Investigation of the putative virulence gene magA in a worldwide collection of 495 Klebsiella isolates: magA is restricted to the gene cluster of Klebsiella pneumoniae capsule serotype K1

Klebsiella pneumoniae is a well-known opportunistic pathogen associated with nosocomial infections such as urinary tract infections, pneumonia and septicaemia (Podschun & Ullmann, 1998). In recent years, a high incidence of community-acquired K. pneumoniae pyogenic liver abscess with a high mortality rate has been reported, especially from Taiwan, and more sporadic cases have been reported from other Asian countries, Europe and North America (Wang et al., 1998; Fung et al., 2002; Okano et al., 2002; Rahimian et al., 2004; Fang et al., 2005). Most recently, K. pneumoniae was reported as the most common organism isolated from pyogenic liver abscesses in two independent USA studies (Rahimian et al., 2004; Lederman & Crum, 2005). Although diabetes mellitus is considered an important risk factor, approximately half the cases occur in otherwise healthy people. Moreover, severe metastatic infections including endophthalmitis and meningitis occur more frequently in patients infected with K. pneumoniae than in patients with liver abscess of other aetiological origins (Wang et al., 1998; Yang et al., 2004).

The pathogenic mechanism of this infectious disease is not well understood. K. pneumoniae characteristically produce vast amounts of capsular polysaccharide covering the bacterial surface. Of the 77 capsular (K) serotypes recognized, the majority of liver abscess isolates belong to the K1 and K2 serotypes (Fung et al., 2002). A new virulence gene magA (mucoviscosity-associated gene) was recently identified in pathogenic strains from Taiwan causing liver abscess (Fang et al., 2004). This gene was detected in the vast majority of K. pneumoniae liver abscess isolates and was associated with hypermucoviscosity, and resistance to killing by human serum and phagocytosis as well as high virulence in an animal model. We investigated the prevalence of the magA gene in our collection of K. pneumoniae at the International Escherichia coli and Klebsiella Reference Centre (WHO), Statens Serum Institut (SSI), Denmark, and found that magA was restricted to the capsular gene cluster of K1.

Initially, eight isolates were checked for the presence of magA by PCR analysis. magA-specific primers MagA-F (5'-TAGGACCGTTAATTTGCTTTGT-3') and MagA-R (5'-GAATATCCACTCCCTCTCC-3') were designed from the published magA sequence (GenBank accession no. AB085741). The eight strains belonged to the most frequent capsule serotypes associated with liver abscess, K1 and K2, and displayed the hypermucoid phenotype associated with magA (Fang et al., 2004). Of the eight isolates, only the three isolates belonging to the K1 serotype, including the K1 serotype reference strain (A5054), were magA positive.

To further investigate the prevalence of magA and the association between magA and capsular serotype, a collection of 495 international K. pneumoniae isolates from the Reference Centre (WHO) was screened by colony blot hybridization. The strain collection included the reference strains of all 77 K. pneumoniae capsule serotypes. The additional 418 clinical isolates included in the collection were serotyped by counter-current immuno-electrophoresis as previously described (Hansen et al., 1998). From the K1 serotype test strain, a digoxigenin (DIG)-labelled nucleotide probe for magA was prepared by use of the PCR DIG Synthesis Kit (Roche), as described by the manufacturer, using the primer pair Maga-F and Maga-R. Colony hybridizations were performed on Hybond-N' membranes (Amersham) under stringent conditions according to the manufacturer's directions. The screening revealed 39 magA-positive isolates (7.8 %). None of the 456 non-K1 serotypes in the strain collection contained magA and all 39 magA-positive isolates were of the K1 capsule serotype, indicating a close relationship between magA and the K1 capsule serotype.

The K. pneumoniae capsule gene cluster contains conserved and serotype-specific regions (Rahn et al., 1999; Brisse et al., 2004). So far, only the serotype-specific region of the K2 serotype has been published (Arakawa et al., 1995). By PCR analysis we established the relationship between magA and the K1 serotype. The primer pair CPS-1 (5'-GCTGTTAGCTGTTAACGCCGGGGTACGG-3') and rCPS (5'-TTATCATCAAGGAGCACCCGACGCAGTTGGAGAGACGCC-3') is specific for conserved regions flanking the K. pneumoniae capsule gene cluster (Brisse et al., 2004). By combining the primers CPS-1 and rCPS with the magA-specific primers UmagA (5'-TTTGGGGCAGAAATGCATTAAAGATAGGA-3') and Dmaga (5'-AAGGGATGTCAAACCTCCGTAGTAGGCGAATGGTCCG-3'), we identified magA as a gene in the serotype-specific region of the K1 capsule gene cluster (Fig. 1).

