Genetically similar isolates of *Klebsiella pneumoniae* serotype K1 causing liver abscesses in three continents

Jane F. Turton, Hilary Engleender, Samantha N. Gabriel, Sarah E. Turton, Mary E. Kaufmann and Tyrone L. Pitt

The *magA* gene was sought in hypermucoviscous isolates of *Klebsiella* spp., the *Klebsiella* K serotype reference strains and in isolates of the K1 serotype of *Klebsiella pneumoniae* from the UK, Hong Kong, Israel, Taiwan and Australia. Only K1 isolates were PCR positive for *magA*; this gene was found in all such isolates tested. Hypermucoviscosity was not confined to *magA* positive isolates, nor was it found in all *magA* positive isolates. Comparison of XbaI PFGE profiles revealed that most (19/23) of the *magA* positive isolates clustered within 72 % similarity, with a further subcluster of isolates, from three different continents, clustering within >80 %. All of the 16 isolates tested within the main cluster had the same sequence type (ST 23) by multilocus sequence typing, with the exception of one isolate, which had a single nucleotide difference at one of the seven loci. This study indicates that a genotype strongly associated with highly invasive disease in Taiwan, where large numbers of cases have been reported, is geographically very widespread.

**INTRODUCTION**

*Klebsiella pneumoniae* subspecies *pneumoniae* is an opportunist nosocomial pathogen, most frequently associated with urinary tract infections, pneumonia and septicaemia (Podschun & Ullmann, 1998). During recent years, highly invasive, community-acquired strains of *K. pneumoniae* of serotype K1, and to a lesser extent K2, have been reported as a cause of liver abscess with bacteraemia, and associated with high mortality, particularly in Taiwan, but also in Europe, North America and Japan (Wang *et al.*, 1998; Fang *et al.* 2004, 2005; Rahimian *et al.*, 2004; Okano *et al.*, 2002). The disease is often complicated by endophthalmitis. Diabetes mellitus is a predisposing risk factor, but approximately half of cases are in otherwise healthy individuals (Fang *et al.*, 2004).

Such *K. pneumoniae* isolates have been associated with hyperproduction of capsular/slime polysaccharide (hypermucoviscosity) and the presence of a putative virulence gene, *magA* (Fang *et al.*, 2004, 2005). It has become clear that this gene is in the serotype-specific region of the K1 capsule gene cluster, and that it is restricted to and present in all of the isolates of this serotype (Struve *et al.*, 2005; Chuang *et al.*, 2006). Other genes in this region unique to the K1 serotype have also been identified, and all provide useful targets for rapid PCR detection of the serotype (Chuang *et al.*, 2006).

The K1 capsule, rather than just the *magA* gene, is the likely virulence factor (Struve *et al.*, 2005).

Comparison, by PFGE, of isolates in Taiwan that had caused liver abscesses revealed that many belonged to a cluster, suggesting a clonal population (Lau *et al.*, 2000). Here we have looked at all isolates of serotype K1 received by the Laboratory of HealthCare Associated Infection (LHCAI) between 2003 and 2005, and compared them with a set of isolates from Taiwan from 2001 by both PFGE and multilocus sequence typing (MLST). MLST identifies clonal lineages by indexing variation within housekeeping genes, which are considered to be selectively neutral, whilst PFGE gives a more general overview of the genome and is particularly useful for investigating outbreaks. Isolates included representatives from Hong Kong, Israel and Australia, as well as from the UK and Taiwan.

