Citywide Clonal Outbreak of Multiresistant Acinetobacter baumannii and Pseudomonas aeruginosa in Brooklyn, NY

The Preantibiotic Era Has Returned

David Landman, MD; John M. Quale, MD; David Mayorga, MD; Adeleyo Adeleji, MD; Kalyani Vangala, MD; Jayshree Ravishankar, MD; Carlos Flores, MD; Steven Brooks, PhD

Background: Carbapenems are important agents for treating nosocomial gram-negative infections. Carbapenem-resistant bacteria have become increasingly problematic in certain regions. This study determined the citywide prevalence and molecular epidemiological features of carbapenem-resistant Acinetobacter baumannii and Pseudomonas aeruginosa in Brooklyn, NY.

Methods: All unique patient isolates of A. baumannii and P. aeruginosa were collected from 15 Brooklyn hospitals from July 1, 1999, through September 30, 1999. Antibiotic susceptibilities, the genetic relatedness of resistant isolates, and the relationship between antibiotic use and resistance rates were determined.

Results: A total of 419 isolates of A. baumannii and 823 isolates of P. aeruginosa were collected. For A. baumannii, 53% were resistant to meropenem and/or imipenem, and 12% were resistant to all standard antibiotics. Ribotyping revealed that a single clone accounted for 62% of the samples and was isolated from patients at all 15 hospitals. The rate of carbapenem resistance was associated with cephalosporin use at each hospital (P = .004). For P. aeruginosa, 24% were resistant to imipenem, 5% to amikacin, and 15% to 29% to other antipseudomonal agents. Ribotyping revealed that 3 clones accounted for nearly half of the isolates and were shared by most hospitals.

Conclusions: Approximately 400 patients were infected or colonized with carbapenem-resistant A. baumannii and P. aeruginosa during a 3-month period in 1999. A few strains have spread widely throughout hospitals in this region. The prevalence of resistant A. baumannii seems to be correlated with cephalosporin use. Multiresistant hospital-acquired bacteria should be viewed as a serious public health issue rather than an individual hospital's problem. An intensive coordinated effort will be needed to effectively address this problem.

Arch Intern Med. 2002;162:1515-1520

THE INTRODUCTION of carbapenem antibiotics in the late 1980s gave clinicians a powerful tool for the management of nosocomial gram-negative infections. Bacterial resistance to the ureidopenicillins, cephalosporins, and fluoroquinolones has been increasing among several gram-negative species, such as Pseudomonas aeruginosa, Klebsiella pneumoniae, Enterobacter cloacae, and Acinetobacter baumannii. Because of this problem, the carbapenems have become increasingly important agents in many areas. Unfortunately, resistance to carbapenems has developed among these species, particularly A. baumannii.1,2 These organisms are frequently resistant to multiple classes of antibiotics, and are often susceptible only to polymyxins. The prevalence of carbapenem-resistant A. baumannii from 158 hospitals around the United States was 3% from 1994 to 1998.3 These organisms have become endemic to hospitals in Brooklyn, NY.2 Pseudomonas aeruginosa is an established nosocomial pathogen and a common cause of bacteremia in patients in the intensive care unit. While hospital outbreaks of resistant strains have been reported, overall imipenem nonsusceptibility has been approximately 15% of isolates in large North American surveillance studies.4,5 Because of the importance of P. aeruginosa as a hospital pathogen, increasing carbapenem resistance would be viewed as a serious problem. In this study, we assessed the ongoing problem of carbapenem resistance in Brooklyn by determining the prevalence of antibiotic-resistant A. baumannii and P. aeruginosa and the molecular epidemiological features of these organisms at Brooklyn hospitals. Finally, the association between carbapenem resistance rates and antibiotic use at the hospitals was evaluated.
MATERIALS AND METHODS

BACTERIAL STRAINS

The 15 hospitals participating in the study were as follows: Beth Israel Medical Center (Kings Highway), Brooklyn Medical Center, Brooklyn Hospital, Coney Island Hospital, Department of Veterans Affairs Medical Center at Brooklyn, Interfaith Medical Center, Kings County Hospital, Kingsbrook Medical Center, Long Island College Hospital, Lutheran Hospital, Maimonides Medical Center, University Hospital, Victory Memorial Hospital, Woodhull Medical Center, and Wyckoff Heights Medical Center. All unique patient isolates of A baumannii and P aeruginosa recovered from routine clinical cultures from July 1, 1999, through September 30, 1999, were collected. Isolates were identified by the participating microbiology laboratories using standard techniques. Isolates were stored at -70°C in Mueller-Hinton broth with 30% glycerol until testing.

