Seroepidemiology of *Klebsiella pneumoniae* in an Australian Tertiary Hospital and Its Implications for Vaccine Development

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The aim of this study was to determine the diversity of *Klebsiella pneumoniae* capsular serotypes in an Australian setting. Consecutive (n = 293) nonrepetitive isolates of *K. pneumoniae* from a large teaching hospital laboratory were analyzed. The majority of isolates were from urinary specimens (60.8%); the next most common source was sputum (14.3%), followed by blood (14%). Serotyping revealed a wide range of capsule types. K54 (17.1%), K28 (4.1%), and K17 (3.1%) were the most common, and K54 isolates displayed a high degree of clonality, suggesting a common, nosocomial source. In vitro, one K54 isolate was more adherent to urinary catheters and HEp-2 cells than four other tested isolates; it was slightly more resistant to chlorhexidine but was more susceptible to drying than heavily encapsulated strains. This is the first seroprevalence survey of *K. pneumoniae* to be performed on Australian isolates, and the high level of diversity of serotypes suggests that capsule-based immunoprophylaxis might not be useful for Australia. In addition there are significant differences in the predominance of specific serotypes compared to the results of surveys performed overseas, which has important implications for capsule-based immunoprophylaxis aimed at a global market.

The aims of this study were to determine the distribution of K serotypes of *K. pneumoniae* among isolates collected in a tertiary hospital and to determine whether the distribution of capsular types was narrow enough that a vaccine based on capsular antigens was a practical option for prevention and therapy and, in addition, to identify whether more infectious “clones” were present and to link the emergence of such clones with a phenotype that might favor their survival.

*K. pneumoniae* is a gram-negative bacillus of the family Enterobacteriaceae with a world-wide distribution and is an important cause of human disease resulting in significant morbidity and mortality. The bacterium most typically causes infections of the urinary tract and pneumonia and bacteremia but, less often, wound infections and meningitis that may be acquired both nosocomially and in the wider community (26). *K. pneumoniae* has been described as an independent predictor for mortality in severe community-acquired pneumonia (24).

*K. pneumoniae* is considered an extracellular pathogen whose virulence is linked with the production of a polysaccharide capsule that provides protection against host defense mechanisms, particularly phagocytosis (8). Immunity against the encapsulated bacterium is largely mediated by antibodies specific for the capsular polysaccharide, an observation that has been exploited to develop prototypic vaccines against the bacterium (13). As with other capsule-based vaccines, e.g., 23-valent pneumococcus vaccine, the efficacy of similar *K. pneumoniae* vaccines will depend on the distribution of capsule or “K” serotypes (5, 6). The capsular distribution for *K. pneumoniae* K types is known to differ worldwide (3, 9, 16, 19, 29), but an acceptable explanation for this phenomenon has yet to be found.

**MATERIALS AND METHODS**

Over a 13-month period (September 2001 and November 2002) all *K. pneumoniae* clinical isolates that were identified at the microbiological laboratory of the Alfred Hospital were collected. The laboratory processes specimens from the main hospital (300-bed tertiary referral University teaching hospital) and three other hospitals (a geriatric hospital, a district community hospital, and a hospice). Isolates were identified using a GNI card (Vitek; bioMerieux, Marcy l’Etoile, France).

Infections with *K. pneumoniae* were considered to be community acquired when the isolate was grown from a specimen taken within the first two complete days of admission to hospital. Urinary isolates were associated with significant bacteriuria (>10⁵ CFU/ml) and the presence of white cells in the urine, i.e., >10 cells per high-powered field, unless the patients were neutropenic. Sputum cultures were included when associated with >25 neutrophils per high-powered field on microscopy. Inclusion of other sites (blood, wounds) required the individual to have signs or symptoms of disease.

