Carbapenemase-producing *Klebsiella pneumoniae* in Brooklyn, NY: molecular epidemiology and *in vitro* activity of polymyxin B and other agents

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Objectives: To describe the molecular epidemiology of carbapenem-resistant *Klebsiella pneumoniae* in Brooklyn, NY and assess the *in vitro* activity of various antibiotic combinations.

Methods: Clinical isolates with suspected carbapenem resistance were referred to the central research laboratory from August 2003 to June 2004. Isolates underwent MIC testing, ribotyping, and were analysed for the presence of KPC carbapenemases. Time–kill studies using various antibiotic(s) were performed on selected isolates.

Results: Ninety-six isolates were referred from 10 Brooklyn hospitals. All isolates were resistant to the carbapenems with most having MICs >32 mg/L. Few were susceptible to fluoroquinolones and cephalosporins; approximately half were susceptible to aminoglycosides, and 90% to polymyxin B. Two-thirds were susceptible to doxycycline, and all were considered susceptible to the investigational glycylcycline antibiotic tigecycline. Virtually all possessed *bla*KPC, and over 80% belonged to one ribotype. In time–kill studies involving 16 isolates, tigecycline demonstrated bacteriostatic activity and polymyxin B concentration-dependent bactericidal activity. The combination of polymyxin B at 0.5 × MIC plus rifampicin had synergic activity against 15/16 isolates, including two polymyxin-resistant strains. The combination of polymyxin B plus imipenem had synergic bactericidal activity against 10/16 isolates, but was antagonistic for three isolates.

Conclusions: Multiresistant *K. pneumoniae* with *bla*KPC are present in multiple hospitals in New York City. The most consistently active agents *in vitro* were tigecycline and polymyxin B, particularly when the latter was combined with rifampicin. The clinical efficacy of these agents remains to be determined.

Keywords: antibiotic resistance, β-lactamases, imipenem, tigecycline

Introduction

Carbapenem antibiotics have been important agents for the management of Gram-negative infections, particularly when caused by difficult nosocomial pathogens. Carbapenems are considered to be the agents of choice for the treatment of infections due to Enterobacteriaceae possessing extended-spectrum β-lactamases (ESBLs). The prevalence of ESBL-producing *Klebsiella pneumoniae* has been rising in the United States, and is approaching 50% of isolates in some regions. When such high rates of ESBL-producing organisms are encountered, carbapenems become an increasingly important therapeutic option.

Over the past few years, a progressive increase in carbapenem-resistant Gram-negative bacteria, particularly *Acinetobacter baumannii* and *Pseudomonas aeruginosa*, has been observed in some areas. In the United States, carbapenem resistance has been largely attributed to expression of a class C cephalosporinase and loss of outer membrane porins in isolates of *A. baumannii*, *P. aeruginosa*, and rarely, *K. pneumoniae*. Carbapenem-hydrolysing β-lactamases have been rarely recovered in *K. pneumoniae*. However, isolates possessing KPC-1, KPC-2, and KPC-3, class A enzymes, have been recently identified in the northeastern United States. These isolates are often resistant to multiple antibiotic classes, presenting clinicians with...
very limited therapeutic options. In this report, the molecular epidemiology of a large number of referred isolates of carbapenem-resistant _K. pneumoniae_ is described, and the _in vitro_ activity of several antibiotic agents is evaluated.

**Methods**

Between August 2003 and June 2004, isolates of _K. pneumoniae_ were referred to the central research laboratory from the microbiology laboratories of 10 Brooklyn, NY hospitals. Isolates were referred because of their unusual resistance to carbapenem antibiotics. MIC testing was performed by the agar dilution or broth microdilution methods according to NCCLS guidelines for the following antibiotics: imipenem, meropenem, ertapenem, cefetetan, ceftazidime, cefepime, piperacillin plus tazobactam (4 mg/L), gentamicin, tobramycin, amikacin, ciprofloxacin, doxycycline, tigecycline, chloramphenicol, polymyxin B and rifampicin. In addition, imipenem was tested in combination with clavulanic acid (2 mg/L). Susceptibility breakpoints were as recommended by the NCCLS; a breakpoint of 2 mg/L has been recommended for polymyxin B and proposed for tigecycline. MIC testing was performed as previously described. The concentrations tested were chosen to reflect clinically relevant levels. Antibiotic carryover was eliminated by diluting the final cultures 200-fold in pour plates. Separate experiments demonstrated no antibiotic carryover effect at this dilution. Bactericidal activity was defined as a decrease of ≥2 log cfu/mL in 24 h. Synergy was defined as a ≥100-fold increase in killing at 24 h by a combination compared with the most active single agent, assuming no significant inhibition by at least one agent. Antagonism was defined as a ≥100-fold decrease in killing at 24 h by a combination compared with the most active single agent.

