemically collected using a computerized data collection format. The study procedures were reviewed and approved by the institutional review board.

Host underlying diseases. We systemically examined the medical records for the existence of underlying diseases, including biliary tract diseases, colorectal pathology, prior trauma or intra-abdominal surgery, malignancy, immunosuppressive therapy, alcoholism, and diabetes mellitus. A diagnosis of diabetes mellitus was made if 2 independent plasma glucose measurements performed while the patient was fasting were $\geqslant$126 mg/dL or if a random plasma glucose measurement was $\geqslant$200 mg/dL [43]. For patients already receiving antidiabetic agents or insulin, a previous diagnosis was considered to be sufficient.

cps *genotyping.* We performed *cps* genotyping by PCR detection of K serotype–specific alleles at *wzy* and *wzx* loci. The primers are listed in table 1. The *K. pneumoniae cps* gene cluster has a conserved organization similar to the *Escherichia coli* group 1 capsule biosynthesis gene cluster, comprising 2 regions separated by a putative stem-loop transcriptional attenuator [39, 44–46]. The 3′ region is serotype-specific and encodes enzymes for a Wzy-dependent biosynthesis system, including enzymes for producing sugar nucleotide precursors, glycosyltransferases, and 2 integral inner membrane proteins—Wzy and Wzx [46]. The polymerase Wzy assembles undecaprenyl diphosphate–linked polymers using lipid-linked repeat units exported by the flippase Wzx [46]. *magA* [38, 39] is the serotype

Table 1. Primers used for *cps* genotyping and for detection of 3 different copies of the *rmpA* gene.

Objective, genotype or copy type, primer	Nucleotide sequence
cps genotyping	
K1	
wzx_K1-F	5′-GTAGGTATTGCAAGCCATGC-3′
wzx_K1-R	5′-GCCCAGGTTAATGAATCCGT-3′
wzy_K1-F	5′-GGTGCTCTTTACATCATTGC-3′
wzy_K1-R	5′-GCAATGGCCATTTGCGTTAG-3′
K2	
wzx_K2-F	5′-GGAGCCATTTGAATTCGGTG-3′
wzx_K2-R	5′-TCCCTAGCACTGGCTTAAGT-3′
wzy_K2-F	5′-GGATTATGACAGCCTCTCCT-3′
wzy_K2-R	5′-CGACTTGGTCCCAACAGTTT-3′
K5	
wzx_K5-F	5′-GCCACCTCTAAGCATATAGC-3′
wzx_K5-R	5′-CGCACCAGTAATTCCAACAG-3′
wzy_K5-F	5′-CAGGGAACTCCTACGCAGATTT-3′
wzy_K5-R	5′-GGGTGATAAGGTATAGCTGACAC-3′
K20	
wzx_K20-F	5′-CCGATTCGGTCAACTAGCTT-3′
wzx_K20-R	5′-GCACCTCTATGAACTTTCAG-3′
wzy_K20-F	5′-CGGTGCTACAGTGCATCATT-3′
wzy_K20-R	5′-GTTATACGATGCTCAGTCGC-3′
K54	
wzx_K54-F	5′-CATTAGCTCAGTGGTTGGCT-3′
wzx_K54-R	5′-GCTTGACAAACACCATAGCAG-3′
wzy_K54-F	5′-GTTACCTCAGAGCGTTGCAT-3′
wzy_K54-R	5′-CGGACTTAATAGCGAGCAAAG-3′
K57	
wzx_K57-F	5′-CGACAAATCTCTCCTGACGA-3′
wzx_K57-R	5′-CGCGACAAACATAACACTCG-3′
wzy_K57-F	5′-CTCAGGGCTAGAAGTGTCAT-3′
wzy_K57-R	5′-CACTAACCCAGAAAGTCGAG-3′
Detection of *rmpA* gene	
rmpA consensus	
Rmp-F	5′-GCAGTTAACTGGACTACCTCTG-3′
Rmp-R	5′-GTTTACAATTCGGCTAACATTTTTCTT-TAAG-3′
*rmpA*_KPP020	
pRmp-F	5′-TACTTTATATGTAACAAGGATGTAAACA-TAG-3′
pRmp-R	5′-CAGTAGGCATTGCAGCACTGC-3′
*rmpA*_KPP302	
RmpA2-F	5′-CTGTGTCCACTATTGGTGGG-3′
RmpA2-R	5′-GATAGTTCACCTCCTCCTCC-3′
*rmpA*_KP3619	
cRmp-F	5′-TGGCAGCAGGCAATATTGTC-3′
cRmp-R	5′-GAAAGAGTGCTTTCACCCCCT-3′

