Rapid Spread of Carbapenem-Resistant 
Klebsiella pneumoniae in New York City

A New Threat to Our Antibiotic Armamentarium

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Background: Carbapenem antibiotics are used to treat serious infections caused by extended-spectrum β-lactamase–carrying pathogens. Carbapenem resistance has been unusual in isolates of Klebsiella pneumoniae. In this study, the prevalence and molecular epidemiologic characteristics of carbapenem-resistant K pneumoniae are analyzed, and the experience involving 2 hospital outbreaks is described.

Methods: A citywide surveillance study was conducted in hospitals in Brooklyn. An observational study involving subsequent outbreaks at 2 hospitals was undertaken. Isolates were genetically fingerprinted by ribotyping and were examined for the presence of KPC-type carbapenem-hydrolyzing β-lactamases.

Results: Of 602 isolates of K pneumoniae collected during the citywide surveillance study, 45% had extended-spectrum β-lactamases. Of the extended-spectrum β-lactamase–producing isolates, 3.3% carried the carbapenem-hydrolyzing β-lactamase KPC-2. Several isolates were reported by the clinical microbiology laboratories as being susceptible to imipenem. Although all the isolates were resistant using agar diffusion methods, minimal inhibitory concentrations of imipenem were substantially lower for several isolates using standard broth microdilution tests and were highly dependent on the inoculum used. Two hospitals experienced the rapid spread of carbapenem-resistant isolates involving 58 patients. Overall 14-day mortality for bacteremic patients was 47%. Most isolates belonged to a single ribotype.

Conclusions: Carbapenem-resistant K pneumoniae isolates are rapidly emerging in New York City. The spread of a strain that possesses a carbapenem-hydrolyzing β-lactamase has occurred in regional hospitals. Because these isolates are resistant to virtually all commonly used antibiotics, control of their spread is crucial. However, automated systems used for susceptibility testing may not accurately identify all these isolates, which will severely hamper control efforts.

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KLEBSIELLA PNEUMONIAE IS a frequent nosocomial pathogen, being the fourth and fifth most common cause of pneumonia and bacteremia, respectively, in intensive care patients. During the 1990s, Klebsiella species possessing extended-spectrum β-lactamases (ESBLs) emerged, conferring resistance to cephalosporins. According to National Nosocomial Infections Surveillance data, cephalosporin resistance has been found in 14% of all isolates of K pneumoniae recovered from intensive care areas, although in some regions this percentage is much higher. Clinical failures have been noted when infections caused by ESBL-possessing isolates were treated with cephalosporins. Because many of these pathogens are also resistant to aminoglycosides and fluoroquinolones, carbapenem antibiotics have been considered the agents of choice to treat serious infections caused by ESBL-carrying pathogens.

To date, carbapenem resistance has been unusual in isolates of K pneumoniae. Occasional studies have documented strains with carbapenem-resistance due to a class C cephalosporinase, which possesses relatively weak carbapenemase activity, combined with reduced porin expression. Carbapenem-hydrolyzing β-lactamases have rarely been reported in Enterobacteriaceae. One particular group of enzymes, designated KPC, has been increasingly reported in several pathogens, including Escherichia coli, Klebsiella species, Enterobacter species, and Salmonella enterica. KPC-type β-lactamases reside on transmissible plasmids. Widespread dissemination of KPC β-lactamases could have

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profound implications regarding the utility of carbapenem antibiotics.

In this study, we describe the epidemiologic characteristics and the mechanisms of resistance of carbapenem-resistant K pneumoniae in New York City. This study includes a citywide prevalence assessment, the experience at 2 hospitals with outbreaks caused by KPC-possessing strains, and a point prevalence survey of colonization with these pathogens in high-risk areas.

METHODS

RESULTS

SURVEILLANCE STUDY

A surveillance study involving 11 hospital-based microbiology laboratories in Brooklyn was conducted between December 1, 2002, and February 28, 2003. All single-patient isolates of K pneumoniae were collected. Bacteria were identified by the participating microbiology laboratories using standard techniques.

