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Full Length Research Paper

Study on capsular serotype of *Klebsiella pneumoniae* from patients with liver abscesses and its clinical characteristics

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Primary pyogenic liver abscess caused by *Klebsiella pneumoniae* has recently become an emerging disease, which is often complicated by septic meningitis and endophthalmitis. *K. pneumoniae* is a significant cause of liver abscess. The invasive *K. pneumoniae* infections can attack healthy individuals, and some of its patient has a predisposing condition such as diabetes mellitus. According to these capsular polysaccharides, *K. pneumoniae* can be classified into 77 serological K antigen types. To date, K serotype distribution of *K. pneumoniae* causing liver abscess is little known in Northeast of China. In our study, we performed polymerase chain reaction (PCR)-based genotyping to identify capsular serotypes, and present the current epidemiologic status of *K. pneumoniae* liver abscess in local area. Among the *K. pneumoniae* from 35 patients with liver abscess, we found 20 (57.1%) isolates of K1 serotype. Clinical characteristics [such as, age, sex, diabetes mellitus, Liver and biliary disease history, extended-spectrum β-lactamases (ESBLs) producing and etc.] of patients with liver abscess of *K. pneumoniae* belonging to K1 or non-K1 serotype were also analyzed, but no significant difference was found. There are only three isolates of *K. pneumoniae* with ESBLs. In conclusion, *K. pneumoniae* K1 serotype is the major serotype causing liver abscess in local region.

Key words: Capsular serotype, liver abscess, *Klebsiella pneumonia.*

INTRODUCTION

Klebsiella pneumoniae is a common Gram-negative pathogen causing both community and nosocomial infections (Podschun and Ullmann, 1998) that cause significant morbidity and mortality. Bacteremia, meningitis, and respiratory and urinary tract infections (Podschun and Ullmann, 1998) are frequently reported. Additionally, *K. pneumoniae* is a significant cause of liver abscess (Cheng et al., 1991), which have been reported in Taiwan, Japan, Europe, North America, Korea, Singapore and Argentina (Cheng et al., 1991; Wang et al., 1998; Ko et al., 2002; Ohmori et al., 2002; Okano et al., 2002; Fang et al., 2004; Rahimian et al., 2004; Chung et al., 2007; Vila et al., 2011; Abate et al., 2012). Several other reports have also observed that this pathogen had become the predominant cause of liver abscess instead of the previously described *Escherichia coli*, Streptococci, anaerobic bacteria and amoebae (Yeoh et al., 1997; Ohmori et al., 2002; Lederman and Crum, 2005). The invasive *K. pneumoniae* infections can attack healthy individuals, and some of its patient has a predisposing condition such as diabetes mellitus (Fang et al., 2004).

K. pneumoniae is enveloped by a polysaccharide capsule that is considered to be a major pathogenicity factor for the species. According to these capsular polysaccharides, *K. pneumoniae* can be classified into 77 serological K antigen types. In Asia, the K1 and K2 serotypes were found to be the most prevalent capsular serotypes in *K. pneumoniae* liver abscess (Wang et al.,

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1998; Fung et al., 2002; Yu et al., 2007), and it has great potential to cause metastasis (Fang et al., 2007; Brisse et al., 2009), this contrast markedly with published surveys from North America and Europe, in which K1 strains were found rare (Blanchette and Rubin, 1980; Smith et al., 1982; Cryz et al., 1986; Thompson et al., 1993). Thus, different region and period would show different distribution of K serotype. Moreover, serotype K1 is the major virulence determinant for *K. pneumoniae* liver abscess (Podschun and Ullmann, 1998; Yeh et al., 2007) either in animals or in humans since they are highly resistant to phagocytosis. In a study in mainland China, Luo (1990) found that serotypes K1 and K33 were the most common serotypes, but were mostly from sputum (73%). Since there were few studies about serotypes of *K. pneumoniae* isolated from liver abscess in China, whether these epidemics reflected local variation or not, are yet to be clarified.