The isolates were nonrepetitive, i.e., only one isolate was included per patient per episode of infection. When *K. pneumoniae* was grown simultaneously from samples from different sites for an individual, only one isolate was included in the analysis unless the organisms were of clearly different phenotypes (e.g., serotype). A second isolate for an individual was included when at least 30 days elapsed between the episodes of infection. All isolates were stored at −70°C in Luria-Bertani (LB) glycerol (22%) broth.

**Adhesion and drying assays.** Five strains of *K. pneumoniae* were investigated for their ability to adhere to HEp-2 cells and urinary catheter plastic and for their
survival after drying in the environment. B5055 (capsule type K2 mouse-lethal strain obtained from the Staten Serum Institut, Denmark), B5055nm (a capsule mutant of B5055 generated in our laboratory; data not shown), and three clinical isolates from the survey, including one of the clonal K54 isolates from a urine specimen and a K1 strain and a K2 strain, both isolated from wound infections, were tested using a GNS card (Vitek; bioMerieux, Marcy l’Etoile, France).

Extended-spectrum beta-lactamase (ESBL)-producing strains. Nonurinary isolates were tested using clavulanic acid disk testing to detect the presence of ESBL activity. A total of 24 of the K54 isolates were randomly selected for analysis by PFGE. Of these, 22 showed a high degree of clonality, suggesting they were clonal in origin (31). Figure 1 shows the PFGE results for 20 of the K54 isolates tested following digestion of the DNA with XbaI.

The K54 isolates were more likely to be isolated from patients in the main tertiary referral hospital than the non-K54 isolates (88.2% versus 75.2%; P = 0.043); however, these patients were no isolates from cerebrospinal fluid in this series. In total 166 isolates (56.7%) were from nosocomial infections.

Serotyping. Antiserum of the recognized 77 serotypes (designated K1 through K74 and K80 through K82 inclusive) were used to analyze the isolates. Fifty-nine serotypes were represented in this series. A total of 151 isolates were positive for just one serotype, and 54 had a positive reaction for more than one serotype; in all, there were 67 serotypes or combinations of serotypes, and 34 of these were represented by just 1 isolate. A total of 88 isolates (30%) were nontypeable (Table 1). This observation indicates that a wide range of different K. pneumoniae strains cause infection in a largely urbanized (i.e., Melbourne) catchment whose populace presents to the Alfred Hospital.

K54 serotype. Over 17% of the isolates expressed the serotype K54; the next largest group was K28, which made up 4.1%. Three of the K54 isolates were found to have ESBL activity. A total of 24 of the K54 isolates were randomly selected for analysis by PFGE. Of these, 22 showed a high degree of similarity, having fewer than three bands different from one another, suggesting they were clonal in origin (31). Figure 1 shows the PFGE results for 20 of the K54 isolates tested following digestion of the DNA with XbaI.

The K54 isolates were more likely to be isolated from patients in the main tertiary referral hospital than the non-K54 isolates (88.2% versus 75.2%; P = 0.043); however, these patients were no isolates from cerebrospinal fluid in this series. In total 166 isolates (56.7%) were from nosocomial infections.
TABLE 1. Distribution of serotypes (or combinations of serotypes) among the 293 isolates consecutively collected from a teaching hospital laboratory in Melbourne, Australia