The magA gene was suggested to be a novel virulence gene in K. pneumoniae isolates causing pyogenic liver abscesses, since 98 % of isolates from patients with liver abscess admitted at the National Taiwan University Hospital were magA positive. In contrast, the magA prevalence in a collection of isolates from patients with septicaemia without spread to the liver was only 29 %. Whereas magA-positive strains are serum resistant, resisted phagocytosis and caused liver abscesses and meningitis in mice, mutants with a deletion of the magA gene are highly serum sensitive, phagocytosis susceptible and avirulent in mice (Fang et al., 2004). In the present study we investigated the prevalence of magA in a collection of 495 K. pneumoniae isolates encompassing all 77 recognized capsular serotypes and found that magA is restricted to isolates of the K1 capsule serotype. Furthermore, we identified magA to be a conserved gene in the serotype-specific
Fig. 1. magA is a gene in the serotype-specific region of the K. pneumoniae K1 capsule serotype gene cluster. (a) Schematic representation of the K. pneumoniae capsule gene cluster containing conserved (grey) and serotype-specific (white) regions. By combination of primers located in conserved regions and magA-specific primers, magA was identified as a gene in the serotype-specific region of the K1 gene cluster. (b) Lanes: M1 and M2, molecular mass markers; 1, PCR product using primer pair pCPS-1 and pUmagA; 2, PCR product using primer pair pDmagA and pCPS; 3, PCR product using primer pair pCPS-1 and pCPS.

region of the K1 capsule gene cluster. Most recently a case of an American patient with a magA-positive K. pneumoniae liver abscess was reported (Fang et al., 2005). In that study magA is described as a novel virulence factor responsible for the increased virulence of certain K. pneumoniae strains. We provide evidence that the magA gene, so far believed to be a specific virulence factor in highly virulent Klebsiella strains, is present in each and every K. pneumoniae isolate of the K1 serotype regardless of virulence.

Our results suggest that the K1 capsule rather than the magA gene per se is an important virulence factor in K. pneumoniae strains causing liver infections. Epidemiological studies have indeed shown a significantly higher prevalence of serotype K1 in K. pneumoniae liver abscess isolates compared to isolates of other clinical origins (Fung et al., 2000, 2002; Cheng et al., 2002). Thus, 63.4 % of 134 cases of K. pneumoniae liver abscess in Taiwan were caused by K1 strains, and in cases complicated by endophthalmitis the prevalence was 85.7 % (Fung et al., 2002). In comparison the prevalence of the K1 serotype in non-selected Taiwanese clinical strains was 21.7 % (Fung et al., 2000), a figure comparable to the 29 % magA-positive (equals the K1 serotype) isolates from patients without liver abscess in the study by Fang et al. (2004).

The capsule is a well-known virulence factor in K. pneumoniae (Podschun & Ullmann, 1998; Cortes et al., 2002; Struve & Krogfelt, 2003). Animal studies have shown that K1 and K2 isolates are more virulent than other serotypes (Mizuta et al., 1983; Simoons-Smit et al., 1984; Kabba et al., 1995). Most recently K1 and K2 isolates were found to be significantly more resistant to phagocytosis than non-K1/K2 isolates (Lin et al., 2004). High resistance of capsule serotype K1 and K2 K. pneumoniae against phagocytosis may explain the high prevalence of these serotypes among liver abscess isolates.

It is striking that the general prevalence of the K1 serotype is significantly higher in Taiwan than that reported in epidemiological studies from Europe and North America (Cryz et al., 1986; Fung et al., 2000; Hansen et al., 1998). The high general prevalence of this virulent serotype in Taiwan may explain the high incidence of K. pneumoniae liver infections in this region. An epidemiological study has recently addressed a global difference in clinical patterns of K. pneumoniae infections (Ko et al., 2002). This difference may be related to a higher prevalence of virulent serotypes in certain regions. Rapid detection of the virulent K1 serotype will be most helpful in diagnosis and treatment to decrease the risk of severe metastatic infections, as well as in epidemiological studies. Traditional serotyping is cumbersome and requires access to high quality antisera. Our results show that magA is restricted to isolates of the K1 serotype. We therefore suggest detection of magA by hybridization or PCR analysis as an easy, fast and highly specific diagnostic method for identification of the K. pneumoniae K1 capsule serotype.

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