NOTE. We originally designed the wzy_K1-F and wzy_K1-R primers for *magA* detection [38]. We designed the primers for genotype K2 from the nucleotide sequence of open-reading frame (ORF) 10 *(wzy)* and ORF11 *(wzx)* in the K2 *cps* gene cluster (GenBank accession number D21242). The prototype tissue-invasive strain *Klebsiella pneumoniae* NTUH-K2044 genome (http://genome.nhri.org.tw/kp/index.php) carries 3 different copies of the *rmpA* gene (GenBank accession numbers AB289642—AB289644): 2 (ORFs KPP020 and KPP302) on the 224-kb large plasmid pK2044 (GenBank accession number AP006726) and 1 (ORF KP3619) on the chromosome. To determine the presence of *rmpA,* we designed primers Rmp-F and Rmp-R from the consensus sequence and verified the presence of each specific copy by PCR, using specific primers.

K1 *wzy* allele (*wzy*_K1; i.e., serotype K1 polymerase gene) in *K. pneumoniae.* The *cps* gene clusters of serotypes K1, K2, K5, K20, K54, and K57 have different alleles at both *wzy* and *wzx* loci.

For the genotyping procedure, we extracted genomic DNA (100 ng/μL) from tested strains as templates. An initial denaturation at 96°C for 3 min was followed by denaturation at 96°C for 30 s, annealing at 56°C for 15 s, and extension at 74°C for 1 min for 30 cycles. There was a final 10-min hold at 72°C. The 77 K-serotype reference strains (Statens Serum Institut; Copenhagen, Denmark) served as positive and negative controls.

***Detection of 20-kb* kfu*/PTS genomic region and* rmpA.** In addition to the *cps* region, the 20-kb *kfu*/PTS region (harboring virulence genes for an iron-uptake system) and *rmpA* (a regulator of exopolysaccharides synthesis) are also essential for full virulence of prototype tissue-invasive strain NTUH-K2044 [38, 40]. We determined the presence of the 20-kb *kfu*/PTS region as originally described [40], using primers kfuB-F1179/kfuC-R649, 7C4-T71/26D6-T32, and PVAR-F/PVAR-R. The primers used to determine the presence of *rmpA* are listed in table 1. PCR conditions were the same as those used in *cps* genotyping.

Serum resistance and colony mucoviscosity assay. We assayed the resistance of clinical *K. pneumoniae* strains to healthy human serum as previously described [38, 47]. We graded resistance by the mean 1-h survival ratio (ratio of colony count after serum treatment for 1 h compared with baseline). Strain NTUH-K2044 (highly serum-resistant) and strain NTUH-K5906 (highly serum-susceptible) were controls. We assayed the colony mucoviscosity as previously described [38], using strain NTUH-K2044 as a control.

Nucleotide sequences. We deposited the *cps* region sequences of Danish K5, K20, K54, and K57 serotype reference strains and Taiwanian clinical strains *K. pneumoniae* NTUH-K9534 (K5), NTUH-KP13 (K20), and NTUH-KP35 (K54) into the GenBank database under accession numbers AB289645–AB289652. We performed annotation and analysis of *wzy* and *wzx* using NCBI protein-BLAST (version 2.2.14; released 7 May 2006), Pfam protein motif database (http://www.sanger.ac.uk/Software/Pfam/), and SMART (http://smart.embl-heidelberg.de/).

Statistical analysis. We performed all statistical tests using S-PLUS 2000 (MathSoft) and SAS software, version 9.1.3 (SAS).

RESULTS

Patient characteristics. We identified 177 patients with *K. pneumoniae* pyogenic liver abscess. The mortality rate was low (5 [2.8%] of 177 patients) because physicians prescribed appropriate antimicrobial therapy for all patients (third-generation cephalosporin in 167 cases [94%]). Table 2 shows the disk susceptibility of the 177 strains. Septic ocular or CNS complications developed in 23 patients (13%), 16 of whom had irreversible catastrophic disability, including loss of vision in the involved eye(s), quadriplegia, paraparesis, or impaired higher cortical function (table 3).

