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Klebsiella pneumoniae liver abscess in Taiwan is not caused by a clonal spread strain

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In Taiwan, the incidence of pyogenic liver abscess caused by *Klebsiella pneumoniae* has been increasing over the past 2 decades. Although most of the patients have no concurrent biliary tract disease, diabetes mellitus is thought to be an important risk factor for the disease. The incidence of metastatic infections in *K. pneumoniae* liver abscess, such as endogenous endophthalmitis and other extrahepatic infections, is also higher than that in liver abscess caused by other microbes. Furthermore, the incidence of metastatic infections in *K. pneumoniae* liver abscess in Taiwan is higher than Western countries. The reasons why *K. pneumoniae* liver abscess is so common in Taiwan and why diabetes mellitus is a risk factor for the disease are not clear. In this study, blood isolates from 40 patients with *K. pneumoniae* liver abscess treated at the Taipei Veterans General Hospital from 1995 through 2000 were randomly selected for study. Pulsed-field gel electrophoresis, ribotyping, and serotyping were used for cluster analysis. A total of 15 strains were of serotype K1 and 25 strains were of a serotype other than K1. No major cluster or a closely related strain of *K. pneumoniae* was found. In conclusion, the results obtained from pulse-field gel electrophoresis and ribotyping of *K. pneumoniae* isolates do not suggest that liver abscess in Taiwan is primarily caused by a single genetically related strain.

Key words: *Klebsiella pneumoniae*, liver abscess, pulsed-field gel electrophoresis, ribotyping

Over the past 20 years in Taiwan, the reported incidence of pyogenic liver abscess caused by *Klebsiella pneumoniae* ranged from 50% to 88% [1-3]. Diabetes mellitus is suggested to be an important risk factor, while intraabdominal infection and biliary tract infection are not predisposing factors [1-3]. Metastatic infection is a characteristic feature of *K. pneumoniae* in Taiwan [1-6]. The demographic characteristics, major pathogenic microbes, and complications are different from those of Western countries [7-11]. The *K. pneumoniae* strains causing liver abscess in Taiwan are community-acquired and susceptible to all cephalosporins and aminoglycosides [1,6,12].

Because of the similar clinical presentations and the same antimicrobial susceptibility pattern, a clonal spread of a specific strain of *K. pneumoniae* has been suspected. Previously, a major cluster of *K. pneumoniae* isolates with a high genetic similarity as shown by pulsed-field gel electrophoresis (PFGE) was reported in liver abscess patients in central Taiwan [13]. However, a study in northern Taiwan found that the endemic nature of liver abscess was not due to any single strain or small groups of strains [12]. This study examined the characteristics of isolates from patients with *K. pneumoniae* liver abscess at the Taipei Veterans General Hospital from 1995 through 2000. Pulsed-field gel electrophoresis, ribotyping, and serotyping were used for cluster analysis.

Materials and Method

Bacterial isolates

K. pneumoniae strains were isolated from blood samples of patients with *K. pneumoniae* liver abscess treated at the Taipei Veterans General Hospital. *K. pneumoniae* liver abscess was diagnosed if a liver abscess was seen on imaging studies (abdominal ultrasonography and/or computerized tomography), or detected during laparotomy; only *K. pneumoniae* was isolated from the

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blood or abscess aspirate; and an amebic serologic test was negative. We randomly selected 40 blood isolates from patients with *K. pneumoniae* liver abscess treated from 1995 through 2000. All patients had community-acquired disease.

Serotyping

All isolates were serotyped by a countercurrent immunoelectrophoresis method. Antisera were provided by the Laboratory of Hospital Infection, Central Public Health Laboratory, London. *K. pneumoniae* ATCC9997 (K2) was used as a control strain.

