

Fecal carriage of serotype K1 *Klebsiella pneumoniae* ST23 strains closely related to liver abscess isolates in Koreans living in Korea

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Abstract We determined the fecal carriage rate of serotype K1 *Klebsiella pneumoniae* in healthy Koreans and studied their genetic relationship with liver abscess isolates. We compared the carriage according to the country of residence. The stool specimens were collected through health promotion programs in Korea. *K. pneumoniae* strains were selected and tested for K1 by PCR. Serotype K1 isolates were characterized by multilocus sequence typing and pulsed field gel electrophoresis. A total of 248 *K. pneumoniae* isolates were obtained from 1,174 Koreans. Serotype K1 was identified in 57 (4.9%), of which 54 (94.7%) were ST 23 and were closely related to the

liver abscess isolates. Participants aged >25 years showed a higher fecal carriage rate than those ≤ 25 ($P=0.007$). The proportion of serotype K1 out of *K. pneumoniae* isolates in foreigners of Korean ethnicity who had lived in other countries was lower compared with those who had lived in Korea (5.6% vs 24.1%, $P=0.024$). A substantial proportion of Koreans >25 years carries serotype K1 *K. pneumoniae* ST23 strains, which are closely related to liver abscess isolates. Differences in carriage rates by country of residence suggests that environmental factors might play an important role in the carriage of this strain.

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Introduction

Klebsiella pneumoniae has become a major pathogen causing liver abscess in Korea and Taiwan over the past two decades [1, 2]. Even though reports on *K. pneumoniae* liver abscess have also been increasing in western countries [3, 4], they are much more common in Asian countries. Furthermore, the serotype K1 strain belonging to sequence type (ST) 23 has been found to be responsible for an increasing incidence of this infection in Korea [5]. Serotypes K1 and K2 have shown the highest virulence in mice out of the 77 serotypes [6], and are more resistant to phagocytosis [7]. Despite its high virulence, however, serotype K1 had been rare in the clinical isolates from western countries [8, 9], although a report from Australia showed that six out of 293 *K. pneumoniae* clinical isolates collected from 2001 to 2002 were serotype K1 [10]. There has been no clear explanation for the geographical difference observed in the epidemiology of *K. pneumoniae* liver abscess.

Various factors including ethnicity, host susceptibility to infection, difference in carriage rates, and environmental factors might be potentially contributing to such a geographical difference in epidemiology of *K. pneumoniae* infection. *K. pneumoniae* can colonize the gastrointestinal tract of humans, and it has been suggested that colonization by the serotype K1 *K. pneumoniae* strains precedes invasion of the intestinal mucosa and portal venous flow or ascending biliary infection, which is followed by the development of liver abscess [11, 12]. However, whether this virulent strain is present in the intestinal microbiota of healthy adults has not been tested yet.

Therefore, in this study the fecal carriage rate of serotype K1 *K. pneumoniae* was investigated in healthy Korean adults. The genetic relationship between the fecal isolates identified in this study and the liver abscess isolates that had been previously reported was examined. In addition, we compared the fecal carriage of this strain among foreigners who are ethnic Koreans according to whether they had lived in Korea or other countries in the previous year in order to understand the roles of ethnic factors and environmental factors in the epidemiology of diseases caused by these strains.

Materials and methods

Study population and bacterial isolates

This study was approved by Samsung Medical Center Institutional Review Board, and met the exemption criteria from the informed consent requirements. The stool specimens from Korean adults were prospectively collected through health promotion programs at 10 university-

affiliated hospitals, which were located nationwide in South Korea in 2007 (one center in 2005). The remaining stool specimens after parasite examination and occult blood test in health promotion programs were used in this study. A total of 65 to 240 stool specimens were collected over 2 to 7 days from each hospital. The subjects were enrolled without any exclusion criteria. Demographic information including age, gender, province of residence, and blood glucose level was extracted from the electronic database of the health promotion programs of the hospitals.