INVESTIGATION OF 2 OUTBREAKS

Patients with carbapenem-resistant K pneumoniae infection or colonization were identified by review of microbiology records at 2 hospitals (hospitals A and B). Epidemiologic and clinical data were extracted retrospectively from paper and electronic medical records. If more than 1 culture yielded carbapenem-resistant K pneumoniae, only the initial culture was used for analysis. Receipt of antibiotics during the 30 days before the positive culture result was noted. Patients were judged to have nosocomial infections based on defined criteria. Outcomes were assessed for bacteremic patients for whom clinical data were available. Clinical outcome was assessed according to survival 14 days after the date the first culture positive for K pneumoniae was obtained. Patients who died during the 14-day period were considered clinical failures. Surviving patients were considered to have microbiologic failure if subsequent culture results remained positive more than 3 days after the start of therapy.

MICROBIOLOGIC CHARACTERIZATION

Minimal inhibitory concentrations (MICs) were determined for all isolates in the central research laboratory using the agar dilution technique, according to National Committee for Clinical Laboratory Standards methods. Susceptibility testing with tigecycline was performed using the microdilution broth technique with Mueller-Hinton broth, as recommended by the manufacturer. The susceptibility of KPC-possessing isolates was also tested using the Etest method (AB Biodisk NA, Inc, Piscataway, NJ). Susceptibility of these isolates to imipenem was tested using the disk diffusion method and using the microdilution broth assay with a variety of inocula. For the latter experiments, susceptibility tests were performed in duplicate.

Genetic Fingerprinting

Selected isolates underwent ribotyping using an automated microbial characterization system (Riboprinter; DuPont Qualicon, Wilmington, Del), with EcoRI as the restriction enzyme. Isolates were considered to be similar if they had a similarity coefficient of 0.90 or greater. Results were verified by visual inspection.

SURVEILLANCE STUDY

During the 3-month study, 602 unique patient isolates of K pneumoniae were collected from the 11 participating hospitals. Overall susceptibility rates are given in Table 1: 45% of the isolates were considered to possess ESBLs (MIC of ceftazidime ≥ 2 µg/mL). Of the 271 isolates producing ESBLs, 265 were available for polymerase chain reaction screening for KPC. Nine isolates (3.3%) were found to carry blake. These 9 isolates originated from 7 different hospitals (Table 2). All these isolates were highly resistant to cephalosporins and carbapenems using the Etest method, and all demonstrated resistance to imipenem using the disk diffusion assay. The

\[ \beta-Lactamase Analysis \]

Isoelectric focusing of \( \beta \)-lactamases was performed according to established techniques. Amplification of \( \text{bla}_{\text{KPC}} \) (KPC-type \( \beta \)-lactamase), \( \text{bla}_{\text{TEM}} \) (TEM-type \( \beta \)-lactamase), and \( \text{bla}_{\text{SHV}} \) (SHV-type \( \beta \)-lactamase) was performed using previously described primers and conditions. Polymerase chain reaction products were resolved using a 1% agarose gel and were visualized using ethidium bromide. Amplified products underwent bidirectional sequencing using the automated fluorescent dye-terminator sequencing system (Applied Biosystems, Foster City, Calif). The following additional internal primers were included for sequencing of \( \text{bla}_{\text{KPC}} \): 5′-AGCTGAACCTCGCCCATCC-3′ and reverse 5′-CCGGCCACTCTTGCACG-3′. Sequences were identified using the BLAST program from the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/BLAST/).

Outer Membrane Protein Analysis

Bacterial outer membrane proteins were examined using established techniques. Isolates were grown in Mueller-Hinton broth; carbapenem-resistant isolates were supplemented with 16 µg/mL of imipenem. Outer membrane proteins were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis using a 13% resolving gel and 4% stacking gels. Gels were then stained with Coomassie blue solution.
MICs of imipenem were substantially lower for 4 of the 9 isolates using the standardized broth microdilution test (Table 3). Moreover, if the standard inoculum was decreased 10-fold (from 10^5 to 10^6 colony-forming units [CFU/mL]), 5 of the 9 isolates had imipenem MICs in the susceptible range. Susceptibility results reported by the clinical microbiology laboratories were available for 8 of the isolates; only 5 were reported as being resistant to imipenem. Of the 3 clinical laboratories that did not correctly identify the resistant isolates, 1 used the Microscan Walkaway System (Dade International Inc, West Sacramento, Calif) and 2 used the Vitek System (bioMerieux Vitek, Hazelwood, Mo).