Table 2. Disk susceptibility of 177 *Klebsiella pneumoniae* strains.

Antimicrobial agent	No. (%) susceptible
Cefazolin	174 (98)
Cefoxitin	175 (99)
Cefotaxime	177 (100)
Imipenem	177 (100)
Gentamicin	177 (100)
Amikacin	177 (100)
Ciprofloxacin	174 (98)

NOTE. The 177 strains were blood isolates (n = 130) or liver abscess isolates (n = 47). The blood isolates were sampled when pyogenic liver abscess was initially diagnosed and had the same antimicrobial susceptibility patterns and genotypes as the liver abscess isolates that were obtained subsequently. The method of drainage was percutaneous aspiration (n = 156) or surgery (n = 21). For 44 patients, drainage of pyogenic liver abscess was performed after 2–14 days of intravenous antimicrobial therapy, and culture of the obtained abscess sample yielded negative results.

cps *genotyping.* Table 4 and figure 1 show the genotype distribution of the 177 strains. The results of *wzx* typing were consistent with *wzy* typing in all tested strains. There was no cross-reaction with the other 76 serotype reference strains. Overall, the K1 genotype was most common (n = 100; 56%), followed by the K2 genotype (n = 36; 20%). The distribution of genotypes differed markedly between primary and secondary liver abscesses (table 4). Most primary abscesses (81%) were caused by K1 strains and most secondary abscesses (58%) were caused by non-K1 strains ($P < .001$). Six genotypes (K1, K2, K54, K5, K20, and K57) accounted for 92% of the 177 strains.

Comparison of K1 versus non-K1 groups. Pyogenic liver abscesses caused by K1 strains differed significantly from those attributed to non-K1 strains in various clinical characteristics (table 5). Patients infected by K1 strains were more likely to be male (73% vs. 44%; $P < .001$) but were less likely to have biliary tract diseases (21% vs. 45%; $P < .001$), a history of intra-abdominal surgery (22% vs. 42%; $P = .008$), diabetes mellitus (54% vs. 70%; $P = .031$; median level of hemoglobin A_{1c} (%), 6.3 vs. 7.9; $P = .026$), malignancy (5% vs. 14%; $P = .038$), or concomitant pathogens (1% vs. 8%; $P = .044$). There were more patients without identifiable underlying diseases in the K1 than in the non-K1 group (32% vs. 5%; $P < .001$).

Of the 19 cases of complications caused by K1 strains, 8 patients (42%) did not have any identifiable underlying medical disease, and nearly all (7 of 8 patients) experienced catastrophic disability (table 3). In contrast, the 4 patients who had complications that were caused by non-K1 strains all had diabetes

Table 3. Twenty-three cases of pyogenic liver abscess with septic ocular or CNS complications.

Patient no.	Year	Age, years (sex)	Bacterial genotype	Serum resistance	Patient's underlying disease(s)	Site(s) of involvement	Outcome
1	1997	50 (M)	K1	R	None	Left eye	Loss of vision
2	1997	65 (F)	K1	HR	DM	Meningitis, lumbar spondylitis and diskitis	No neurological deficit
3	1998	55 (M)	K1	HR	DM	Right eye	Limited vision
4	1998	33 (M)	K1	HR	None	Right eye, periorbital cellulitis, septic lung emboli, right orchitis	Loss of vision
5	1999	43 (M)	K1	HR	None	Right eye, meningitis, pneumonia	Loss of vision, impaired higher cortical function
6	2002	73 (F)	K1	S	DM	Left eye	Loss of vision
7	2002	70 (M)	K1	HR	DM, biliary tract stone	Meningitis	Living and well
8	2003	67 (F)	K1	HR	Corticosteroid for ITP	Both eyes	Limited vision
9	2003	58 (F)	K1	HR	DM	Right eye	Preserved vision
10	2003	75 (M)	K1	HR	DM, lung malignant fibrotic tumor, resected	Lumbar spondylitis and diskitis	Paraparesis
11	2003	61 (F)	K1	HR	None	Left eye	Preserved vision
12	2004	46 (F)	K1	HR	None	Left eye	Loss of vision
13	2004	64 (M)	K1	HR	Peptic ulcer	Both eyes	Preserved vision
14	2004	35 (M)	K1	HR	None	Left eye	Loss of vision
15	2004	44 (M)	K1	HR	None	Both eyes, cervical spondylitis	Quadriplegia, loss of vision
16	2005	50 (M)	K1	HR	DM	Meningitis, pneumonia, bacteriuria	Living and well
17	2005	52 (M)	K1	HR	None	Meningitis, cervical spondylitis	Quadriparesis
18	2005	72 (M)	K1	HR	DM	Both eyes, brain abscess	Loss of vision
19	2005	58 (M)	K1	HR	DM	Left cerebellum abscess, left thigh and right leg necrotizing fasciitis	Dysmetria
20	2004	70 (M)	K2	HR	DM, acute leukemia	Right eye	Died
21	2004	63 (F)	K2	R	DM, colonic polyp and submucosal tumor	Meningitis	Hydrocephalus
22	2004	54 (M)	NT	R	DM, history of head injury	Meningitis and brain abscess	Quadriplegia, impaired higher cortical function
23	2005	66 (F)	K54	R	DM, history of head injury, otitis media	Meningitis	Living and well