**Results**

A total of 96 isolates were referred to the central research laboratory during the study period. The results of susceptibility testing are shown in Table 1. The isolates were highly resistant to the carbapenems with nearly all having an MIC > 32 mg/L. The addition of clavulanic acid to imipenem lowered the MIC twofold in approximately one-third of isolates. Whereas 45% and 66% were susceptible to amikacin and doxycycline, respectively, nearly all had MICs at the respective breakpoints of 16 and 4 mg/L. Over 90% remained susceptible to polymyxin B, and all were inhibited by tigecycline 2 mg/L (Table 1).

PCR testing revealed the presence of a _blaKPC_ gene in 95 of 96 isolates. Ribotyping of the 95 KPC-positive isolates revealed eight distinct ribotypes (Figure 1). However, 78/95 (82%) were a single type (ribotype 1), 12/95 (13%) were a second type (ribotype 2), and the remaining six isolates were unique types. Isolates belonging to ribotypes 1 and 2 were detected at 9/10 and 7/10 hospitals, respectively. PFGE generally correlated with the ribotyping results, but only identified five of eight strain types (Figure 1).

Sixteen isolates were selected for further _in vitro_ testing, including the six unique strains and 10 representative isolates of ribotypes 1 and 2. The ribotype results for the 16 isolates are shown in Table 2.

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**Table 1. Susceptibility results for 96 isolates of _K. pneumoniae_ from 10 Brooklyn, NY hospitals**

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Susceptible</th>
<th>Intermediate</th>
<th>Resistant</th>
<th>MIC&lt;sub&gt;50&lt;/sub&gt;</th>
<th>MIC&lt;sub&gt;90&lt;/sub&gt;</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Imipenem</td>
<td>0%</td>
<td>1%</td>
<td>99%</td>
<td>&gt;32</td>
<td>&gt;32</td>
<td>8–&gt;32</td>
</tr>
<tr>
<td>Meropenem</td>
<td>1%</td>
<td>0%</td>
<td>99%</td>
<td>&gt;32</td>
<td>&gt;32</td>
<td>4–&gt;32</td>
</tr>
<tr>
<td>Ertapenem</td>
<td>0%</td>
<td>0%</td>
<td>100%</td>
<td>&gt;32</td>
<td>&gt;32</td>
<td>8–&gt;32</td>
</tr>
<tr>
<td>Cefotetan</td>
<td>59%</td>
<td>18%</td>
<td>23%</td>
<td>16</td>
<td>&gt;64</td>
<td>1–&gt;64</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>2%</td>
<td>0%</td>
<td>98%</td>
<td>&gt;32</td>
<td>&gt;32</td>
<td>2–&gt;32</td>
</tr>
<tr>
<td>Cefepime</td>
<td>40%</td>
<td>30%</td>
<td>30%</td>
<td>&gt;32</td>
<td>&gt;32</td>
<td>2–&gt;32</td>
</tr>
<tr>
<td>Piperacillin/tazobactam</td>
<td>0%</td>
<td>1%</td>
<td>99%</td>
<td>&gt;128</td>
<td>&gt;128</td>
<td>64–&gt;128</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>61%</td>
<td>6%</td>
<td>33%</td>
<td>2</td>
<td>&gt;16</td>
<td>≤0.25–&gt;16</td>
</tr>
<tr>
<td>Tobramycin</td>
<td>3%</td>
<td>3%</td>
<td>94%</td>
<td>&gt;16</td>
<td>&gt;16</td>
<td>0.25–&gt;16</td>
</tr>
<tr>
<td>Amikacin</td>
<td>45%</td>
<td>52%</td>
<td>3%</td>
<td>32</td>
<td>32</td>
<td>0.5–&gt;64</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>2%</td>
<td>0%</td>
<td>98%</td>
<td>&gt;8</td>
<td>&gt;8</td>
<td>≤0.125–&gt;8</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>66%</td>
<td>10%</td>
<td>24%</td>
<td>4</td>
<td>&gt;32</td>
<td>1–&gt;32</td>
</tr>
<tr>
<td>Tigecycline</td>
<td>100%</td>
<td>0%</td>
<td>0%</td>
<td>0.5</td>
<td>1</td>
<td>0.25–2</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>4%</td>
<td>7%</td>
<td>89%</td>
<td>16</td>
<td>&gt;16</td>
<td>≤0.25–&gt;16</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>7%</td>
<td>0%</td>
<td>93%</td>
<td>&gt;32</td>
<td>&gt;32</td>
<td>≤1–&gt;32</td>
</tr>
<tr>
<td>Polymyxin B</td>
<td>91%</td>
<td></td>
<td>9%</td>
<td>2</td>
<td>2</td>
<td>0.5–16</td>
</tr>
</tbody>
</table>
in Figure 1. Representative isolates from ribotypes 1 and 2 were selected to include some that were susceptible and resistant to gentamicin and doxycycline. Two of the isolates were resistant to polymyxin B (MICs 8 and 16 mg/L) and the remainder had MICs of 0.5–2 mg/L. Sequencing of the \( bla_{KPC} \) gene revealed that all 16 possessed KPC-2.