In our study, we performed PCR-based genotyping to identify capsular serotypes (Pan et al., 2008) and present the current epidemiologic status of *K. pneumoniae* liver abscess in local area, and analyze the impact factors of the clinical characteristics of *K. pneumoniae* liver abscess patients by K1 and non-K1 serotypes.

MATERIALS AND METHODS

Study population and bacterial isolates

We examined 35 *K. pneumoniae* clinical isolates from liver abscess patients during February 2009~November 2011 at affiliated First Hospital of China Medical University, a 2500-bed hospital in northeastern China. All isolates were identified by the VITEK 2 system (Bio-Merieux, France) or API 20E system (Bio-Merieux, France). Infections were considered to be community-acquired if *K. pneumoniae* was isolated from cultures within 48 h of admission and hospital acquired if organism was isolated over 48 h of admission. To avoid duplication, only 1 isolate from each patient was chosen from each episode of infection. All isolates were stored in brain heart infusion broth at -70°C until use.

Capsular antigen typing

The serotype K1 was determined by PCR using a primer pair specific for wzy_{KpK1} (*K. pneumoniae* wzy-K1 gene for serotype K1 polymerase), which is previously known as *magA* (mucoviscosity-associated gene A). Since it is a gene specific for the K1 antigen, it has been used as a molecular marker for K1 serotype in *K. pneumoniae* (Yeh et al., 2010). The primers were chosen as previously described: forward, 5' -GGTGCTCTTTACATCATTGC-3', and reverse, 5'-GCAATGGCCATTTGCGTTAG-3' (Fang et al., 2007). For the genotyping procedure, we extracted genomic DNA from the tested strains as templates. Initial denaturation at 94°C for 5 min was followed by denaturation at 94°C for 30 s, annealing at 51°C for 30 s, and extension at 72°C for 30 s and 30 cycles. There was a final extension of 7 min at 72°C.

Sequences

Fragments were also sequenced for confirmation. Amplified DNA was purified using the Ultra clean PCR Clean-up kit (Biospin). Sequencing was performed in a thermal cycler with the use of the dRhodamine Dye Terminator Cycle sequencing Ready Reaction Kit (Applied Biosystems), which is the same primers as for the PCR, and 2~4 μL of the purified DNA.

Statistical analysis

Categorical variables were compared using the Fisher's exact test. A two-tailed P-value <0.05 was considered to be statistically significant. Statistical analyses were performed using SPSS software (version 13).

RESULTS

Bacterial strains and serotyping

Of the 35 isolates, 33 (94.3%) were from community-acquired infections, and only 2 (5.7%) were from hospital-acquired infections. 9 (25.7%) isolates were from blood specimens, 17 (48.6) from the liver abscess pus and 9 (25.7) were from liver draining of postoperation.

K1 serotypes were found predominantly in patients with liver abscess [20 (57.1%) patients infected with K1 serotype compared with 15 (42.9%) patients with non-K1 serotype]. Isolate serotype was verified by sequence and electrophoretic identification of the PCR amplified wzy_{KpK1} gene (Figure 1.)

Clinical characteristic of *K. pneumoniae* liver abscess patients

Clinical characteristics of these patients with liver abscess are presented in Table 1. There are trending that K1 serotype was more common in male patients [65% versus 40%; odds ratio (OR) 2.79, 95% confidence interval (CI) 0.7 to 11.1] and a lower percentage of patients infected with K1 serotype had diabetes mellitus (55% versus 80%; OR 0.31, 95% CI 0.07 to 1.43), although there were no significant differences between them. No differences were found in age, liver and biliary disease history, ESBLs and community-acquired for the patients. We also analyzed factors like, patients that have had operation, those that are admitted in the hospital, the use of antibiotic, and those that had drainage in the past three month, no significant difference were found.

Out of 35 isolates *K. pneumoniae*, only three were ESBLs producer, which account for 8.6%. In the past three month only, 13 (37.1%) patients have used antibiotic, which include penicillin, first or second generation cephalosporins.