**METHODS**

**Isolates.** Originally, any *Klebsiella* isolates displaying hypermucoviscosity (i.e. that formed a ‘string’ when a colony was touched with a loop) were selected for study. However, since all (25/25) of the first batch of isolates tested (of serotypes other than K1) were PCR negative for the *magA* gene, K1 isolates received between 2003 and 2005, and a set of isolates from Taiwan (of both K1 and K2 serotypes) collected during 2001, were selected for further investigation. All isolates were from different patients. All *Klebsiella* K serotype strains held by the LHCAI, representing 77 serotypes, were also screened for the *magA* gene.
PCR. Detection of the magA gene by PCR was carried out as described by Fang et al. (2004). Conditions were: 94°C for 1 min, followed by 30 cycles of 94°C for 30 s, 59°C for 45 s, 72°C for 2 min, and a final extension at 72°C for 6 min. PCRs were carried out in 25 μl volumes containing 3 μl extracted DNA, 12.5 pmole each primer, 200 μM each dNTP, 1 × PCR buffer (Qiagen) and 1 U Taq DNA polymerase. The final MgCl₂ concentration was 1.5 mM. The original method describes a nested PCR but, in our experience, only isolates that had already given a positive result in the first PCR gave a band in the second, and we therefore found the second PCR to be unnecessary. PCR reactions from all isolates that were negative in the first PCR were subjected to the second PCR, but none gave a positive result.

MLST. MLST was carried out as described by Diancourt et al. (2005). Sequencing reactions were performed using Beckman Coulter CEQ dye terminator cycle sequencing with a Quick Start kit (Beckman Coulter) and analysed using a Beckman Coulter CEQ8000 sequencer. Sequences were compared with those on the MLST web site (http://pubmlst.org/kpneumoniae/) developed by Keith Jolley and hosted by the University of Oxford (Jolley et al., 2004). The development of this site has been funded by the Wellcome Trust. Alleles and sequence types (STs) were assigned accordingly. Sequence traces of alleles found that were not already on the database were submitted to the curator, and the new allele (rpoB-19, gapA-18, mdh-25, mdh-26, pgi-22, pgi-23, phoE-31, infB-21, infB-22, tonB-49, tonB-50) and ST (137–139) numbers given are included here.

PFGE. PFGE of XbaI-digested genomic DNA was carried out as described previously (Turton et al., 2004) using a CHEF DRII apparatus (Bio-Rad) at 12°C. A linear ramp of 5–35 s was used, and gels were run for 30 h at 6 V cm⁻¹. Gel images were analysed by BioNumerics (Applied Maths) and the percentage similarity of profiles calculated by the Dice coefficient. UPGMA was used for clustering.

Serotyping. Isolates were serotyped using a combination of counter-current immunoelectrophoresis and capsule swelling reactions with K antisera (Ayling-Smith & Pitt, 1990).

**RESULTS AND DISCUSSION**

Most isolates of Klebsiella received by the LHCAI displaying hypermucoviscosity were PCR negative for the magA gene. Of an initial set of 25 such isolates tested, none were PCR positive for the gene. Moreover, 6 of 23 K1 isolates