<table>
<thead>
<tr>
<th>Serotype or combination (K type[s])</th>
<th>No. of isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>3/68</td>
<td>5</td>
</tr>
<tr>
<td>11</td>
<td>2</td>
</tr>
<tr>
<td>11/21</td>
<td>5</td>
</tr>
<tr>
<td>11/21/33</td>
<td>3</td>
</tr>
<tr>
<td>13</td>
<td>2</td>
</tr>
<tr>
<td>15</td>
<td>2</td>
</tr>
<tr>
<td>16</td>
<td>3</td>
</tr>
<tr>
<td>17</td>
<td>9</td>
</tr>
<tr>
<td>19</td>
<td>2</td>
</tr>
<tr>
<td>22/23</td>
<td>2</td>
</tr>
<tr>
<td>22/37/46</td>
<td>4</td>
</tr>
<tr>
<td>24</td>
<td>2</td>
</tr>
<tr>
<td>25</td>
<td>2</td>
</tr>
<tr>
<td>26</td>
<td>4</td>
</tr>
<tr>
<td>27</td>
<td>2</td>
</tr>
<tr>
<td>27/46</td>
<td>5</td>
</tr>
<tr>
<td>28</td>
<td>12</td>
</tr>
<tr>
<td>30</td>
<td>6</td>
</tr>
<tr>
<td>38</td>
<td>3</td>
</tr>
<tr>
<td>47</td>
<td>5</td>
</tr>
<tr>
<td>48</td>
<td>2</td>
</tr>
<tr>
<td>54</td>
<td>51</td>
</tr>
<tr>
<td>55</td>
<td>4</td>
</tr>
<tr>
<td>57</td>
<td>2</td>
</tr>
<tr>
<td>60</td>
<td>3</td>
</tr>
<tr>
<td>61</td>
<td>6</td>
</tr>
<tr>
<td>64/14</td>
<td>3</td>
</tr>
<tr>
<td>64/14/62</td>
<td>4</td>
</tr>
<tr>
<td>64/62</td>
<td>3</td>
</tr>
<tr>
<td>64/62/61</td>
<td>2</td>
</tr>
<tr>
<td>Isolates of individual or combination serotypes appearing only once eacha</td>
<td>34</td>
</tr>
<tr>
<td>Nontypeable</td>
<td>88</td>
</tr>
<tr>
<td>Total</td>
<td>293</td>
</tr>
</tbody>
</table>

a These K-types (or combinations of K-types) were represented by just one isolate in this series: 3, 10, 31, 44, 49, 51, 53, 56, 59, 69, 71, 80, 81, 82, 16/14/59/64, 11/26/54, 7/11/26/74, 6/27/46, 30/32, 12/29/42, 5/6/7, 5/6/33, 9/36/51, 2/30/60, 2/13, 2/69; 30/69, 13/30, 21/26/33, 42/64, 47/56, 71/72, 61/63/64.

Isolation for some types of infection and not others: of the 18 isolates from central venous catheter tips, 10 (55.6%) were K54 and 62.4% of the non-K54 isolates were of urinary origin whereas only 52.1% of the K54 isolates were cultured from urine (though neither result was significant).

Susceptibility testing. A total of 285 isolates were tested against at least five antibiotics by one of the two methods outlined above. The percentages which were susceptible were as follows: for amoxicillin-ampicillin, 15.5%; for amoxicillin-ampicillin with clavulanate, 94.3%; for gentamicin, 93.3%; for trimethoprim, 83%; for norfloxacin, 91%.

ESBL production was detected in 8.2% of the isolates. ESBLs were present in serotypes 5, 11, 14, 16, 21, 25, 26, 30, 54, and 64 and in nontypeable isolates. Three (6.5%) of the 46 K54 isolates tested were found to have ESBLs, a result which was not significantly different from the percentage seen with the non-K54 isolates (8.3%; P = 0.43). By multivariate analysis the ESBLs were significantly more likely to be isolated in the intensive care unit (OR, 5.22 [CI, 1.17 to 23.25]; P = 0.03). Similarly, there was a trend showing that the ESBLs were more likely to be nosocomial in origin, although this did not quite reach statistical significance (OR, 2.61 [CI, 0.67 to 10.21]; P = 0.082). There was no carbapenem resistance found in this survey’s isolates, though metallo-beta-lactamase-producing K. pneumoniae bacteria have subsequently been detected in our laboratory; however, the serotypes of these organisms have not been determined (C. Franklin, personal communication).