The results of time–kill studies for various antibiotics and antibiotic combinations are summarized in Table 2. At 24 h, imipenem, rifampicin, doxycycline, cefotetan, and the combination of imipenem plus clavulanate produced no significant inhibition. Tigecycline was bactericidal against two isolates, and generally produced a bacteriostatic effect. Gentamicin was rapidly bactericidal against gentamicin-susceptible isolates. The combinations of gentamicin with imipenem, doxycycline, or tigecycline produced no interaction. Polymyxin B demonstrated concentration-dependent killing, and was bactericidal against most strains at 2 or 4 mg/L. The combination of polymyxin B plus rifampicin was bactericidal against 15/16 isolates at 1 mg/L polymyxin, was synergic for 15/16 isolates at 0.5 \( \times \) MIC of polymyxin, and was consistently the most active regimen. For the isolate with a polymyxin B MIC of 16 mg/L, a decrease of \( \sim 2 \) log cfu/mL was demonstrated with the combination of rifampicin with subinhibitory concentrations of polymyxin B. The combination of polymyxin B (0.5 \( \times \) MIC) with imipenem was synergic for 10/16 isolates but was antagonistic for three isolates. The addition of imipenem to the combination of polymyxin B plus rifampicin had no effect.

Discussion

Over the past decade, carbapenem resistance has become a serious problem that primarily affected non-lactose-fermenting bacteria. Carbapenem-resistant Enterobacteriaceae have remained uncommon. In a previous report involving \( \sim 600 \) isolates of \( K. \) pneumoniae collected during a citywide survey in Brooklyn in 2003, nine KPC-producing isolates were recovered. The current report provides evidence that these multiresistant pathogens are an increasing problem. Isolates from nearly 100 unique patients were referred during an 11 month period. The isolates were selected at the discretion of the individual microbiology laboratories and did not necessarily include all carbapenem-resistant isolates. In addition, the presence of carbapenem resistance in KPC-producing \( K. \) pneumoniae may be missed by automated microdilution susceptibility testing. These factors suggest that an even greater number of patients may have been affected during the study period.

The carbapenem-hydrolysing enzymes KPC-1, KPC-2 and KPC-3 have been demonstrated to reside on plasmids, facilitating the transmission of carbapenem resistance among strains. Indeed, ribotyping of this collection revealed that eight unrelated strains possessed KPC-2. KPC-2 has also been found in two strains of Enterobacter sp. and one strain of \( K. \) oxytoca in New York City. Nevertheless, the fingerprinting data demonstrate that the problem is primarily clonal in nature. The presence of this highly resistant clone in most regional hospitals suggests that joint efforts

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**Figure 1.** Ribotyping (top) and PFGE (bottom) results for 16 isolates of carbapenem-resistant \( K. \) pneumoniae possessing KPC-2.
at patient identification and infection control will be important to contain the spread of this problem.

The isolates collected in this study were not only broadly resistant to β-lactams, but also to fluoroquinolones and variably to aminoglycosides. In many cases, none of the commonly used antibiotics are likely to be useful and effective therapeutic regimens have not been defined. Although many isolates were susceptible to doxycycline, the MICs of these isolates were at the NCCLS breakpoint of 4 mg/L. In time–kill studies, doxycycline was ineffective at a clinically relevant concentration. Therefore, the use of doxycycline against these strains cannot be recommended. Gentamicin demonstrated bactericidal activity for gentamicin-resistant isolates, and may be considered for infections likely to respond to aminoglycoside therapy. Tigecycline, an experimental glycylic cycle antibiotic, was generally bacteriostatic at 2 mg/L and may prove to be a clinically relevant concentration. Therefore, the use of doxycycline in this setting are lacking.

Increasingly, clinicians are using polymyxins as agents of last resort for multiresistant Gram-negative pathogens. Preliminary data on the use of colistin for carbapenem-resistant A. baumannii and P. aeruginosa suggest that this agent is useful. A single case has also been reported of the successful use of colistin for sepsis due to carbapenem-resistant K. pneumoniae. This study provides evidence of concentration-dependent bactericidal activity of polymyxin B against a collection of multiresistant K. pneumoniae isolates. The combination of polymyxin B plus rifampicin was synergic against nearly all isolates, including two isolates that were resistant to polymyxin B. Polymyxin B plus rifampicin may remain a treatment option for such strains. Whether this combination proves to be clinically effective, and whether use of the combination can prevent the emergence of polymyxin resistance, remains to be determined.

Carbapenem-resistant K. pneumoniae possessing KPC enzymes appear to be spreading through hospitals in New York City. The outbreak is characterized by the presence of multiple clones, with one dominant strain affecting most hospitals. In vitro data suggest that polymyxin B ± rifampicin, or possibly tigecycline alone, may prove to be useful therapeutic options. Coordinated infection control efforts will be important to help contain the spread of these pathogens.

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References


