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 sepsicaemia (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'-T A G A C C G T T A A T T T G C T T T G T 3') and MagA-R (5'-G A A T T A T T C C A C T C C T C T C C-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.

Our results suggest that the K1 capsule serotype is significantly higher in Taiwan than that reported 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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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.

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 K1 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.