Adhesion, drying, disinfectant, and mucoviscosity assays. The K54 isolate was found to be significantly more adherent to HEp-2 cells (P < 0.005) and urinary catheter plastic (P < 0.005) than each of the other four strains tested (Fig. 2 and 3). Each of the five strains tested was increasingly vulnerable to drying as time progressed (Fig. 4). However, B5055 was significantly (P < 0.005) more resistant to drying than K54 and K1 strains at all three time points and was significantly (P < 0.005) more resistant to drying than both B5055nm and K2 at 4 and 24 h, as well. All five K. pneumoniae strains were fully susceptible to both disinfectant products at a concentration of 0.0078%, which suggests that these preparations would be probably successful in killing these organisms during hand washing. However, the K54 strain was able to grow in 15 of the 16 wells at a 0.0019% concentration of chlorhexidine whereas the other strains were more susceptible at that concentration; however, this difference was not significant (Table 2).

B5055, K1, and K2 demonstrated a high degree of mucoviscosity and produced viscous string lengths greater than 0.5 cm (B5055 up to 10 cm, K1 up to 3 cm, and K2 up to 8 cm). B5055nm and K54 strains produced no measurable “string” when touched with a metal loop.

DISCUSSION

This novel survey of K. pneumoniae isolates from a variety of hospitals served by a tertiary hospital microbiology laboratory revealed that the bacterium was responsible for at least 293 infections in a 13-month period, with most of the disease manifestations being nosocomial in origin. K. pneumoniae is recognized as a cause of serious morbidity and can increase lengths of hospital stay and, therefore, cost (1, 22, 30). A seroprevalence study of capsule types has not been performed on a large collection of Australian isolates before. The aim of this study was to determine whether the range of capsular serotypes of K. pneumoniae found in a large urban hospital was sufficiently narrow to support the use of immunoprophylaxis (targeting capsular antigens) as a viable therapeutic option.

The isolates were analyzed for capsular serotype by CIE, a test that is only available in a restricted number of reference laboratories worldwide. Of the 205 isolates that gave a positive reaction for capsule in CIE, 67 patterns (single serotypes or combinations of serotypes) were observed. Some of the isolates reacted in combinations of patterns that have been noted in previous studies (19, 25) (Table 1). Isolates which show cross-reactivity with several anticapsular sera have been thought to have greater resistance to antimicrobials (19); however, this was not the case in this series. Despite the use of enriched media to encourage capsule growth, 30% of the isolates were nontypeable and many of these appeared as small,
dry colonies and therefore seemed to lack a capsule entirely. There were other isolates that appeared mucoid but did not react with the antiserum panel, and it is possible these are capsule types not included in the 77 standard K types used by most *Klebsiella* reference laboratories. Clearly, a wide range of serotypes (and nontypeable isolates) exists in this population and argues against the practicality of vaccine- or immunotherapy-based disease prevention where such a course is based on the capsular polysaccharide. There have been no similar surveys prior to this one in Australia, so the range and distribution of serotypes in other parts of the country are unknown.

A similarly wide range of capsular serotypes has been demonstrated in other studies; however, there are differences in the serotypes that appear most frequently in some countries. In Taiwan the most common serotypes isolated were K1, K2, and K57 (16), whereas in Melbourne K54, K28, and K17 were the commonest capsular types observed. The clinical picture was different too: only 14% of the Melbourne isolates were cultured from the blood, whereas 41.2% of Taiwanese *K. pneumoniae* isolates were from blood specimens. In Taiwan *K. pneumoniae* urinary tract infection isolates comprised just 19.3% of the total, whereas over 60% of the Australian isolates

![FIG. 1. PFGE of 20 *K. pneumoniae* serotype K54 isolates (18 from nosocomial infections) from different individuals. Eighteen isolates are closely related (having fewer than three bands of difference), indicating clonality. Lane 4 has up to four bands and lane 6 more than six bands different from the other lanes. Lanes 1, 7, 13, 19, and 25 show molecular markers in 48.5-kb increments.](image1)