SUSCEPTIBILITY TESTING

Minimal inhibitory concentrations were determined for all isolates by the agar-dilution method, as recommended by the National Committee for Clinical Laboratory Standards. Ampicillin and sulbactam were tested at a 2:1 ratio, and the concentration of tazobactam was fixed at 4 µg/mL. For polymyxin B sulfate, the susceptibility break point was considered to be 4 µg/mL.6 The susceptibility break point for the other agents were as recommended by the National Committee for Clinical Laboratory Standards. For study purposes, isolates with intermediate susceptibility or resistance were defined as resistant.

GENETIC STUDIES

Genetic fingerprinting was performed using one system (RiboPrinter Microbial Characterization System; Qualicon, Wilmington, Del). Automated ribotyping was done on nearly all meropenem- or imipenem-resistant A baumannii isolates (minimal inhibitory concentration, ≥8 µg/mL) and on nearly all imipenem-resistant P aeruginosa isolates (minimal inhibitory concentration, ≥16 µg/mL). Typing was also done on a smaller number of carbapenem-susceptible isolates of A baumannii randomly selected from 5 different hospitals. Ribosomal DNA was digested with EcoRI and PvuII for A baumannii and P aeruginosa, respectively, according to the manufacturer’s instructions. Pulsed-field gel electrophoresis (PFGE) was performed on a select number of carbapenem-resistant isolates of A baumannii selected from different hospitals using Smal and NotI (New England Biolabs, Beverly, Mass), as previously described. The PFGE patterns were interpreted according to the recommendations of Tenover et al.5

ANTIBIOTIC USE DATA

Information on antibiotic use during the study period was available from the pharmacies of 12 of the 15 participating hospitals. In most cases, the actual number of units of each parenteral antibiotic dispensed during the study period was used. In some cases, only the number of units purchased during the 6-month period from April 1, 1999, through September 30, 1999, was available. Using the average daily census for each hospital, antibiotic use was expressed as the number of defined daily doses6 of each antibiotic class dispensed (or purchased) per 1000 patient-days.

STATISTICAL ANALYSES

The relationship between parenteral antibiotic use and carbapenem resistance rates at each hospital was assessed by a stepwise multiple linear regression analysis using computer software (Statistical Product and Service Solutions; SPSS Inc, Chicago, Ill). The following antibiotic classes were included in the analysis: aminoglycosides, β-lactamase inhibitor combinations, carbapenems, all cephalosporins plus aztreonam, and fluoroquinolones. The average length of stay and the average daily census for each hospital were also included as independent variables. For categorical variables, χ2 and Fisher exact tests were used. Statistical significance was defined as P≤.05.

This study was approved by the Institutional Review Board at State University of New York Downstate Medical Center.

RESULTS

ACINETOBACTER BAUMANNII

Susceptibility Studies

A total of 419 unique patient isolates of A baumannii were collected during the 3-month period. The sources of the isolates were as follows: blood and body fluids, 14%; respiratory, 46%; urine, 19%; wounds, 17%; catheters, 2%; and unavailable, 2%. The results of the susceptibility testing are shown in Table 1. Overall, more than 50% of the isolates were resistant to 1 or both carbapenems and 12% were resistant to all commonly used antibiotics. Carbapenem-resistant isolates were found in all 15 hospitals, with resistance rates ranging from 20% to 76% of all isolates. The activity of imipenem was generally 2-fold greater than that of meropenem, and isolates with intermediate susceptibility to meropenem were often susceptible to imipenem at the break point (4 µg/mL). Approximately two thirds of the isolates were susceptible to amikacin and sulbactam, and only about one third were susceptible to ceftazidime and a combination of piperacillin and tazobactam. Among the carbapenem-resistant isolates, only half were susceptible to amikacin and sulbactam. Most of these isolates (97%) were susceptible to polymyxin B sulfate and resistant to the remaining antibiotics. Susceptibility did not differ by site of isolation. Five isolates were resistant to all the antibiotics tested.