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Country of isolation</th>
<th>Date received</th>
<th>PFGE profile*</th>
<th>Hypermucoviscosity†</th>
<th>Clinical details</th>
</tr>
</thead>
<tbody>
<tr>
<td>TW1</td>
<td>Taiwan</td>
<td>2001</td>
<td>1</td>
<td>Yes</td>
<td>Liver abscess without endophthalmitis or other infections</td>
</tr>
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<td>TW2</td>
<td>Taiwan</td>
<td>2001</td>
<td>1</td>
<td>Yes</td>
<td>Liver abscess with endophthalmitis</td>
</tr>
<tr>
<td>TW3</td>
<td>Taiwan</td>
<td>2001</td>
<td>1</td>
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<td></td>
</tr>
<tr>
<td>TW4</td>
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<td>2001</td>
<td>1</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>TW5</td>
<td>Taiwan</td>
<td>2001</td>
<td>1</td>
<td>Yes</td>
<td></td>
</tr>
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<td>TW6</td>
<td>Taiwan</td>
<td>2001</td>
<td>1</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>TW7</td>
<td>Taiwan</td>
<td>2001</td>
<td>Main</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>TW8</td>
<td>Taiwan</td>
<td>2001</td>
<td>Main</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>TW9</td>
<td>Taiwan</td>
<td>2001</td>
<td>Main</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>TW10</td>
<td>Taiwan</td>
<td>2001</td>
<td>Main</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>AS1</td>
<td>Australia</td>
<td>2003</td>
<td>1</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>AS2</td>
<td>Australia</td>
<td>2003</td>
<td>Unique</td>
<td>No</td>
<td>Not known</td>
</tr>
<tr>
<td>AS3</td>
<td>Australia</td>
<td>2003</td>
<td>Unique</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>AS4</td>
<td>Australia</td>
<td>2003</td>
<td>Unique</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>HK1</td>
<td>Hong Kong</td>
<td>May 2003</td>
<td>1</td>
<td>Yes</td>
<td>Blood culture isolate</td>
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<tr>
<td>HK2</td>
<td>Hong Kong</td>
<td>May 2003</td>
<td>Main</td>
<td>No</td>
<td>Blood culture isolate</td>
</tr>
<tr>
<td>HK3</td>
<td>Hong Kong</td>
<td>May 2003</td>
<td>1</td>
<td>Yes</td>
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<tr>
<td>UK1‡</td>
<td>UK</td>
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<td>1</td>
<td>Yes</td>
<td>Not known</td>
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<tr>
<td>UK2‡</td>
<td>UK</td>
<td>Nov 2003</td>
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<td>Yes</td>
<td>From Chinese patient presenting with chronic lung (? and liver) abscess and pneumonia</td>
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<tr>
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<td>UK</td>
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<td>Unique</td>
<td>No</td>
<td>Blood isolate</td>
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<tr>
<td>UK4‡</td>
<td>UK</td>
<td>Jan 2005</td>
<td>1</td>
<td>Yes</td>
<td>From patient with haematogenous endophthalmitis in both eyes; isolated from blood and vitreous humour</td>
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<tr>
<td>UK5‡</td>
<td>UK</td>
<td>Mar 2005</td>
<td>Main</td>
<td>No</td>
<td>? Urinary tract infection</td>
</tr>
<tr>
<td>IS1</td>
<td>Israel</td>
<td>Jun 2005</td>
<td>1</td>
<td>Yes</td>
<td>Liver abscess with ophthalmic involvement</td>
</tr>
</tbody>
</table>

*1 indicates isolates belonging to subcluster 1 within the main PFGE cluster (see Fig. 1).
†Isolates were grown on both nutrient agar and MacConkey agar.
‡Isolates from the UK were from five different hospitals.
Fig. 1. Comparison of PFGE and MLST profiles of K1 isolates. Isolates in subcluster 1 within the main cluster and the remaining isolates in the main cluster are indicated by ● and ■, respectively. Dotted and dashed lines indicate 72% (for the main cluster) and 82% (for the subcluster) similarity, respectively. MLST alleles are in the order rpoB, gapA, mdh, pgi, phoE, infB, tonB. NT, Not tested.

Isolate | Country | MLST alleles | ST
--- | --- | --- | ---
UK3 | UK | 19 18 25 22 11 22 50 | 139
AS2 | Australia | 13 18 26 23 31 22 49 | 138
● AS1 | Australia | 4 2 1 1 9 1 12 | 23
● TW3 | Taiwan | NT |
● TW5 | Taiwan | NT |
● HK1 | Hong Kong | 4 2 1 1 9 21 12 | 137
● UK4 | UK | 4 2 1 1 9 1 12 | 23
● IS1 | Israel | 4 2 1 1 9 1 12 | 23
● TW1 | Taiwan | 4 2 1 1 9 1 12 | 23
● UK1 | UK | 4 2 1 1 9 1 12 | 23
● TW2 | Taiwan | 4 2 1 1 9 1 12 | 23
● TW4 | Taiwan | NT |
● HK3 | Hong Kong | 4 2 1 1 9 1 12 | 23
■ UK5 | UK | 4 2 1 1 9 1 12 | 23
■ TW7 | Taiwan | 4 2 1 1 9 1 12 | 23
■ HK2 | Hong Kong | 4 2 1 1 9 1 12 | 23
■ UK2 | UK | 4 2 1 1 9 1 12 | 23
■ TW6 | Taiwan | 4 2 1 1 9 1 12 | 23
■ TW8 | Taiwan | 4 2 1 1 9 1 12 | 23
■ TW9 | Taiwan | 4 2 1 1 9 1 12 | 23
■ TW10 | Taiwan | 4 2 1 1 9 1 12 | 23
AS4 | Australia | NT |
AS3 | Australia | NT |
possessing magA did not have this property (Table 1). We therefore suggest that, despite recommendations to the contrary (Fang et al., 2005), hypermucoviscosity is not a reliable indicator of the presence of the magA gene.