Ribotyping

Ribotyping was performed using the automated Riboprinter Microbial Characterization System (Qualicon, Wilmington, DE, US) according to the manufacturer's instructions. Colonies were picked and loaded into the Riboprinter microbial characterization unit (MCU). Within the MCU, total DNA was digested with *Eco*RI enzyme, separated by electrophoresis, and transferred directly to nylon membranes. Ribopatterns were expressed by hybridization with a chemiluminescence-labeled DNA-probe containing an rRNA operon (rrnB) from *E. coli*. The patterns were automatically imaged and stored in the MCU computer. The positions of standard markers were used to correct for both lane-to-lane and membrane-to-membrane variations in band position. The ribopattern for each isolate was compared with other patterns in the Riboprinter database. Assignment to a particular ribogroup was based upon differences in band numbers, band position, and signal intensity at a given banding position [14].

Pulsed-field gel electrophoresis

Total DNA was prepared and PFGE was performed as described in the literature [15]. The restriction enzyme *Xba*I (New England Biolabs, Beverly, MA, US) was used. Restriction fragments were separated by PFGE in 1% agarose gels (Bio-Rad, Hercules, CA, US) in 0.5x Tris-Boric acid-EDTA (TBE) buffer (45 mM Tris, 45 mM boric acid, 1.0 mM EDTA, pH 8) using a Bio-Rad CHEF-Mapper apparatus (Bio-Rad Laboratories, Richmond, CA, US). Gels were stained with ethidium bromide and photographed under ultraviolet light. Band patterns were visually compared and classified as indistinguishable (clonal), closely related (clonal variants, ≥3 band differences), possibly related (4-6 band differences), and unrelated (>6 bands differences) according to previously described criteria [16].

Results

Among the 40 isolates from bacteremic patients with liver abscess, 15 strains belonged to serotype K1; 5 to K16; 3 each to K2 and K21; 2 each to K5, K28, and K57; and 1 each to K3, K8, K9, K29, K32, K38, K39, and K54. Further subtyping using PFGE and ribotyping revealed that among the 15 isolates belonging to K1, only a few individual pairs had less than 3 band differences. The dendrogram of the PFGE pattern for the 15 isolates is illustrated in Figure 1. For isolates with various serotypes, a high degree of genetic polymorphism was observed in PFGE. The dendrogram