![FIG. 2. Histogram representing the number of bacteria adherent to HEp-2 cells for five strains of *K. pneumoniae*, expressed as a percentage of the initial inoculum for each strain (log scale). The K54 strain was significantly (*, $P < 0.005$) more adherent than the other four strains.](image2)

![FIG. 3. Histogram representing the number of bacteria adherent to 0.5-cm sections of urinary catheter of five strains of *K. pneumoniae* after 3 h of incubation. The data for adherent bacteria are expressed as a percentage of the initial inoculum for each strain (log scale). The K54 strain was significantly (*, $P < 0.005$) more adherent than the other four strains.](image3)
were from this site (16). The K54 serotype was clearly the
commonest single strain found, and it was overwhelmingly
clonal, potentially indicating a common source. However, during
the period of the survey there was no “outbreak” of K. pneumoniae
infection noted and the overall numbers of infections caused by K. pneumoniae were relatively stable in the
Alfred Hospital from 1998 to the present (data not shown).
Antimicrobial susceptibility testing was carried out using a
Vitek GNS card or disk susceptibility testing methodology
adapted from CLSI recommendations. Although 15.5% were
found to be “resistant” to ampicillin-amoxicillin, these agents
would not be recommended for therapeutic use, as the result
provides indications of poor induction of the beta-lactamase in
vitro. The majority of our series were from nosocomial infec-
tions; however, relatively few of the isolates produced ESBLs

![Figure 4](http://jcm.asm.org/)  
**FIG. 4.** Histogram representing the number of bacteria recovered after drying five strains of K. pneumoniae for 1 h, 4 h, and 24 h. After 1 h of drying strain B5055 was significantly (*, P < 0.005) more resistant to drying than the K54 and K1 strains, and at 4 and 24 h B5055 was significantly (**, P < 0.005) more resistant to drying than the other four strains.

### TABLE 2. Percentage of wells (n = 16 for each strain and each dilution) allowing growth (turbidity) of five strains of K. pneumoniae in the presence of increasing concentrations of chlorhexidine

<table>
<thead>
<tr>
<th>Conc of chlorhexidine (% 10^(-7) [vol/vol])</th>
<th>% Of wells with growth of indicated K. pneumoniae serotype</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B5055</td>
</tr>
<tr>
<td>0.049</td>
<td>100</td>
</tr>
<tr>
<td>0.098</td>
<td>100</td>
</tr>
<tr>
<td>0.19</td>
<td>50</td>
</tr>
<tr>
<td>0.39</td>
<td>0</td>
</tr>
<tr>
<td>0.78</td>
<td>0</td>
</tr>
<tr>
<td>1.56</td>
<td>0</td>
</tr>
</tbody>
</table>

*a K54 grew in 15 of the 16 wells at a chlorhexidine concentration of 0.009% (B5055 and K1 grew in 8 wells and B5055nm and K2 grew in 2 wells at 0.009%; none of these four grew at 0.0039%). K54 tended to survive better than the other strains in the presence of chlorhexidine, but this was not significantly different from the other four strains. and these were susceptible to most antibiotics tested for. In neighboring Pacific countries the SENTRY study has determined that in some locations, more than 40% of K. pneumoniae isolates are ESBL positive (2). This has led to research into the use of vaccines for the management of disease (especially nosocomial) caused by this bacterium.

Based on European seroprevalence data for bacteremic isola-
lates, a Swiss group developed a 24-valent capsule-based vac-
cine that was investigated in a clinical trial of 10 patients who were victims of acute trauma. The vaccine elicited a fourfold rise in antibody titer (compared to baseline) in 80% or more of the patients for 21 of the 24 capsular antigens, but its efficacy was unclear (7). This vaccine did not contain a K54-derived antigen. This, and the fact that 30% of the Australian isolates were nontypeable, would make the usefulness of such a vaccine in an urban Australian setting highly questionable.