Genetic Studies

Ribotype profiles were obtained for 224 carbapenem-resistant isolates of A baumannii (Table 2). A total of 10 unique ribotypes were identified. However, 4 strains (Aci-
Netobacter types 1-4) (Table 2) accounted for 97% of the isolates and 1 strain (Acinetobacter type 1) accounted for 62% of all isolates. Isolates from Acinetobacter type 1 were recovered at all 15 Brooklyn hospitals, and isolates from types 2 through 4 were recovered at 5 to 8 of the hospitals. Isolates from Acinetobacter type 1 were less likely to be susceptible to ampicillin or sulbactam than other carbapenem-resistant isolates (39% vs 71%; \( P < 0.001 \)). The 5 panresistant isolates included 2 belonging to type 1, 2 belonging to type 3, and 1 unique strain. Ribotype profiles were also obtained for 48 carbapenem-susceptible isolates randomly selected from 5 of the hospitals. In contrast to the genetic similarity of most carbapenem-resistant isolates, 35 of the 48 susceptible isolates were unique types, and only 2 were shared by more than a single hospital.

Pulsed-field gel electrophoresis was performed on 20 carbapenem-resistant isolates with 4 distinct ribotype profiles. Ribotype profiles and PFGE results of a sample of resistant isolates are shown in the Figure. Pulsed-field gel electrophoresis revealed 3 unique clones. Automated ribotyping and PFGE gave comparable results for 19 of the 20 isolates; a single isolate from PFGE clone C had a unique ribotype.

**Antibiotic Use Data**

Information regarding antibiotic use, average length of stay, and average daily inpatient census was available at 12 of the 15 hospitals. Antibiotics were grouped into classes as follows: aminoglycosides, fluoroquinolones, \( \beta \)-lactamase inhibitor combinations, carbapenems, and all cephalosporins plus aztreonam (Table 3). In a multivariate analysis, only the use of cephalosporins plus aztreonam was directly associated with the rate of carbapenem-resistant A baumannii and ceftazidime-resistant...
A *baumannii* at each hospital (*P* = .004 and *P* = .03, respectively).

**Relation Between Cephalosporin and Carbapenem Susceptibility**

Ceftriaxone sodium–resistant isolates exhibited reduced susceptibility to the carbapenems, even among the carbapenem-susceptible subgroup. For example, among the meropenem-susceptible isolates, ceftriaxone-resistant isolates were more likely to have a meropenem minimal inhibitory concentration of 1 µg/mL than ceftriaxone-susceptible isolates (48% vs 17%; *P* < .001). Ceftriaxone susceptibility also predicted the molecular characterization of strains. Of the 48 carbapenem-susceptible isolates that were typed, 11 were resistant to ceftriaxone. Of these 11, 8 had ribotype profiles belonging to *Acinetobacter* types 1 to 4, compared with only 1 of 37 ceftriaxone-susceptible isolates (*P* < .001).

Surveillance cultures were performed from environmental surfaces at 10 participating hospitals. Cultures were positive for carbapenem-resistant *A baumannii* at 7 of the 10 hospitals, particularly involving bed rails and respirator equipment (data not shown). At one hospital, surveillance cultures were taken from soap dispensers (containing 2% chlorhexidine gluconate) in 28 rooms in various patient care areas. Carbapenem-resistant strains of *Acinetobacter*, *Pseudomonas*, and *K pneumoniae* were isolated from 4 of the 28 dispensers.