NOTE. All cases of septic ocular complications were diagnosed and followed up by ophthalmologists. Loss of vision was defined as blindness of the involved eye at the end of follow-up. None of these patients had recorded blurred vision before the episode of *Klebsiella pneumoniae* infection, although there was no baseline vision assessment. There was no other identifiable reason for the loss of vision. DM, diabetes mellitus; HR, highly serum-resistant; ITP, immune thrombocytopenic purpura; NT, not belonging to K1, K2, K5, K20, K54 or K57; R, serum-resistant; S, serum-susceptible.

mellitus and additional predisposing condition(s) (such as acute leukemia, head injury, or chronic otitis media).

Risk factors for septic ocular/CNS complications. Patients infected with K1 strains had a significantly higher risk than did those infected with non-K1 strains for the development of septic ocular or CNS complications (19% vs. 5%; $P = .007$). Logistic regression analyses showed that the K1 genotype was the only significant predictor of septic ocular or CNS complications

Table 4. Genotype distribution of 177 strains associated with pyogenic liver abscess.

Genotype	Type of abscess		Total[c]
	Primary[a] (n = 53)	Secondary[b] (n = 76)	
K1	43 (81)	32 (42)	100 (56)
K2	6 (11)	21 (28)	36 (20)
K54	2 (4)	3 (4)	8 (5)
K5	0 (0)	5 (7)	7 (4)
K20	0 (0)	2 (3)	6 (3)
K57	1 (2)	3 (4)	5 (3)
Other	1 (2)	10 (13)	15 (8)

NOTE. Data are no. (%) of strains.

[a] Cryptogenic cases, culture of liver abscess positive for *Klebsiella pneumoniae*.

[b] Cases with intra-abdominal predisposing factors [32–37]; culture of liver abscess positive for *K. pneumoniae*.

[c] Includes an additional 44 cases with *K. pneumoniae* bacteremia despite negative results of liver abscess culture, 2 hospital-acquired cases, and 2 cases unclassified due to lack of CT study.

(table 6). Its effect remained significant (adjusted OR, 4.8; 95% CI, 1.5–15.7; $P = .009$), even after diabetes mellitus and immunosuppressive therapy were controlled for (table 7). The effect was not significant for the K2 genotype (adjusted OR, 0.8; 95% CI, 0.1–6.9; $P = .821$) or diabetes mellitus (adjusted OR, 1.0; 95% CI, 0.4–2.5; $P = .997$).

Difference in virulence among* K. pneumoniae *strains. The serum resistance assay indicated that virulence was highly variable among *K. pneumoniae* strains that have the same *cps* genotype. Strains of genotype K2, K20, K5, and K54 could be highly resistant or highly susceptible to serum (table 8). On average, K1 strains ($n = 100$) were significantly more resistant to serum from healthy humans than were strains of K2 ($n = 36$), K20/K5/K54 ($n = 21$), or K57/other genotypes ($n = 20$) (group median of 1-h survival ratio, 7.5 vs. 3.2, 2.0, or 0.3, respectively; $P < .001$, by the Mann-Whitney test, for each comparison). There was no significant difference in resistance to serum between K2 strains and K20/K5/K54 strains ($P = .150$). Nevertheless, K2 strains and K20/K5/K54 strains were significantly more serum-resistant than were K57/other strains ($P < .001$ and $P = .002$, respectively).