A K54 isolate was compared to other isolates for phenotypic
traits that might render it more transmissible in the hospital setting. The bacterium was avirulent for mice (intravenous
50% infectious dose, >10^7 CFU), whereas B5055 and the K1
and K2 clinical isolates (the strains which displayed mucoviscosi-
city in the “string” test) were found to be lethal for mice at
an inoculum of approximately10^4 CFU in a bacteremic infec-
tion model (data not shown). The B5055nm strain did not display mucoviscosity and, like K54, was avirulent. It has been
reported that although the capsule is an important virulence
factor, not all encapsulated strains of the same serotype are
equally virulent. One group found that that a virulence plasmid
(also coding for the siderophore aerobicin) conferred a “large
colony, viscid” phenotype to a particular strain of K2. This
phenotype, although it “glistened,” did not produce excess
amounts of capsule but was 1,000-fold more lethal to Swiss
mice than the same K2 strain lacking this plasmid (23). This
phenomenon has also been described as “hyperviscosity” and
has been associated with clinical isolates that cause invasive
disease; microscopically, the bacterial colonies produce an
exopolsaccharide web that is attached to the capsule (14). Cap-
sule polysaccharides are recognized as virulence determinants
and have the ability to protect the bacterium from phagocytosis
(26); they also provide protection against desiccation (27).
However, capsules may impede other cellular functions such as
adhesion. The K54 isolate was found to be more adherent to
surfaces, including plastic and human epithelial cells, than
other strains but was not more resistant to drying than the others
strains tested. It has been noted that a reduction in capsule can increase the adhesive abilities of K. pneumoniae
(15); this may explain why K54 was more adherent than B5055.
However, K54 was also significantly more adherent to both
HEP-2 cells and plastic than B5055nm, a defined, constructed
nonencapsulated bacterium from the parent strain B5055, in-
dicating that more than just the presence of a thick capsule
can increase the adhesive abilities of K. pneumoniae (15); this may explain why K54 was more adherent than B5055.
However, K54 was also significantly more adherent to both
HEP-2 cells and plastic than B5055nm, a defined, constructed
nonencapsulated bacterium from the parent strain B5055, in-
dicating that more than just the presence of a thick capsule
prevented B5055 from adhering to surfaces to the same degree
as K54. However, these conclusions are cautiously drawn, as
only one clonal K54 isolate was tested against these K1 and K2
(and K2 mutant) strains.

As well as having type 1 fimbriae, most K. pneumoniae iso-
lates have type 3 fimbriae, the major subunit being the Mrk A
polypeptide that allows adsorption to abiotic polymers of med-
dical devices. Some strains also possess the Mrk D adhesin,
which enables the bacteria to adhere and replicate on these

polymers (11, 17, 28). There are several reports linking adhesive phenotypes of K. pneumoniae to nosocomial infection (10, 12, 20). Adhesion to fomites is an advantage for survival in the hospital environment and establishment of infection in medical devices, and this may have been a factor in the high proportion of central venous catheter infections found to be due to K54. In addition, adhesion to epithelial cells might allow for persistent colonization of the gastrointestinal tract, supporting resistance to the powerful waves of peristalsis (21). The K54 isolate was slightly more resistant to common hospital disinfectants, though whether this helps to explain the prevalence of this serotype has yet to be determined.

K. pneumoniae is an important cause of morbidity worldwide and shows a wide range of capsular serotypes in surveys from many countries, including this Australian study. Studies should continue to determine why some isolates of bacteria such as K. pneumoniae persist in causing disease in hospital environments. The increase in ESBL-producing and carbapenem-resistant K. pneumoniae isolates means that attempts should be made to define noncapsular targets which might form the basis of vaccines or immunoprophylaxis to protect frequently hospitalized patients against this bacterium. If a vaccine were to be developed, it would be infeasible to base it on capsular antigens, as the targets required are too numerous.

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