**PSEUDOMONAS AERUGINOSA**

**Susceptibility Studies**

A total of 823 unique patient isolates of *P aeruginosa* were collected during the 3-month period. The sources of the isolates were as follows: blood and body fluids, 10%; respiratory, 38%; urine, 28%; wounds, 20%; catheters, 1%; and unavailable, 3%. The results of the susceptibility testing are shown in Table 1. Greater than 90% susceptibility was found only for amikacin. Overall, only 76% of the isolates were susceptible to imipenem. Carbapenem-resistant isolates were recovered at all 15 hospitals, with rates ranging from 12% to 58% of *P aeruginosa* isolates. Meropenem was approximately 4-fold more active than imipenem. Six isolates, recovered at 6 different hospitals, were susceptible to none of the tested antibiotics. Among the 196 imipenem-resistant isolates, the susceptibility rates to amikacin, piperacillin-tazobactam, ceftazidime, cefepime, meropenem, and ciprofloxacin were 87%, 61%, 60%, 42%, 31%, and 28%, respectively. Susceptibility did not differ by site of isolation.

**Genetic Studies**

Ribotype profiles were obtained for 136 imipenem-resistant isolates of *P aeruginosa*. The results are shown in Table 4. A total of 47 unique ribotypes were identified. However, 3 strains (*Pseudomonas* types 1–3) accounted for nearly half of all isolates and were recov-

<table>
<thead>
<tr>
<th>Hospital No.</th>
<th>Average Length of Stay, d</th>
<th>Aminoglycosides</th>
<th>Fluoroquinolones</th>
<th>β-Lactamase Inhibitors</th>
<th>Carbapenem Resist. %</th>
<th>Cefepim. Plus Aztreonam†</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8.75</td>
<td>30</td>
<td>25</td>
<td>57</td>
<td>7</td>
<td>69</td>
</tr>
<tr>
<td>2</td>
<td>6.63</td>
<td>27</td>
<td>31</td>
<td>22</td>
<td>11</td>
<td>66</td>
</tr>
<tr>
<td>3</td>
<td>6.32</td>
<td>9</td>
<td>37</td>
<td>33</td>
<td>19</td>
<td>145</td>
</tr>
<tr>
<td>4</td>
<td>NA</td>
<td>55</td>
<td>51</td>
<td>65</td>
<td>6</td>
<td>155</td>
</tr>
<tr>
<td>5</td>
<td>8.24</td>
<td>35</td>
<td>15</td>
<td>18</td>
<td>7</td>
<td>165</td>
</tr>
<tr>
<td>6</td>
<td>6.22</td>
<td>52</td>
<td>24</td>
<td>33</td>
<td>12</td>
<td>186</td>
</tr>
<tr>
<td>7</td>
<td>9.04</td>
<td>42</td>
<td>46</td>
<td>27</td>
<td>3</td>
<td>196</td>
</tr>
<tr>
<td>8</td>
<td>8.77</td>
<td>36</td>
<td>39</td>
<td>107</td>
<td>13</td>
<td>105</td>
</tr>
<tr>
<td>9</td>
<td>5.53</td>
<td>NA</td>
<td>20</td>
<td>37</td>
<td>15</td>
<td>136</td>
</tr>
<tr>
<td>10</td>
<td>5.97</td>
<td>28</td>
<td>47</td>
<td>5</td>
<td>11</td>
<td>218</td>
</tr>
<tr>
<td>11</td>
<td>6.22</td>
<td>17</td>
<td>20</td>
<td>85</td>
<td>10</td>
<td>173</td>
</tr>
<tr>
<td>12</td>
<td>5.73</td>
<td>49</td>
<td>32</td>
<td>46</td>
<td>16</td>
<td>228</td>
</tr>
</tbody>
</table>

*NA* indicates data not available.
†*P* = .004.
*P* values are for hospital comparison.