Genomic background and* rmpA *copy number. The genomic background of K1 strains differed significantly from non-K1 strains. Genotype K1 was strongly associated with the presence of the 20-kb *kfu*/PTS region in the bacterial genome (97 of 100 vs. 13 of 77; $P < .001$). None of the K2, K54, and K57 strains had this region (table 8). *rmpA* was present in nearly all (98%) of the 177 strains tested, and most (88%) had >1 copy. The mean number of *rmpA* copies per strain of K1 strains (2.1 copies) was significantly higher than that of K2 strains (1.9 copies), K20/K5/K54 strains (1.8 copies), or strains of K57/other genotypes (1.6 copies; for each comparison, $P < .05$, by Student's t test; $P < .001$, by 1-way analysis of variance). The mean number of *rmpA* copies was not significantly different among the latter 3 groups ($P = .110$, by 1-way analysis of variance).

DISCUSSION

Twenty-three (13%) of our patients with *K. pneumoniae* pyogenic liver abscess developed septic ocular or CNS complications, and 16 of those patients (70%) experienced long-term disability due to visual or neurological deficits. We found that genotype K1 was the only significant risk factor for this type of septic complication, even after the effects of host immunity (i.e., diabetes mellitus and immunosuppressive therapy) were adjusted for. None of the other bacterial genotypes or underlying conditions were related to this outcome.

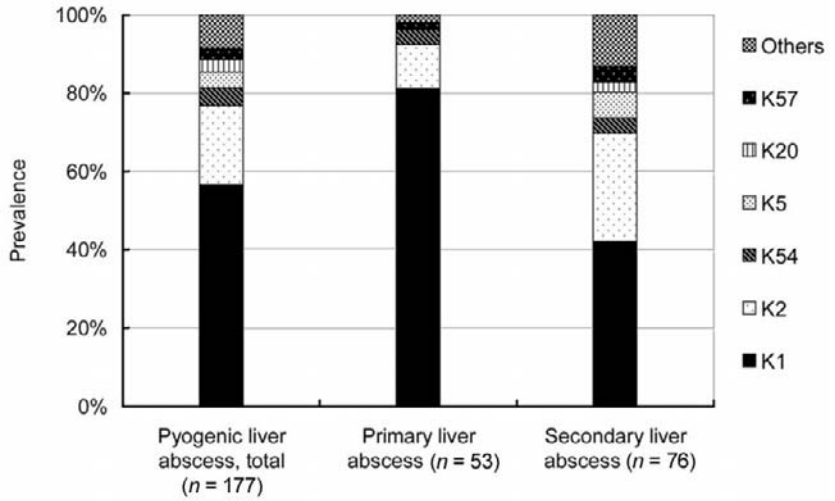

Figure 1. Genotype distribution of *Klebsiella pneumoniae* strains, by type of pyogenic liver abscess

Table 5. Comparison of pyogenic liver abscess in patients infected with *Klebsiella pneumoniae* genotype K1 and non-K1 strains.

Characteristic	K1 (n = 100)	Non-K1 (n = 77)	P
Age, years, median (range)	58 (24–87)	61 (33–92)	.050
Male sex	73 (73)	34 (44)	<.001[a]
Host predisposing factor[b]			
Biliary tract diseases[c]	21 (21)	35 (45)	<.001[a]
Colorectal pathology[d]	3 (3)	6 (8)	.180
History of intra-abdominal surgery[e]	22 (22)	32 (42)	.008[a]
Blunt trauma	0 (0)	1 (1)	.435
Diabetes mellitus	54 (54)	54 (70)	.031[a]
Hemoglobin A_{1c} (%), median (range)	6.3 (4.7–14.5)	7.9 (5.2–15.6)	.026[a]
Alcoholism	1 (1)	0 (0)	1.000
Malignancy[f]	5 (5)	11 (14)	.038[a]
Immunosuppressive therapy[g]	2 (2)	3 (4)	.654
Other[h]	0 (0)	2 (3)	.188
No predisposing factors	32 (32)	4 (5)	<.001[a]
Concomitant pathogens[i]	1 (1)	6 (8)	.044[a]
Septic ocular or CNS complications	19 (19)	4 (5)	.007[a]
Other sites of involvement[j]	7 (7)	8 (10)	.431
Severity of sepsis syndrome[k]			
Sepsis	99 (99)	76 (99)	1.000
Sepsis-related hypotension	27 (27)	22 (29)	.866
Sepsis-related organ dysfunction	15 (15)	16 (21)	.327
Intensive care in ICU	21 (21)	22 (29)	.290
Infection-related mortality[l]	3 (3)	2 (3)	1.000