DISCUSSION

K. pneumoniae is a Gram-negative pathogen that causes a variety of infections. Primary pyogenic liver abscess

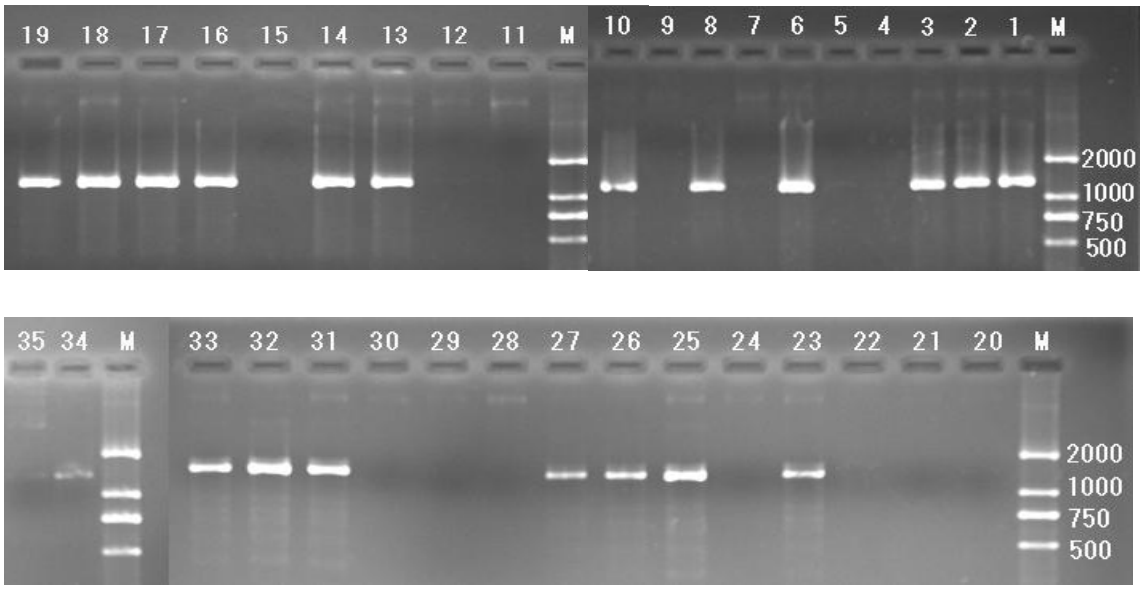

Figure 1. Electrophoresis patterns of *magA*. All isolates of the patients are shown in the figure and numbered according to the patients (*magA* is 1283 bp).

Table 1. Clinical characteristics of 35 patients with *K. pneumoniae* liver abscess caused by K1 and non-K1 serotypes.

Characteristic	K1 serotype (n=20) (%)	Non-K1 (n=15) (%)	OR (95% CI)	P value
Age, years, median (range)	58 (15 to 88)	57(37 to 79)		0.914
Male	13 (65)	6 (40)	2.79 (0.7 to 11.1)	0.182
Diabetes mellitus	11 (55)	12 (80)	0.31 (0.07 to 1.43)	0.163
Liver and biliary disease history	4 (20)	3 (20)	1 (0.19 to 5.33)	1
ESBLs producer	1 (5)	2 (13.3)	0.34 (0.03 to 4.18)	0.565
Community-acquired	19 (95)	14 (93.3)	1.36 (0.08 to 23.61)	1
last 3 month				
Operation	1 (5)	1 (6.7)	0.74 (0.04 to 12.82)	1
In hospital	1 (5)	1 (6.7)	0.74 (0.04 to 12.82)	1
Antibiotic using	7 (35)	6 (40)	0.81 (0.2 to 3.22)	1
Drainage	18 (90)	13 (86.7)	1.48 (0.60 to 3.65)	1

Data are numbers (%) of patients; OR, odds ratio; CI, confidence interval; ESBLs, extended-spectrum β-lactamases.

caused by community-acquired *K. pneumoniae* has recently become an emerging disease. Many cases of *K. pneumoniae* liver abscess with septic metastatic infection have been reported in Taiwan, but only few cases have been reported in China, South America, North America and Europe (Cheng et al., 1991; Chang et al., 2000; Fang et al., 2005; Lee et al., 2008; Vila et al., 2011), Dispite advances in intensive care medicine, liver abscess is still a catastrophic illness with a high morbidity and mortality rate (Chen et al., 2008).