<table>
<thead>
<tr>
<th>Ribotype No.</th>
<th>No. (%) of Isolates†</th>
<th>No. of Hospitals†</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>26 (19)</td>
<td>12</td>
</tr>
<tr>
<td>2</td>
<td>20 (15)</td>
<td>10</td>
</tr>
<tr>
<td>3</td>
<td>17 (12)</td>
<td>5</td>
</tr>
<tr>
<td>4</td>
<td>6 (4)</td>
<td>4</td>
</tr>
<tr>
<td>5</td>
<td>5 (4)</td>
<td>3</td>
</tr>
<tr>
<td>6</td>
<td>5 (4)</td>
<td>3</td>
</tr>
<tr>
<td>7</td>
<td>4 (3)</td>
<td>4</td>
</tr>
<tr>
<td>8</td>
<td>4 (3)</td>
<td>3</td>
</tr>
<tr>
<td>9</td>
<td>4 (3)</td>
<td>2</td>
</tr>
<tr>
<td>10</td>
<td>3 (2)</td>
<td>3</td>
</tr>
<tr>
<td>11</td>
<td>3 (2)</td>
<td>2</td>
</tr>
<tr>
<td>12</td>
<td>2 (1)</td>
<td>2</td>
</tr>
<tr>
<td>13</td>
<td>2 (1)</td>
<td>1</td>
</tr>
<tr>
<td>14</td>
<td>2 (1)</td>
<td>1</td>
</tr>
<tr>
<td>15-47</td>
<td>33 (24)</td>
<td>1 Each</td>
</tr>
</tbody>
</table>

*Percentages may not total 100 because of rounding.
†Those with patients having cultures positive for a given ribotype.
ered in 5 to 12 of the participating hospitals. Isolates from *Pseudomonas* type 1 were more likely to be susceptible to ceftazidime (81% vs 50%; *P* = .01) and meropenem (31% vs 9%; *P* = .01) and less likely to be susceptible to ciprofloxacin (8% vs 29%; *P* = .04) than other imipenem-resistant isolates. Isolates from *Pseudomonas* type 2 were more likely to be susceptible to ceftazidime (80% vs 52%; *P* = .04) and piperacillin/tazobactam (100% vs 57%; *P* < .001) than other imipenem-resistant isolates. Finally, isolates from *Pseudomonas* type 3 were less likely to be susceptible to ceftazidime (24% vs 64%; *P* = .01) and ciprofloxacin (0% vs 29%; *P* = .02) than other imipenem-resistant isolates.

Among the 73 imipenem-resistant isolates not belonging to types 1 to 3, 44 unique ribotypes were identified. While 9 of these strains were present in 2 to 5 of the hospitals, most were single unshared isolates. Six isolates were resistant to all of the tested antibiotics. Ribotype profiles were obtained for 5 of these 6 isolates, and all 5 were unique strains.

**Antibiotic Use Data**

For *Pseudomonas*, a multivariate analysis revealed no association between use of any particular antibiotic class, average length of stay, or average daily inpatient census with the carbapenem resistance rates at the hospitals.

**COMMENT**

Carbapenem-resistant *A baumannii* are becoming a worldwide problem, with outbreaks reported in several European countries, 10-14 southeast Asia, 15,1617 South America, 10,11,12,16 Kuwait, 12 and the United States. 1,2,17 From 1997 to 1998, 2.8% to 5.8% of *A baumannii* from hospitals across the United States were nonsusceptible to carbapenems. 3,4

In most reports, these strains were limited to select hospitals and tended to be isolated primarily in patients in the intensive care unit. In 1997, however, carbapenem-resistant *A baumannii* were endemic to most hospitals in Brooklyn. 2

In this survey of clinical isolates from 1999, carbapenem resistance rates remained high at all the area hospitals. A retrospective review at several of the hospitals revealed that more than 35% of *A baumannii* isolates and 30% of *P aeruginosa* isolates represented Centers for Disease Control and Prevention–defined infection and not colonization (data not shown). Approximately half of the patients with *A baumannii* harbored multiresistant strains, including 12% that were resistant to all commonly used antibiotics. Only polymyxin B sulfate, a fairly toxic older agent, retained activity against nearly all isolates. Ribotyping of these isolates demonstrated that 1 strain accounted for two thirds of the isolates and was present in all the hospitals. The PFGE studies demonstrated excellent correlation with automated ribotyping. The spread of the dominant clone among all Brooklyn hospitals may be due to the sequential hospitalization of colonized patients at multiple hospitals. The rotation of medical staff and students among the hospitals may also contribute to the spread. Once this multiresistant strain is introduced into a hospital’s environment, it seems to be difficult to eradicate. *Acinetobacter* species are well-known to contaminate environmental surfaces and patient care items 18 and may spread through the air as well. 19,20 In fact, surveillance cultures of environmental surfaces were positive for carbapenem-resistant *A baumannii* at most participating hospitals. The finding of carbapenem-resistant organisms on soap dispensers at one hospital suggests that the hands of health personnel may be an important vector for nosocomial transmission. Extensive environmental contamination and hand colonization are likely to be important factors in the persistence of this problem.

