magA is not a specific virulence gene for *Klebsiella pneumoniae* strains causing liver abscess but is part of the capsular polysaccharide gene cluster of *K. pneumoniae* serotype K1

Klebsiella pneumoniae is an important emerging pathogen in community-acquired liver abscesses worldwide, especially in Taiwan, Asia and the USA (Chang & Chou, 1995; Okano *et al.*, 2002; Lim *et al.*, 2003; Lederman & Crum, 2005). The prevalence of *K. pneumoniae* in cases of pyogenic liver abscess is as high as 78 % in Taiwan and 41 % in the USA (Tsay *et al.*, 2002; Rahimian *et al.*, 2004). Our group has observed that among our *K. pneumoniae* liver abscess cases, 85 of 134 (63.4 %) were caused by serotype K1 *K. pneumoniae* and 12 of the 14 (85.7 %) cases with complications of endophthalmitis were serotype K1, including K1 isolates which are highly resistant to neutrophil phagocytosis (Fung *et al.*, 2002; Lin *et al.*, 2004).

Further investigation by Fang *et al.* (2004) has identified a novel virulence gene, *magA*, that causes *K. pneumoniae* liver abscess and septic metastatic complications. Among the isolates in Fang's study, 52 of 53 (98 %) liver abscess isolates carried this specific virulence gene and the presence of one *magA*-negative isolate was suggested to be due to the patient's underlying disease of liver cirrhosis and hepatic failure. Thus Fang *et al.* (2004) concluded that *magA* is an essential virulence gene for *K. pneumoniae* strains causing liver abscess and could be used as a diagnostic tool. Unfortunately, the authors did not discuss the correlation of virulence between the novel gene and serotype specificity.

In a recent article in the *Journal of Medical Microbiology*, Struve *et al.* (2005) tried to investigate the above correlation and found that, of the 495 *Klebsiella* isolates from a worldwide collection isolated from unknown different sites, all 39 *magA*-positive isolates were of serotype K1 and none of the 456 non-K1 serotypes contained *magA*. They concluded that

magA is restricted to the capsular gene cluster of serotype K1. Since all the 495 isolates were non-liver abscess isolates, the presence of *magA* in non-K1 liver abscess isolates has not been studied. One may argue that all liver abscess isolates may have the *magA* virulence gene. Our group has sequenced the whole K1 capsular gene cluster (GenBank accession no. AY762939; Fig. 1). Thus we have tried to investigate the prevalence of *magA* among serotypes K1, K2 and other serotypes from liver abscess patients. Our results further confirmed those of Struve *et al.* (2005) indicating that *magA* is only present in serotype K1 liver and non-liver abscess isolates.

In the present study, we investigated the incidence of *magA* among 134 *K. pneumoniae* liver abscess isolates (Fung *et al.*, 2002) and 4 strains of *K. pneumoniae* from the ATCC. Nested PCRs for *magA* detection were designed according to Fang *et al.* (2004). The sequence of the two primer sets were as follows: outer forward 5'-GGTGCTCTTTACATCATTGC-3' and outer reverse 5'-GCAATGGCCATTT-GCGTAAG-3'; and inner forward 5'-CGCCGCAAATACGAGAAGTG-3' and inner reverse 5'-GCAATCGAAGTGAA-GAGTGC. Control strains of serotype K1 (ATCC 4208), K2 (ATCC 9997), K3 (ATCC 13883) and K6 (ATCC 700603) were included for PCR. Results obtained from PCR amplification showed that only K1 isolates were positive for *magA* (Table 1). All other capsular serotypes were negative for *magA*. Thus we strongly agree with Struve *et al.* (2005) that the K1 capsule rather than *magA* is the important virulence factor for *K. pneumoniae* strains causing liver abscess and that *magA* is a part of the gene cluster involved in K1 capsule formation. According to studies done on our collection of *K. pneumoniae* liver abscess isolates, more than 36 % of the cases would have been missed if *magA*

was used as a diagnostic tool. Although 86 % of the cases with complications were caused by serotype K1 and tested positive for *magA*, only 14 cases were collected in our previous study (Fung *et al.*, 2002). The usefulness of *magA* for the detection of liver abscess and septic metastatic complications needs to be studied further.

We conclude that *magA* is a component of K1 capsule formation, but is not an independent virulence gene in *K. pneumoniae* strains causing liver abscess. The K1 capsule rather than *magA* is an important virulence factor.

Table 1. PCR amplification of *magA* among different K serotypes of *K. pneumoniae* isolated from liver abscess patients

Serotype* (K)	No. of isolates	Isolates positive for <i>magA</i> (%)
K1	86	86 (100)
K2	20	0
K16	6	0
K21	4	0
K28	4	0
K54	3	0
K5	2	0
K29	2	0
K38	2	0
K39	2	0
K57	2	0
K68	1	0
K3	1	0
K6	1	0
NT	2	0
Total	138	86 (63.4)†

*Isolates included control strains of serotype K1 (ATCC 4208), K2 (ATCC 9997), K3 (ATCC 13883) and K6 (ATCC 700603).

†Percentage excluded ATCC control strains.

GalF: UTP-glucose-1-phosphate uridylyltransferase

ORF2: Hypothetical protein

ORF3: Hypothetical protein

Wza: Putative capsule polysaccharide export protein

Wzb: Protein-tyrosine-phosphatase

Wzc: Tyrosine-protein kinase

ORF7: Hypothetical protein

ORF8: Hypothetical protein

MagA: Hypothetical protein

ORF10: Hypothetical protein

ORF11: Hypothetical protein

Gmd: GDP-D-mannose dehydratase

WcaG: GDPfucose synthetase

WcaH: GDPmannose mannosylhydrolase

WcaI: Glycosyltransferase

RfbP: Glycosyltransferase

Gnd: Phosphogluconate dehydrogenase

ManC: Mannose-1-phosphate guanylyltransferase

Fig. 1. Gene cluster for K1 capsular polysaccharide (GenBank accession no. AY762939), showing genes with known and unknown functions.

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