The stool specimens from the foreigners who are ethnic Koreans were collected through a health promotion program for foreigners at Samsung Medical Center from September 2009 to March 2010. The information including nationality, ethnicity, and country of residence in the previous year was collected through the printed questionnaires. For the subjects who did not return the questionnaires and carried *K. pneumoniae* in feces, information was further collected by telephone questionnaires.

Stool specimens were inoculated into the culture media within 24 to 72 h from sample collection. In 2009, we used enrichment media before we inoculated into the selection media. *K. pneumoniae* strains were selected using MC1K (MacConkey-inositol-potassium tellurite) media, as described previously [13] and five colonies were tested from an individual subject. *K. pneumoniae* was identified using the API-20E test kits (bioMérieux, Hazelwood, MO, USA).

Microbiological and genotypic characterization

The serotype K1 was determined by PCR using a primer pair specific for *magA*, which is a gene specific for the K1 antigen. The primers were chosen as previously described: forward, 5'-GGTGCTCTTTACATCATTGC-3', and reverse, 5'-GCAATGGCCATTTGCGTTAG-3' [14].

Multilocus sequence typing (MLST) was performed on the isolates of serotype K1 *K. pneumoniae* by determining the nucleotide sequences of seven housekeeping genes (*gapA*, *infB*, *mdh*, *pgi*, *phoE*, *rpoB*, and *tonB*) as described previously [15]. Sequence types were assigned using the *K. pneumoniae* MLST database (<http://www.pasteur.fr/mlst/>).

For pulsed field gel electrophoresis (PFGE), agarose-embedded bacterial genomic DNA was digested with 20 U of *Xba*I. The restriction fragments were separated by electrophoresis in 0.5× Tris-borate-EDTA buffer. Electrophoresis was performed using a CHEF Mapper XA (BioRad Laboratories, Hercules, CA, USA). The PFGE patterns were analyzed using molecular analysis software (BioRad, Hercules, CA, USA). Eight isolates of serotype K1 *K. pneumoniae* from a previous study on liver abscess [5] and two ATCC reference strains of serotype K1 (ATCC8044 and ATCC35593) were also typed for comparison of the PFGE patterns.

Table 1 Demographic characteristics of Korean adults who were tested for fecal carriage of serotype K1 *K. pneumoniae*

	Number (%)			P value ^b
	<i>K. pneumoniae</i>		No <i>K. pneumoniae</i>	
	Serotype K1	Serotype non-K1		
Number (%)	57 (4.9)	191 (16.3)	926 (78.9)	
Age, median (range)	48 (26–85)	45 (18–83)	47 (16–90)	0.3648
Male gender	30 (52.6)	100 (52.4)	497 (53.7)	0.865
Serum glucose (mg/dl), median (range)	88 (68–167)	85 (49–142)	90 (61–285)	0.4540
Province of residence ^a				0.129
Seoul	17 (4.5)	80 (21.2)	280 (74.3)	
Taegu	17 (5.7)	40 (13.3)	243 (81.0)	
Gyeonggi	9 (6.7)	38 (28.1)	88 (65.2)	
Jeju	2 (2.0)	11 (11.0)	87 (87.0)	
Jeolla	1 (1.0)	11 (11.3)	85 (87.6)	
Busan	8 (8.0)	7 (7.0)	85 (85.0)	
Chungcheong	3 (4.6)	4 (6.2)	58 (89.2)	

^a Expressed as number of strains in %/ number of all the strains isolated in the province

^b Compared between group of serotype K1 and group of no *K. pneumoniae*

Statistical analyses

Continuous variables with non-normal distribution were compared using rank sum test. Categorical variables were compared using the Chi-squared test or Fisher's exact test. A two-tailed *P* value of <0.05 was considered to be statistically significant. Stata software (version 11.1, StataCorp, College Station, TS, USA) was used for statistical analysis.