Various classes of antibiotics have been associated with single-hospital outbreaks of resistant *Acinetobacter*, including cephalosporins, 21-22 fluoroquinolones, 23-24 amikacin, 24 and imipenem. 1 We found a strong association between cephalosporin use and rates of carbapenem-resistant *A baumannii* at hospitals throughout Brooklyn. The ribotyping data suggest that most cephalosporin-resistant *A baumannii* belong to one of the dominant carbapenem-resistant clones. Therefore, it is understandable that higher levels of cephalosporin use (Table 3) might fuel the spread of these organisms. Controlling antibiotic use, particularly cephalosporins, may be an important component of strategies to limit the spread of these pathogens.

The emergence of carbapenem-resistant *P aeruginosa* presents another serious public health problem. The National Nosocomial Infections Surveillance system reported imipenem resistance rates of 17% for intensive care unit areas, 11% for non–intensive care unit inpatient areas, and 7% for outpatient areas in US hospitals in 1999. 25 The imipenem resistance rate of 24% for all patient areas in Brooklyn hospitals in this study is substantially higher. Unlike *A baumannii*, which has been an uncommon nosocomial pathogen in the past, *P aeruginosa* is a well-recognized cause of nosocomial infection. Only amikacin retained reliable activity against the imipenem-resistant *P aeruginosa* isolates in this study. Of obvious concern is the presence of a few isolates that are resistant to all tested antibiotics.

In a previous report, 26 increased imipenem use following an outbreak of extended-spectrum β-lactamase–producing *K pneumoniae* at one hospital resulted in an increase in imipenem-resistant *P aeruginosa*. Hospitals in this region are also facing a high prevalence of resistant *K pneumoniae*, 27 which might cause clinicians to use carbapenems more frequently. However, we were unable to demonstrate any relation between carbapenem use and resistance rates for *P aeruginosa*. Ribotyping revealed that while multiple strains were detected, 3 comprised the bulk of the isolates and were shared by most hospitals. As with *A baumannii*, nosocomial and interhospital transmission of these strains is an important part of the ongoing problem of carbapenem resistance.

In summary, carbapenem-resistant *A baumannii* and *P aeruginosa* are common nosocomial pathogens in hospitals throughout Brooklyn. In just a 3-month period, approximately 400 patients were colonized or infected with carbapenem-resistant strains at our hospitals. In 56 of these cases, the isolate was resistant to all commonly used antibiotics. The rate of resistant *A baumannii* is correlated with cephalosporin use at the hospitals. A few strains...
of each species account for most isolates and are shared by most or all hospitals. This problem should be viewed as a serious regional public health issue rather than an individual hospital problem. Only a coordinated strategy of intense surveillance, infection control measures, and control of inappropriate antibiotic use is likely to effectively deal with this problem.

Accepted for publication November 15, 2001.

This study was supported by AstraZeneca, Wilmington, Del; Bayer Corp, West Haven, Conn; Dura, San Diego, Calif; Merck, West Point, Pa; Roche, Nutley, NJ; and Wyeth-Ayerst Laboratories, Philadelphia, Pa.

This study was presented in part at the 40th Annual Interscience Conference on Antimicrobial Agents and Chemotherapy, Toronto, Ontario, September 17-20, 2000; and the 38th Infectious Diseases Society of America Annual Meeting, New Orleans, La, September 7-10, 2000.

We thank the members of the microbiology laboratories of the 15 participating hospitals for their cooperation with this study.

Corresponding author and reprints: John M. Quale, MD, Department of Medicine, State University of New York Downstate Medical Center, 450 Clarkson Ave, Campus Box 77, Brooklyn, NY 11203 (e-mail: jquale@downstate.edu).

REFERENCES