### INVESTIGATION OF 2 OUTBREAKS

At hospital A, preexisting infection control procedures included contact precautions for any patient with a culture growing a multiresistant gram-negative bacillus, defined as resistance to 3 or more antibiotics. In August 2003, 2 patients with imipenem-resistant *K pneumoniae* were recognized. Additional infection control measures instituted in August 2003 included placing patients in private rooms and holding educational sessions for health care workers to reinforce contact precautions. Between September 1, 2003, and February 29, 2004, a total of 30 additional patients were identified as having imipenem-resistant *K pneumoniae*, prompting notification of the New York State Department of Health. Thirty of the 32 isolates were considered to be acquired nosocomially, with median length of hospital stay before the positive culture result of 18.5 days. Two nursing home residents had positive culture results within 48 hours of hospital admission, suggesting carriage in the long-term care facility. Although 18 of the 32 patients had spent time in an intensive care area before the culture, most cases were widely scattered throughout the hospital, and an epidemiologic link for the cases was not identified. Of the 32 cultures with positive results, 12 originated from urine, 7 from sputum, 9 from blood, and 4 from wound specimens.

At hospital B, preexisting infection control practices included placing patients with cultures that revealed any gram-negative bacillus resistant to ceftazidime or imipenem on contact precautions. The initial imipenem-resistant *K pneumoniae* was recovered in December 2003. In the ensuing 3 months, another 24 patients were identified. All isolates were considered to be acquired nosocomially, with median length of hospital stay before the positive culture result of 18 days. All the patients were placed on contact precautions; screening stool surveillance cultures were not performed. Patients with cultures that yielded carbapenem-resistant *K pneumoniae* were found on 6 different floors in hospital B; again, epidemiologic links were not identified. Of the 27 cultures with positive results, 6

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### Table 1. Susceptibility Results of 602 Isolates of *Klebsiella pneumoniae* Collected During a Citywide Surveillance Conducted in 2003

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Susceptible, %</th>
<th>Susceptibility Break Point, µg/mL</th>
<th>MIC&lt;sub&gt;50&lt;/sub&gt;, µg/mL</th>
<th>MIC&lt;sub&gt;90&lt;/sub&gt;, µg/mL</th>
<th>Range, µg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cefoxitin</td>
<td>67</td>
<td>≤8</td>
<td>8</td>
<td>&gt;32</td>
<td>1 to &gt;32</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>61</td>
<td>≤8</td>
<td>0.125</td>
<td>&gt;64</td>
<td>0.015 to &gt;64</td>
</tr>
<tr>
<td>Cefazidime</td>
<td>60</td>
<td>≤8</td>
<td>0.5</td>
<td>&gt;32</td>
<td>0.03 to &gt;32</td>
</tr>
<tr>
<td>Cefepime</td>
<td>87</td>
<td>≤8</td>
<td>0.125</td>
<td>16</td>
<td>0.008 to &gt;32</td>
</tr>
<tr>
<td>Imipenem</td>
<td>98</td>
<td>≤4</td>
<td>0.25</td>
<td>1</td>
<td>0.03 to &gt;32</td>
</tr>
<tr>
<td>Meropenem</td>
<td>98</td>
<td>≤4</td>
<td>0.03</td>
<td>0.125</td>
<td>0.004 to &gt;32</td>
</tr>
<tr>
<td>Ertapenem</td>
<td>94</td>
<td>≤2</td>
<td>0.03</td>
<td>0.5</td>
<td>0.002 to &gt;32</td>
</tr>
<tr>
<td>Piperacillin-tazobactam</td>
<td>66</td>
<td>≤16/4</td>
<td>8</td>
<td>&gt;256</td>
<td>0.5/4 to &gt;256/4</td>
</tr>
<tr>
<td>Amikacin</td>
<td>75</td>
<td>≤16</td>
<td>2</td>
<td>32</td>
<td>0.25 to 64</td>
</tr>
<tr>
<td>Tigecycline*</td>
<td>96</td>
<td>≤2</td>
<td>0.5</td>
<td>2</td>
<td>0.06 to &gt;8</td>
</tr>
<tr>
<td>Trimethoprim-sulfamethoxazole</td>
<td>52</td>
<td>≤≤2/36</td>
<td>2</td>
<td>&gt;4</td>
<td>0.5/9.3 to &gt;8/76</td>
</tr>
</tbody>
</table>

Abbreviations: MIC<sub>50</sub>, minimal inhibitory concentration for 50% of isolates; MIC<sub>90</sub>, minimal inhibitory concentration for 90% of isolates.

*Investigational antibiotic.