NOTE. Data are no. (%) of patients, unless otherwise indicated. ICU, intensive care unit.

[a] Statistically significant by Fisher's exact test or the Mann-Whitney test.

[b] Some patients had >1 predisposing factor.

[c] Biliary tract stone (n = 37), cholecystectomy (n = 9), acute cholecystitis (n = 6), gallbladder sludge (n = 4), cholangitis (n = 3), cholangiocarcinoma (n = 3), dilatation of intrahepatic ducts (n = 2), metastatic carcinoma (n = 1), gallbladder cancer (n = 1), hepatocellular carcinoma (n = 1). Some patients had >1 condition.

[d] Colorectal cancer (n = 2), tubulovillous adenoma (n = 2), tubular adenoma (n = 1), ischemic colitis (n = 1), hemorrhagic colitis (n = 1), hemorrhoid ligation complication (n = 1), colonic polyp and submucosal tumor (n = 1).

[e] Appendectomy (n = 24), cholecystectomy (n = 9), abdominal hysterectomy (n = 8), Cesarean section (n = 5), laparotomy for traumatic intestinal perforation (n = 2), right hemicolectomy for ruptured appendicitis (n = 1), total gastrectomy for gastric cancer (n = 1), lower anterior resection for colon cancer (n = 1), operations for ectopic pregnancy (n = 3), endometriosis (n = 1), ureter stone (n = 2), or ectopic pancreas (n = 1). Some patients had >1 condition.

[f] Cholangiocarcinoma (n = 3), colorectal cancer (n = 2), gastric cancer (n = 2), cervical cancer (n = 2), lung malignant fibrous tumor (n = 1), laryngeal cancer (n = 1), gallbladder cancer (n = 1), acute leukemia (n = 1), hepatocellular carcinoma (n = 1), metastatic adenocarcinoma (n = 1), prostate cancer (n = 1).

[g] Corticosteroids for eosinophilic fasciitis (n = 1), immune thrombocytopenic purpura (n = 1), rheumatoid arthritis (n = 1); chemotherapy for acute leukemia (n = 1), and rectal cancer with liver metastasis (n = 1).

[h] Nephrotic syndrome (n = 1), liver cirrhosis (n = 1).

[i] *Aeromonas sobria* and *Enterobacter* species (blood; n = 1), *Escherichia coli* and *Bacteroides fragilis* (blood; n = 1), *E. coli* (liver abscess; n = 1), *Enterococcus* species and *Enterobacter cloacae* (liver abscess; n = 1), *Klebsiella oxytoca* (liver abscess; n = 1), *Mycobacterium tuberculosis* (liver abscess; n = 1), gram-positive cocci (liver abscess, by Gram staining; n = 1). The 6 polymicrobial non-K1 cases were all associated with genotype K2 strains.

[j] Pneumonia (n = 5), bacteriuria (n = 5), septic pulmonary emboli (n = 3), empyema (n = 1), lung abscess (n = 1), orchitis (n = 1), necrotizing fasciitis (n = 1).

[k] Sepsis, sepsis-related hypotension, and organ dysfunction were defined by the American College of Chest Physicians/Society of Critical Care Medicine [48].

[l] Death was considered to be related to *K. pneumoniae* if it occurred before resolution of signs and symptoms or within 14 days from the onset of *K. pneumoniae* infection and there was no evidence for another cause.

Table 6. Univariate logistic regression analysis of risk factors for septic ocular or CNS complications.