Results

Fecal carriage of serotype K1 *K. pneumoniae* among Koreans

A total of 1,174 stool specimens were collected from 10 hospitals and screened for the presence of *K. pneumoniae*. *K. pneumoniae* was found in 248 (21.1%) and serotype K1 *K. pneumoniae* was identified in 57 (4.9%), which was 23.0% of *K. pneumoniae* isolates. There was no difference in age, gender, province of residence, and serum glucose level between the group carrying serotype K1 *K. pneumoniae* and the group carrying no *K. pneumoniae* (Table 1). Median age of the subjects carrying serotype K1 *K. pneumoniae* was 48 years (range 26–85), and 52.6% were male. The fecal carriage rates of serotype K1 *K. pneumoniae* varied from 1.0% to 8.0% according to the province of residence, although there was no significant difference. Serotype K1 was not found in 27 *K. pneumoniae* isolates from the feces of 104 Koreans in the age range of 16 to 25 years (0/104); however, 5.6% (57/

1,013) of the subjects older than 25 years carried serotype K1 *K. pneumoniae* (*P*=0.007; Fig. 1).

Genotypic characterization of serotype K1 *K. pneumoniae* fecal isolates from Koreans

The MLST revealed that 54 (94.7%) of the isolates with serotype K1 belonged to ST23. Two isolates were ST260, double-locus variants (DLV) of ST23. The other isolate, ST424, was totally unrelated. The PFGE patterns of the serotype K1 *K. pneumoniae* isolates from the feces of healthy Korean adults, 8 isolates from liver abscess, and 2 ATCC strains, which are ST82, were analyzed (Fig. 2). Most isolates of ST23 were clustered into a single group with >69% similarity regardless of whether they were fecal isolates from healthy subjects or

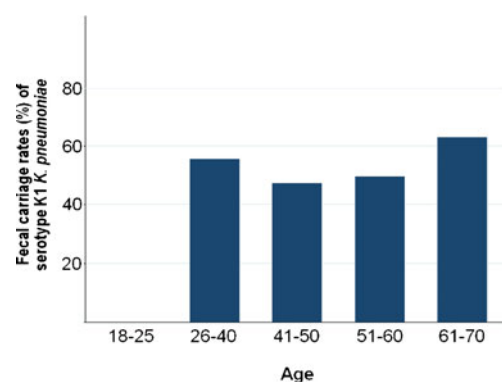


Fig. 1 Distribution of fecal carriage rates of serotype K1 *K. pneumoniae* among Koreans according to age