All the isolates that were magA positive belonged to serotype K1, and all K1 isolates tested were magA positive. This is in agreement with the findings of Struve et al. (2005) and Chuang et al. (2006) that the magA gene is part of the serotype-specific region of the K1 capsule gene cluster. The magA gene was not detected in type strains of any of the other serotypes (K2–K72 inclusive, K74 and K79–82 inclusive).

We do not have clinical details of the disease manifestations associated with all of the K1 isolates, but, where they are known, they mostly include liver abscess, with or without optic involvement (Table 1). Isolates were from the UK (5), Taiwan (10), Israel (1), Hong Kong (3) and Australia (4). Despite their geographically distant origins, many isolates were similar by PFGE (Fig. 1). A main group of isolates clustered within 72% similarity; a subgroup within it, with representatives from three different continents, clustered with a similarity of >80%. The main group probably corresponds to the major cluster, designated cluster A, described by Lau et al. (2000). PFGE profiles shown in that article appear similar to those of the main cluster in the present study. The K1 type strain was not a representative of this cluster. Isolates belonging to the main cluster were of MLST ST 23, having the rpoB, gapA, mdh, pgi, phoE, infB and tonB alleles 4, 2, 1, 1, 9, 1 and 12, respectively, with the exception of one of the isolates from Hong Kong (HK1) within the cluster, which differed by one nucleotide in the infB gene (T instead of G at nt 156). The high similarity of isolates by PFGE, and that they were identical by MLST, reveals a clonal population. Their presence in different continents supports this.

One of the five isolates from the UK and three of the four Australian isolates had unique PFGE profiles. The two unique isolates (UK3, AS2) subjected to MLST (one from each country) had different alleles of the housekeeping genes from the other isolates, with most not previously having been described. They shared the same allele at two of the loci (gapA-18, infB-22); these had 5 (out of 450) and 10–11 (out of 318) nucleotide differences, respectively, from the corresponding allele (gapA-2, and infB-1 or infB-21) in the main cluster isolates. Their alleles at all but one of the other loci were more similar to one another than they were to the main cluster isolates, from which they differed considerably; for example, there was only 1 nucleotide difference between their alleles at both the rpoB and mdh loci, but 11–12 (out of 501) and 20 (out of 477) nucleotide differences, respectively, from the corresponding allele in the other isolates. Biochemical tests suggest that these isolates are K. pneumoniae and are not a separate entity.

Invasive disease due to K1 serotypes of K. pneumoniae is fortunately rare in the UK, and the LHCAI only received 5 isolates of this serotype from UK hospitals between 2003 and 2005 (<1% of isolates), each from a different hospital. However, this is an emerging disease, and the finding of a genotype strongly associated with invasive disease in Taiwan, where it has affected large numbers of individuals, in the UK and elsewhere is of great concern. Whether ethnicity contributes to susceptibility to this organism remains to be established. The magA gene provides a convenient and reliable target to detect these organisms, and we will continue to monitor their presence. Other markers have also been found, which may prove useful for diagnosis and for further characterization of these tissue invasive strains (Chuang et al., 2006; Ma et al., 2005).

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REFERENCES