Variable	Septic ocular or CNS complications: Yes (n = 23)	Septic ocular or CNS complications: No (n = 154)	OR (95% CI)	P
Age >65 years	7 (30)	47 (31)	1.0 (0.4–2.6)	.993
Male	16 (70)	91 (59)	1.6 (0.6–4.1)	.341
Diabetes mellitus	13 (57)	95 (62)	0.8 (0.3–2.0)	.636
Malignancy	2 (9)	14 (9)	1.0 (0.2–4.5)	.951
Immunosuppressive therapy	2 (9)	3 (2)	4.8 (0.8–30.4)	.096
Third-generation cephalosporin therapy				
Empirical use	17 (74)	95 (62)	1.8 (0.7–4.7)	.261
All use	23 (100)	144 (94)	…	
Klebsiella pneumoniae bacteremia[a]	21 (91)	130 (84)	1.9 (0.4–8.8)	.392
Genotype K1	19 (83)	81 (53)	4.3 (1.4–13.2)	.011[b]
Genotype K2	2 (9)	34 (22)	0.3 (0.1–1.5)	.154
No. of *rmpA* copies[c]	2.1	1.9	2.1 (0.8–5.2)	.109
*rmpA*_KPP020	22 (96)	140 (91)	2.2 (0.3–17.6)	.457
*rmpA*_KPP302	22 (96)	139 (90)	2.4 (0.3–18.9)	.414
*rmpA*_KP3619	5 (22)	20 (13)	1.9 (0.6–5.6)	.267

NOTE. Data are no. (%) of patients, unless otherwise indicated.

[a] When pyogenic liver abscess was initially diagnosed.

[b] Statistically significant.

[c] Mean per strain.

Our clinical finding of an independent pathogenicity for genotype K1 is supported by the serum resistance assay. Because resistance to human serum is a prerequisite for bacterial hematogenous invasion to vital organ systems, a significantly higher serum resistance for genotype K1 supports its higher pathogenicity for septic ocular or CNS complications. It is noteworthy that >40% of the complicated cases (8 of 19 cases) caused by K1 strains were in patients who were free from diabetes mellitus or other underlying medical diseases. This is not a result of underdiagnosis due to lack of data, because we used a computerized data collection form to systemically obtain information about predefined medical conditions. Furthermore, of the nondiabetic patients, all but 1 (who had a normal hemoglobin A_{1c} level of 5.3% and negative result of urine sugar test) underwent blood glucose tests that yielded negative results, indicating a genuine absence of host diabetes. This highlights the need for clinical awareness about the possibility of catastrophic septic ocular or CNS complications from pyogenic liver abscess in previously healthy persons.

The high serum resistance of K1 strains is possibly related to K1 capsular polysaccharides [49], although the mechanism requires further investigation. In addition to the bacterial capsule, the genomic background of genotype K1 strains also differs significantly from that of non-K1 strains regarding the presence of pathogenicity-associated large chromosomal fragments such as the 20-kb *kfu*/PTS region. These findings suggest that *K. pneumoniae* genotype K1 should be considered a distinctive pathogen.

Although *K. pneumoniae* has been the most-common [34, 37, 50] or second–most-common [33, 35, 36] pathogen isolated from pyogenic liver abscess in the United States since the 1970s, septic ocular or CNS complications have appeared to be rare, with only a few case reports [7]. This rarity could be due to a low prevalence of genotype K1 strains, although we cannot exclude the possibility that host susceptibility factors such as HLA type or ethnicity may also be involved. Further studies are required to clarify these issues.

The prevalence of diabetes mellitus was 61% (108 of 177 patients) among our patients with pyogenic liver abscess and 57% (13 of 23 patients) in those with ocular or CNS complications, similar to that reported in other studies from Taiwan [3, 4, 14–17] except that by Fung et al. [10]. Our data do not show diabetes mellitus to be a significant risk factor for septic ocular or CNS complications. This probably indicates that the bacterial virulence of K1 strains alone is sufficient for causing this syndrome, even without the presence of compromised host immunity.