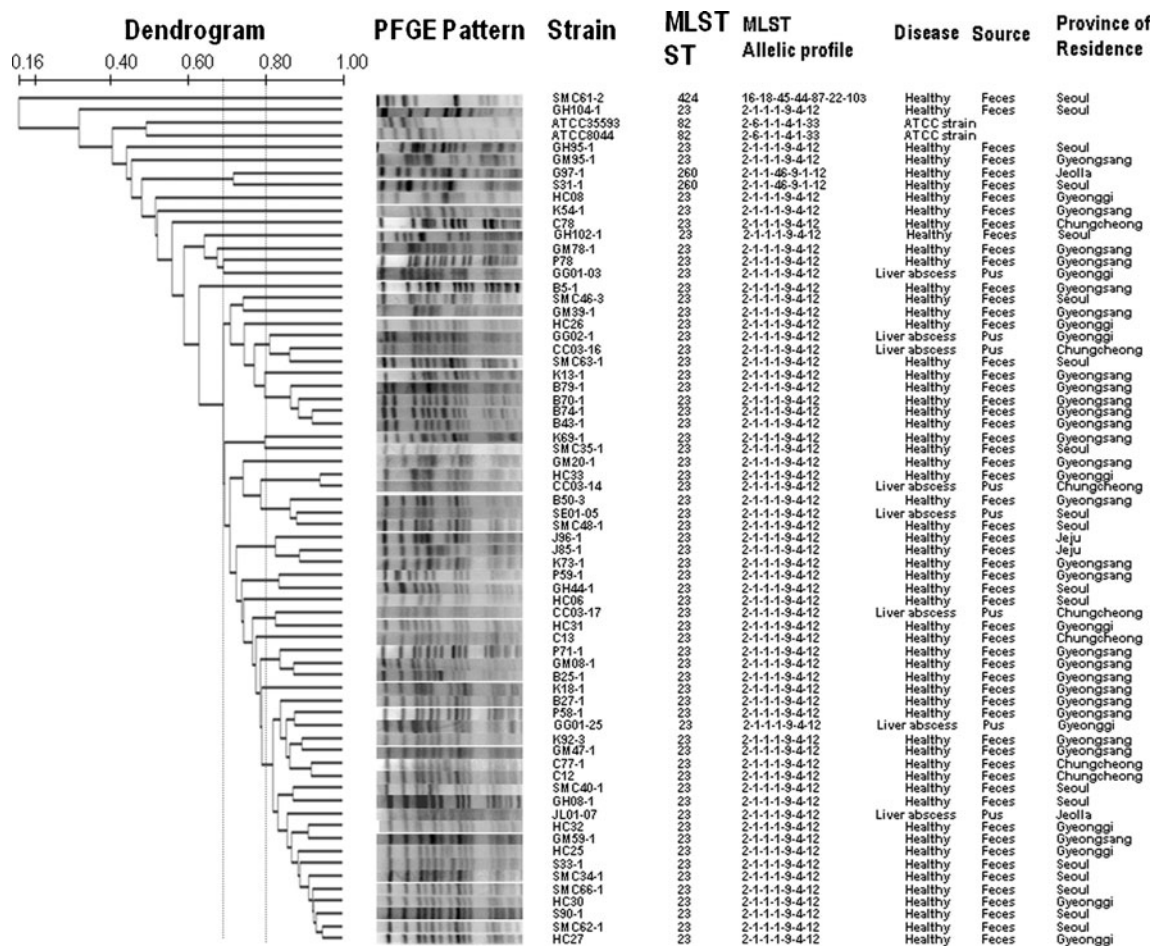


Fig. 2 Dendrogram of PFGE profiles and MLST sequence types of the serotype K1 *K. pneumoniae* fecal isolates from Koreans and liver abscess isolates

liver abscess isolates. A large clonal group and small groups were clustered with >80% similarity.

Comparison of fecal carriage of serotype K1 *K. pneumoniae* in foreigners who are ethnic Koreans by country of residence

A total of 130 (48.3%) isolates of *K. pneumoniae* were isolated from 269 foreigners who are ethnic Koreans. For 94 isolates with the information of the place of residence in the previous year, the proportions of serotype K1 *K. pneumoniae* were compared according to whether they had lived in Korea or not in the previous year (Table 2). The countries in which they had lived in the previous year include Korea (61.7%), USA (26.6%), Canada (8.5%), Australia (1.1%), England (1.1%), and Germany (1.1%). There was no difference in age and gender between the groups. Serotype K1 accounted for only 5.6% (2 out of 36) of *K. pneumoniae* fecal isolates from the subjects who had been resident in countries other than Korea, whereas this comprised 24.1% (14 out of 58) in the subjects who had been resident in Korea ($P=0.024$). Fifteen out of 16 isolates

of serotype K1 *K. pneumoniae* were ST23, and the other one was ST249. Both of the isolates of serotype K1 from subjects who had been resident in the other countries were ST23. The countries of residence were the USA and Canada respectively.