### Table 2. Susceptibility Results for 29 Isolates of *Klebsiella pneumoniae* Possessing the KPC-2 β-Lactamase

<table>
<thead>
<tr>
<th>Ribotype</th>
<th>Isolates, No.</th>
<th>Hospitals, No.</th>
<th>Amikacin</th>
<th>Tigecycline</th>
<th>Polymyxin</th>
<th>Ciprofloxacin</th>
<th>Piperacillin-Tazobactam</th>
<th>Cefotetan</th>
<th>Ceftriaxone</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1</td>
<td>21</td>
<td>5</td>
<td>32-96</td>
<td>0.5-3</td>
<td>1-48</td>
<td>&gt;32</td>
<td>&gt;256</td>
<td>8 to &gt;256</td>
<td>&gt;32</td>
</tr>
<tr>
<td>R2</td>
<td>6</td>
<td>5</td>
<td>2-48</td>
<td>1-2</td>
<td>2-8</td>
<td>&gt;32</td>
<td>&gt;256</td>
<td>&gt;256</td>
<td>&gt;32</td>
</tr>
<tr>
<td>R3</td>
<td>1</td>
<td>1</td>
<td>32</td>
<td>2</td>
<td>2</td>
<td>8</td>
<td>&gt;256</td>
<td>32</td>
<td>&gt;32</td>
</tr>
<tr>
<td>R4</td>
<td>1</td>
<td>1</td>
<td>≤≤1</td>
<td>0.5</td>
<td>2</td>
<td>0.06</td>
<td>&gt;256</td>
<td>8</td>
<td>&gt;32</td>
</tr>
</tbody>
</table>

Abbreviation: MIC, minimal inhibitory concentration.
The medical records of 60 patients were reviewed (5 patients identified in the surveillance study, 32 patients from hospital A, and 23 patients from hospital B). Virtually all the patients had severe debilitating illnesses and had received multiple antibiotic agents before the initial culture. Previous antibiotic therapy included fluoroquinolones in 36 patients (60%), β-lactam/β-lactamase inhibitor antibiotics in 36 (60%), aminoglycosides in 15 (25%), cephalosporins in 15 (25%), and carbapenems in 12 (20%). Forty-three patients (72%) were considered to have nosocomial infections. Particular attention was paid to the outcome of patients with bacteremia with imipenem-resistant *K pneumoniae*. Of the 22 patients with bacteremia, 19 had complete medical records available for review. The source of bacteremia was considered to be the urinary tract in 4 patients, intravenous catheters in 4, the respiratory tract in 3, and skin and soft tissue in 1. In 7 patients, an obvious source could not be determined. After 14 days, 9 (47%) of the 19 patients had died. Two patients died the same day of the initial blood culture. Nine patients received combinations of antibiotics to which the isolates were resistant; 6 died, 2 were microbiologic failures who died after 14 days, and 1 survived. The remaining 8 patients received therapy with agents to which the isolate was deemed susceptible: polymyxin B sulfate (n=4), doxycycline hyclate (n=2), ciprofloxacin (n=1), and gentamicin sulfate (n=1). One patient died, 1 was considered a microbiologic failure and subsequently died, and 6 survived.

**MICROBIOLOGIC CHARACTERIZATION**

The 9 isolates from the citywide surveillance study, 11 isolates from the outbreak in hospital A, and 9 isolates from hospital B were available for characterization. Of the 29 isolates, 21 belonged to ribotype 1, 6 to ribotype 2, and 2 to unique ribotypes (Table 2). Of the 11 isolates from hospital A, 10 belonged to ribotype 1. Similarly, of the 9 isolates from hospital B, 8 belonged to ribotype 1. Isoelectric focusing revealed the presence of a β-lactamase with an isoelectric point (pI) of 6.7 in all isolates. Nucleotide sequencing identified the gene encoding KPC-2 in all 29 isolates. All isolates had other β-lactamases characterized, and nucleotide sequences encoding TEM-1 and SHV-11 were found in all 5.

Twelve isolates, including 6 from ribotype 1 and 6 from ribotype 2, underwent analysis of outer membrane pro-
teins. An obvious loss of an outer membrane protein was evident in only some of the carbapenem-resistant isolates (data not shown). In addition, susceptibility to carbapenems did not change after 20 subcultures on antibiotic-free media, suggesting a stable mechanism of carbapenem resistance.

