Polymerase Chain Reaction Analysis for Detecting Capsule Serotypes K1 and K2 of Klebsiella pneumoniae Causing Abscesses of the Liver and Other Sites

To the Editor—The mucoviscosity-associated gene A (magA) of Klebsiella pneumoniae was first reported to highly correlate hypermucoviscosity phenotype with liver abscess strains [1]. In the 1 March 2006 issue of the Journal, Chuang et al. [2] identified an operon containing magA that is responsible for capsular serotype K1 of K. pneumoniae. After screening magA for 74 strains using polymerase chain reaction (PCR), all 36 magA-positive strains were serotype K1, and the 38 magA-negative strains were not. Having sequenced the magA flanking region of K. pneumoniae NTUH-K2044 (K1) and compared it with the capsular polysaccharide (cps) loci in both K. pneumoniae MGH78578 (K52) and the Chedid strain (K2), Chuang et al. demonstrated that magA is located within an operon that is specific to serotype K1 cps gene clusters. Similarly, Struve et al. [3] investigated 495 worldwide isolates and Yeh et al. [4] screened 134 K. pneumoniae strains causing liver abscess; both studies found that magA is restricted to the gene cluster of K. pneumoniae capsule serotype K1 and that all the non-K1 strains were magA negative. Thus, PCR analysis for magA is a rapid and accurate method to detect capsule K1 strains.

Can PCR analysis be applied to detect capsular serotype K2 of K. pneumoniae? This question addresses the second common clinical occurrence in liver abscess strains and has not been well studied, even though the serotype-specific cps region of the K. pneumoniae K2 serotype has been reported [5]. On the basis of the above findings, we postulated that PCR analysis for the open reading frame (ORF)–9 region (the K2 capsule-associated gene A [k2A]) of K. pneumoniae Chedid strain (K2), which corresponds to the magA region in the cps gene clusters of K1 strain [2, 5], could be used as a highly specific diagnostic method to identify the cps of K. pneumoniae capsule K2 serotype. Therefore, we assessed the prevalence of magA and k2A in our collection of 61 non-repetitive K. pneumoniae isolates causing primary liver abscess (n = 44) and other abscesses (n = 17), which were collected at 2 medical centers in southern Taiwan. Abscesses other than the liver involved the neck (n = 4), psoas muscle (n = 3), lung (n = 3), pleural cavity (n = 2), and 1 each for pericardial cavity, kidney, pancreas, paraspinal muscle, and abdominal wall. All isolates were serotyped using a counter-current immunoelectrophoresis method [6]. Antisera were provided by the Gram Negative Serotyping Unit, Laboratory of HealthCare Associated Infection, Health Protection Agency (London, UK). K. pneumoniae ATCC9997 (K2) was used as a control strain. Using crude genomic DNA as the template, PCRs were performed by using the magA-specific primers (forward, 5'-GGTGCTTTGA- CATCATTGC-3'; and reverse, 5'-GCA- ATGGCCATTGCGTTAG-3') [7] and k2A-specific primers (forward, 5'-CAACC- ATGGCGTCGATTAG-3'; and reverse, 5'-TGATGCATACCGTTTGG-3'). PCR products (experiments were performed >3 times) from 1 represented strain KP344 (capsule serotype K2) were repeatedly DNA sequenced using k2A-specific primers and were confirmed as the same sequence. The target sequence was cloned into Escherichia coli DH5α for further sequencing, which showed a high level of identity (99% homology) with the published target sequence of ORF9 region of K. pneumoniae Chedid (K2) strain [2, 5]; this was selected as the positive control for the subsequent k2A PCR experiments (GenBank accession number ER221827).

We found that all the K1 and K2 strains were magA and k2A positive, respectively, and that all the non-K1/K2 strains were negative to magA and k2A, regardless of isolates recovered from abscesses of the liver or other sites (table 1). The occurrence of K. pneumoniae liver abscess with

<table>
<thead>
<tr>
<th>Abscess, capsule K serotype</th>
<th>Isolates, no. (%)</th>
<th>magA+ isolates</th>
<th>k2A+ isolates</th>
<th>Septic meningitis</th>
<th>Septic endophthalmitis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any</td>
<td>61</td>
<td>28</td>
<td>15</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>K1</td>
<td>28 (45.9)</td>
<td>28</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>K2</td>
<td>13 (21.3)</td>
<td>0</td>
<td>13</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Non-K1/K2</td>
<td>20 (32.8)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Liver</td>
<td>44</td>
<td>23</td>
<td>10</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>K1</td>
<td>23 (52.3)</td>
<td>23</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>K2</td>
<td>10 (22.7)</td>
<td>0</td>
<td>10</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Non-K1/K2</td>
<td>11 (25.0)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Others</td>
<td>17</td>
<td>5</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>K1</td>
<td>5 (29.4)</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>K2</td>
<td>3 (17.6)</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Non-K1/K2</td>
<td>9 (52.9)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

The table illustrates the prevalence of capsule serotypes magA and k2A in 61 isolates causing abscesses of the liver and other organs identified in southern Taiwan.
metastatic infections was 13% (3/23 isolates) for K1 strains and 10% (1/10 isolates) for K2 strains, but there was none for non-K1/K2 strains, similar to a previous report [8]. The prevalence of serotype K1 (52.3%) and serotype K2 (22.7%) among liver abscess strains was comparable to previous data for K1 (63.4%) and K2 (14.2%) [8] but was rather different from the data for K1 (83.3%) and K2 (2.4%) reported by Chuang et al. [2].

Compared with non-K1/K2 strains, K1 and K2 isolates are generally more virulent and are highly resistant to phagocytosis [9–11], which may explain their high prevalence in liver abscess strains, particularly with a propensity to cause distant septic metastasis [8]. The substantial prevalence of K2 isolates in our results suggests a need for rapid detection of K2 serotype in addition to K1 serotype. In addition, K2 isolates ranked as the most prevalent serotype K2 (14.2%) [8] but was rather different from previous data for K1 (63.4%) and K2 (22.7%) [5], given that K1 and K2 are the 2 major strains causing this disease [6].

References


Potential conflicts of interest: none reported.

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Reply to Yu et al.

To the Editor—Our studies are in agreement with Yu et al.’s [1] observation that polymerase chain reaction (PCR) is sensitive and specific for detecting both se- rotypes K1 [2] and K2 (data not shown). Studies from other researchers have also shown similar results that PCR and PCR–restriction fragment length polymorphism can be used to differentiate the capsular serotypes [3, 4]. We also find that by PCR rmpA is present in several serotypes of K. pneumoniae strains, including K1 and K2 (data not shown). That may be the reason why rmpA appeared to more sensitive but less specific than magA in detecting primary liver abscess caused by K. pneumoniae [5], given that K1 and K2 are the 2 major strains causing this disease [6].

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