Table 2 Fecal carriage of serotype K1 *K. pneumoniae* among the foreigners who are ethnic Koreans according to their country of residence in the previous year

	Number (%)		P value
	Resident in other country (n=36)	Resident in Korea (n=58)	
Age in years, median (range)	53.5 (29–71)	51.5 (23–82)	0.5938
Male gender	14 (38.9)	30 (51.7)	0.225
Proportion of serotype K1 among <i>K. pneumoniae</i> fecal isolates	2 (5.6)	14 (24.1)	0.024

Discussion

The results of this study showed that a substantial proportion of healthy Korean adults carried serotype K1*K. pneumoniae* ST23, which is a major etiological organism of liver abscess in Korea, as intestinal flora. Fecal carriage rate of serotype K1*K. pneumoniae* in Korean adults older than 25 was 5.3%; most of them were ST23 and were closely related genotypically. Furthermore, they were closely related to the liver abscess isolates previously reported. The finding that younger adults below the age of 25 had a lower rate of fecal carriage of serotype K1*K. pneumoniae* suggests the possibility that these strains are acquired after the age of 25 in Korea through unknown external sources. Interestingly, such age distribution seen in intestinal colonization by serotype K1*K. pneumoniae* in our study is consistent with that in *K. pneumoniae* liver abscess. The mean age of the patients with *K. pneumoniae* liver abscess in a previous nationwide prospective study in Korea was 60 years (SD 13), and only one of the 262 patients was younger than 25 [1].

A much lower proportion of the foreigners of Korean ethnicity who had lived in the countries other than Korea in the previous year carried serotype K1*K. pneumoniae* strains, whereas foreigners of Korean ethnicity who had lived in Korea carried serotype K1*K. pneumoniae* in a proportion comparable to that of native Koreans. These findings suggest a potential role of environmental factors in the intestinal colonization of these strains. Possible environmental sources for acquisition of these strains might include diets such as meats, uncooked plants, and fermented foods. In fact, it has been reported that *K. pneumoniae* is ubiquitous in nature and the isolates of environmental origin are identical to clinical isolates with respect to phenotypic properties and pathogenic potentials to humans [16, 17].

In addition to the probable role of environmental factors, virulence factors of serotype K1*K. pneumoniae* strains might contribute to favorable colonization of these strains in human intestinal tracts. Capsular polysaccharides have been reported to play an important role in gut colonization by *K. pneumoniae* [18] as well as in the pathogenesis of invasive infections caused by this organism. Furthermore, serotypes K1 and K2 have been reported to be highly resistant to phagocytosis [7]. These factors might contribute the serotype K1*K. pneumoniae* ST23 strain to be adapted to colonize the human intestinal tract. Other factors including socioeconomic status might affect the difference in colonization of these strains.

Our study has some limitations. First, in comparative analysis for the foreigners who are ethnic Koreans, we presented the proportion of serotype K1 among *K. pneumoniae* fecal isolates rather than the true fecal carriage rate of

this strain. Only those persons who were determined to carry *K. pneumoniae* in their feces were further contacted by telephone to obtain the answers to questionnaires, so the fecal carriage rate of *K. pneumoniae* could be falsely high in those about whom we have information concerning their place of residence in the previous year. Second, the time period of enrollment was different between the two populations compared, and it could make a bias in comparison. Third, to strengthen our suggestion that environmental factors are important for the intestinal colonization of serotype K1*K. pneumoniae* rather than ethnic factors, a study on the foreigners of white ethnicity who had lived for a long time in Korea would also be of value; however, we could not include those in our study. Fourth, information on underlying diseases or conditions, such as diabetes mellitus or antibiotic exposure, was not collected. Those factors could affect the results of our study.

This is the first report to document fecal carriage of the serotype K1*K. pneumoniae* ST23 in healthy adults living in the region with a high prevalence rate of *K. pneumoniae* liver abscess, and to demonstrate that serotype K1*K. pneumoniae* isolates from intestinal carriers and liver abscess patients are closely related genotypically. Additionally, our findings suggest that environmental factors might play an important role in the intestinal colonization of these strains in Korean ethnicity. These findings may partially explain the geographical difference in the epidemiology of *K. pneumoniae* liver abscess.

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Potential conflict of interest None of the authors had any conflicts.

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