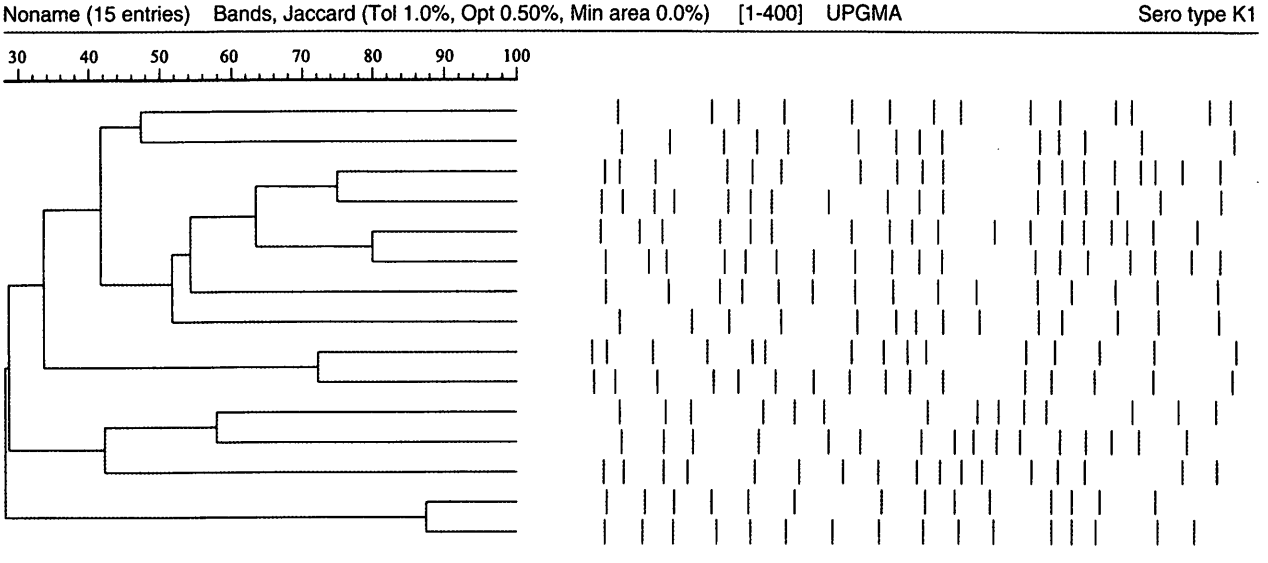

Fig. 1. Dendrogram of pulsed-field gel electrophoresis patterns for the 15 isolates of *K. pneumoniae* serotyped as K1.

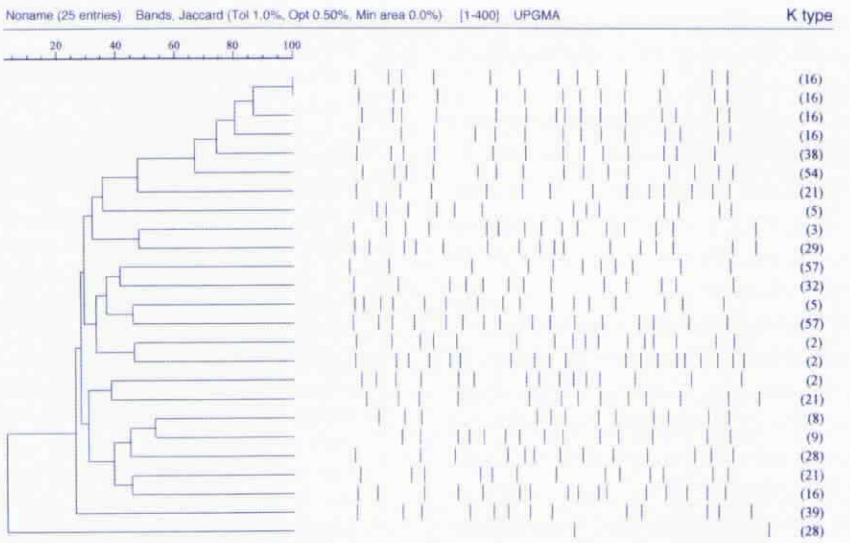

Fig. 2. Dendrogram of pulsed-field gel electrophoresis pattern for the 25 non-K1 *K. pneumoniae* isolates.

of another 25 isolates is shown in Figure 2. Figures 1 and 2 clearly demonstrate different PFGE patterns for these *K. pneumoniae* isolates. Even among those isolates with the same serotype K1, no major cluster of strains could be found.

Figure 3 shows the results of ribotyping for the 15 isolates identified as serotype K1. Similarly, only a few of these isolates had the same ribotype and no major cluster was identified. Figure 4 shows the results of ribotyping for another 25 isolates, again with no genetically related strains being found.

Discussion

Pyogenic liver abscess in Taiwan in the past 20 years has been reported to be caused by a single organism, *K. pneumoniae*, in 50% to 80% of cases [1-3]. Lau *et al* [13] used PFGE to identify a major cluster of *K. pneumoniae* isolates from patients with liver abscess in central Taiwan. They typed 96 isolates from patients with *K. pneumoniae* liver abscess and demonstrated the presence of pathogenic clones of *K. pneumoniae* in 62.5% of isolates. Thus, they postulated that *K. pneumoniae* liver abscess may be due to the spread of a single clone or closely related strains.