The important capsular serotype and *magA* of *K. pneumoniae* in virulence and phagocytosis resistance have been reported (Simoons-Smit et al., 1984; Fang et al., 2004; Lin et al., 2004). Initially, the *magA* was provisionally named because it was associated to form a capsular-associated mucopoly-saccharide web, and increase resistance to phagocytosis (Fang et al., 2004). Subsequently, *magA* has been confirmed to be located in capsular polysaccharide synthesis (*cps*) gene cluster of serotype K1 isolates (Chuang et al., 2006; Yeh et al., 2006). Furthermore, experiments have showed that *magA* is the serotype K1 allele of the polymerase gene wzy in the *cps* gene cluster and that it is responsible for capsular serotype K1 (Fang et al., 2007), and so *magA* was designated as wzy_{KpK1} (Yeh et al., 2010), which has been defined as "*Klebsiella pneumoniae wzy_K1* gene for serotype K1 polymerase" (Fang et al., 2010). So that all wzy_{KpK1} (*magA*)-positive strains were capsular serotype

K1 (Chuang et al., 2006). A previous survey on the serotype of 293 *K. pneumoniae* isolates (Jenney et al., 2006) reported that 88 isolates (30%) were nontypeable by counter current immunoelectrophoresis (Palfreyman, 1978), while 54 isolates had a positive reaction for more than one serotype. Therefore, *cps* PCR genotyping seems to be a more sensitive and specific way for detecting this serotype (Fang et al., 2007; Pan et al., 2008). Thus, we determined our strains to be serotype K1 by investigating only the genotype, but not the serotype of *K. pneumoniae*.

Our study examined the K serotype of *K. pneumoniae* from 35 patients with liver abscess by PCR and found 57.1% K1 serotype of *K. pneumoniae*. The result indicated that K1 is the major serotype of *K. pneumoniae* causing liver abscess in local region. Our data are similar with Seoul in Korea (53%) (Chung et al., 2007) and Taiwan (54.5%) (Yeh et al., 2007), which were different from Europe and North American (Cryz et al., 1986; Thompson et al., 1993).

Pyogenic liver abscess caused by *K. pneumoniae* serotype K and its catastrophic consequence are little known. K1 serotyping was found in high frequency in patients with liver abscess in most area of Asia and the difference in characteristics of patients infected with K1 serotype and non-K1 serotype have not been cleared. In our study, clinical characteristics of patients with liver abscess of *K. pneumoniae* belong to K1 or non-K1 serotype were also analyzed, which included factors of age, sex, diabetes mellitus, Liver and biliary disease history, ESBLs production and so on. We found the trending that the male patients are more prone to K1 serotype of *K. pneumoniae* and have a lower prevalence of diabetes mellitus than those with non-K1 serotype, despite the fact that no significant difference was found. Further detailed study on serotype and adding factors of investigation and survey on quantity of patients would contribute to finding significant factors.

Susceptibility test for the strains isolated from our patients indicated good susceptibility to antimicrobial drugs. Only 3 *K. pneumoniae* with ESBLs cases were found. This was associated with most patients who have not used antibiotic recently or adopted narrow-spectrum antibiotics. Moreover, most of patients (94.3%) were community-acquired infection, thus the rate of ESBLs producing were lower than that from hospital-acquired infection (41.6%, data not shown). However, as ESBLs producing *K. pneumoniae* is increasing, an appropriate antimicrobial drug should be choosing according to the situation.

At present, *K. pneumoniae* serotype does not affect the choice of treatment, but it is important to investigate the *K. pneumoniae* serotype because in the future, treatment may be based on the serotype. Recently, monoclonal protected antibodies against *magA* (*wzy*$_{KpK1}$)-positive *K. pneumoniae* was reported (Wu et al., 2009). With a rapid diagnosis method for the detection of the K1 serotype (*magA*), the administration of monoclonal antibodies would become the main therapy for serotype K1 *K. pneumoniae* causing liver abscess.

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