The effect of genotype K2 on the risk of ocular or CNS complications was also nonsignificant, although the lack of statistical significance might be due to a small sample size for this subgroup. Nevertheless, the in vitro serum assay showed a significantly higher serum resistance on average for K1 than K2 strains, indicating that K1 and K2 strains have unequal virulence. Our results raise doubt about the idea that capsular serotype K1 or K2 could be viewed as a major virulence determinant [13]. In the previous study that reported K1 and K2 strains to be "equally more phagocytosis resistant and virulent" (p. 470) than non-K1/K2 strains, the data actually showed K1 strains to be more resistant to phagocytosis than K2 strains, although the difference was statistically insignificant (P = .052) [13]. It should be noted that a lack of statistical significance does not establish equivalence [51], as the study could have been underpowered due to a small sample size (34 K1 vs. 15 K2 strains in the phagocytosis assay; 4 K1 vs. 4 K2 strains in the mice lethality test) [13]. In the current study, we used a larger sample size (100 K1 vs. 36 K2 strains) and demonstrated a significant ($P < .001$) and meaningful difference. Our data

Table 7. Multiple logistic regression analysis of risk factors for developing septic ocular or CNS complications.

Variable	OR (95% CI)	P
Genotype K1	4.8 (1.5–15.7)	.009[a]
Diabetes mellitus	1.0 (0.4–2.5)	.997
Immunosuppressive therapy	7.4 (0.97–56.1)	.053

[a] Statistically significant.

Table 8. Distribution of 20-kb *kfu*/PTS region, *rmpA* copy number, and indicators of virulence among strains of different genotypes.

Factor	Genotype						
	K1 (n = 100)	K2 (n = 36)	K20 (n = 6)	K5 (n = 7)	K54 (n = 8)	K57 (n = 5)	Other (n = 15)
20-kb *kfu*/PTS region	97 (97)	0 (0)	3 (50)	4 (57)	0 (0)	0 (0)	6 (40)
Mean no. of *rmpA* copies per strain	2.1	1.9	2.0	1.7	1.8	2.0	1.5
Hypermucoviscosity	95 (95)	35 (97)	4 (67)	7 (100)	7 (88)	5 (100)	11 (73)
Serum resistance (graded by 1-h survival ratio)							
Highly resistant (>3.0)	89 (89)	21 (58)	3 (50)	3 (43)	2 (25)	0 (0)	0 (0)
Resistant (0.9–3.0)	9 (9)	10 (28)	1 (17)	2 (29)	5 (63)	0 (0)	5 (33)
Susceptible (0.1–0.9)	1 (1)	2 (6)	0 (0)	1 (14)	1 (13)	4 (80)	8 (53)
Highly susceptible (<0.1)	1 (1)	3 (8)	2 (33)	1 (14)	0 (0)	1 (20)	2 (13)
Group median	7.5	3.2	5.3	1.4	1.8	0.2	0.3

NOTE. Data are no. (%) of strains, unless otherwise indicated.

suggest that K1 and K2 strains should be treated separately in future research.

Studies from ⩾10 years ago reported 10%–11% mortality in patients with *K. pneumoniae* pyogenic liver abscess who were treated with drainage of pus and administration of cefazolin plus gentamicin [16]. In the present study, physicians administered pus drainage and more potent third-generation cephalosporins [52] to nearly all patients and achieved a mortality rate of 2.8% (0% in nondiabetic patients). This low mortality is similar to that of 2 recent reports from the United States [50, 53]. Thus, the main concern is no longer mortality, but catastrophic disability due to irreversible ocular or neurological complications. The advance in the *cps* genotyping method, based on PCR detection of *magA* (*wzy*_K1), provides an accurate, rapid, and convenient molecular diagnosis for highly pathogenic K1 strains. This procedure is simple and inexpensive and can be easily replicated in general clinical microbiology laboratories. Although K1 strains can also be identified by capsular K serotyping using countercurrent immunoelectrophoresis, this procedure is relatively complex, requires expensive high-quality antisera, and is available only in a few reference laboratories worldwide [29, 54].

There has been considerable attention given to the increasing frequency of *K. pneumoniae* isolated from pyogenic liver abscess over the past 3 decades in Taiwan and other geographic areas [50–53]. The present study shows that *K. pneumoniae* genotype K1 is an emerging pathogen capable of causing catastrophic septic ocular or CNS complications from pyogenic liver abscess independent of host underlying diseases. Our results highlight the need to further investigate the epidemiology of this virulent organism.

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