Chang *et al* [12] used PFGE to analyze the molecular typing of 51 *K. pneumoniae* strains from patients with community-acquired liver abscess. They concluded that the endemic status of *K. pneumoniae* liver abscess in Taiwan was not caused by a few particular strains, but by many genetically distinct

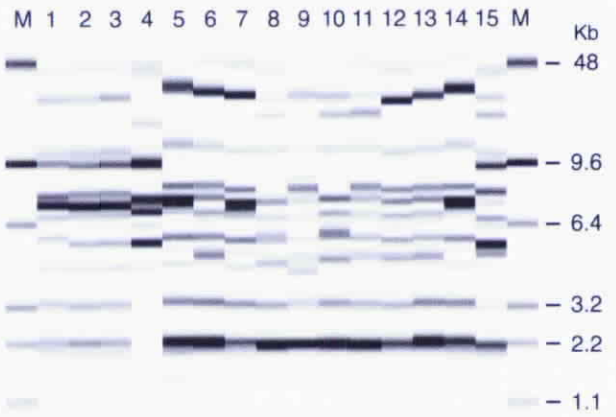

Fig. 3. Ribotyping for the 15 isolates of *K. pneumoniae* serotyped as K1.

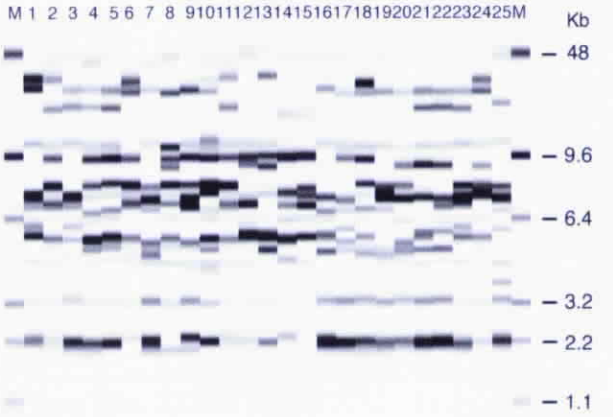

Fig. 4. Ribotyping for the 25 non-K1 *K. pneumoniae* isolates.

strains instead. The overall similarity of isolates on PFGE and ribotyping in this study indicates that these isolates are non-clonally related.

In this study, 2 molecular typing methods were used to analyze the genetic relationship of the bacteremic *K. pneumoniae* strains isolated from patients with liver abscess. Neither ribotyping nor PFGE showed any clonal spread among *K. pneumoniae* strains even for isolates with the same serotype.

Pulsed-field gel electrophoresis is a useful method for analysis of potential outbreaks spanning relatively short periods [16]. Its patterns will not change if the genetic events are relatively minor, and the technique provides a simple overview of the population structure of *K. pneumoniae*. The reason for the discrepancy in the molecular epidemiological pattern of *K. pneumoniae* strains in liver abscess between this study and that of Lau *et al* [13], despite the fact that both studies used PFGE method, remains unclear. It is possible that there might be a small spread of similar strains of *K. pneumoniae* in central Taiwan during that period. However, it cannot be attributed to the whole molecular epidemiology of *K. pneumoniae* in liver abscess in Taiwan, according to this study and that of Chang *et al* [12].

Seroepidemiologic surveys of *K. pneumoniae* isolated from community-acquired infection in Taiwan showed a high prevalence of capsular serotype K1 [17,18]. Chang *et al* [12] found no genetically related strains in a study of isolates from 51 patients with community-acquired liver abscess. Also, a single genetically related strain does not exist in this study of isolates from 40 randomly selected patients. Thus, close contact and clonal spread do not appear to be the major pathogenic factors to *K. pneumoniae* liver abscess in Taiwan. The reason for the high prevalence of *K. pneumoniae* liver abscess, especially in diabetic patients, is still unknown. Environmental or host factors resulting in a special invasiveness for liver may be involved.

In conclusion, this study showed that *K. pneumoniae* liver abscess in Taiwan is not caused by a single strain or genetically related clone. Environmental or host factors might have important roles in the pathogenesis of the disease and may explain its differences to that observed from Western countries. Further study of the pathogenesis of *K. pneumoniae* liver abscess is required.

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