**COLONIZATION STUDY**

A point prevalence survey that involved patients in 3 intensive care areas was conducted at 2 other hospitals; an outbreak of carbapenem-resistant *K pneumoniae* had been ongoing in these units. Of the 36 patients included in the study, 14 (39%) were found to have gastrointestinal colonization with carbapenem-resistant *K pneumoniae*. Only 2 (14%) of the 14 colonized patients had a previous clinical culture that identified a carbapenem-resistant *K pneumoniae*. In addition, 2 of 39 cultures from intravenous poles, 5 of 35 cultures from blood pressure cuffs, and 2 of 37 cultures from bed rails grew KPC-possessing *K pneumoniae*.

In this study, 45% of all *K pneumoniae* isolates collected from 11 hospital-based microbiology laboratories were found to possess ESBLs. Carbapenems are considered the therapeutic agents of choice to treat serious infections caused by these pathogens. To date, carbapenem resistance among significant nosocomial pathogens has been largely confined to *Pseudomonas aeruginosa* and *Acinetobacter baumannii*; the finding of efficient carbapenem-hydrolyzing β-lactamases remains unusual in the United States.

KPC-type β-lactamases are class A β-lactamases that efficiently hydrolyze carbapenem antibiotics. The first description of a KPC enzyme was in 2001 in an isolate of *K pneumoniae* from North Carolina. Although KPC-type enzymes have been described in sporadic isolates of *E coli*, *Salmonella enterica*, and *Enterobacter* species, most studies have involved *K pneumoniae*, including an outbreak involving 24 intensive care patients in 1 New York City hospital. The ribotype of a representative isolate from this outbreak in Manhattan did not match any of the ribotypes of the Brooklyn isolates (data not shown). The finding of KPC-2 in 3.3% of ESBL-possessing *K pneumoniae* from Brooklyn was disconcerting. The subsequent outbreaks at area hospitals 1 year later suggest that these pathogens are spreading rapidly.

Most patients in this study had been heavily treated with antibiotics before the culture that revealed imipenem-resistant *K pneumoniae*. Although case-cohort studies are necessary to accurately identify risk factors, only 20% of the patients had recent previous therapy with a carbapenem. In contrast, virtually all patients had received previous therapy with a β-lactam or a fluoroquinolone. Treatment of infections caused by KPC-possessing *K pneumoniae* will be difficult because most isolates were resistant to all β-lactam antibiotics, fluoroquinolones, and aminoglycosides. Several isolates were also resistant to polymyxin B sulfate, an antibiotic considered to be a last resort for many gram-negative infections. Many isolates were susceptible to tigecycline sodium, an investigational glycyclcline. Whether this agent demonstrates clinical success remains to be determined. Although the attributable mortality remains to be defined, the fact that 47% of bacteremic patients in this study died within 14 days of the initial positive culture emphasizes the need for an effective therapeutic regimen for these pathogens.

Controlling the spread of KPC enzymes will be difficult. Previous studies have documented that these enzymes reside on transmissible plasmids. The fact that different genera of bacteria, and different strains of *K pneumoniae* identified in this study, have been found to carry KPC β-lactamases is a testament to their ease of transmission. Detection of isolates that carry these enzymes may be especially difficult. Using standard broth microdilution methods, some isolates have MICs of imipenem that are below the break point for resistance. Several of the clinical microbiology laboratories, which use automated broth microdilution systems, failed to identify some KPC-possessing isolates as being resistant to imipenem. For these isolates, a moderate decrease in the inoculum can lead to an inaccurate susceptibility result when using the broth microdilution technique. All of the isolates were highly resistant to carbapenems when tested using Etest or disk diffusion methods, which use much higher inoculum concentrations than the broth microdilution method. For laboratories that use automated systems, additional testing using agar diffusion methods may be needed to identify all KPC-possessing isolates. Because KPC-possessing *K pneumoniae* reported to date have all been resistant to third-generation cephalosporins, confirming carbapenem susceptibility for cephalosporin-resistant Enterobacteriaceae using an agar diffusion method may be necessary.

Our results also suggest that clinical cultures cannot be relied on to identify patients colonized with these pathogens. In high-risk situations, targeted surveillance to identify patients with gastrointestinal colonization will be needed. No commercially available media is appropriate for the screening of carbapenem-resistant *K pneumoniae*. Finally, it is likely that intensive regional efforts, similar to those used to control vancomycin-resistant enterococci, are needed to identify and contain the spread of resistant *Klebsiella* species in New York City.

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