Antimicrobial resistance in Enterobacteriaceae in Brooklyn, NY: epidemiology and relation to antibiotic usage patterns

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In November 1997, all Enterobacteriaceae isolated at 15 hospitals in Brooklyn were collected. Extended-spectrum β-lactamases (ESBLs) were present in 44% of 409 Klebsiella pneumoniae isolates. Six isolates had reduced susceptibility to carbapenems, including two that were not susceptible to any of the antibiotics tested. Pulsed field gel electrophoresis revealed a commonality of resistant isolates within and between hospitals. The occurrence of ESBL-containing isolates was associated with cephalosporin usage (P = 0.055). ESBLs were present in 4.7% of Escherichia coli and 9.5% of Proteus mirabilis isolates. It is concluded that ESBL-producing Enterobacteriaceae are endemic in Brooklyn, are spread between hospitals, and may be associated with cephalosporin usage.

Introduction
Enterobacteriaceae that produce extended-spectrum β-lactamases (ESBLs) are an increasing problem world-wide. Many of the outbreaks reported have been associated with cephalosporin usage, and controlling third-generation cephalosporin usage appears to have been useful in limiting some outbreaks.1,2 Whereas localized outbreaks with ESBL-producing Enterobacteriaceae are well described, the overall prevalence of these organisms is unclear. In this paper, the susceptibilities of all Klebsiella pneumoniae, Escherichia coli and Proteus mirabilis isolated during 1 month at 15 hospitals in Brooklyn, New York, USA, were determined. In addition, the relationship between antimicrobial usage and isolation of ESBL-producing bacteria was evaluated, and the molecular epidemiology of these resistant isolates was examined.

Materials and methods
During the month of November 1997, all aerobic bacteria isolated by the microbiology laboratories at 15 hospitals in Brooklyn, New York, were collected. Bacteria that are often considered to be contaminants, such as coagulase-negative staphylococci, were excluded. The 15 hospitals have an average daily census of 354 in-patients (range 154–614), and include nearly all the hospitals in Brooklyn. The laboratories at the hospitals were encouraged to avoid submitting repeat patient isolates.

MICs were tested by the agar dilution method as recommended by the NCCLS.3 Antibiotic powders were obtained from their manufacturers as follows: ampicillin and gentamicin (Sigma Chemical Co., St Louis, MO, USA), ampicillin–sulbactam and trovafloxacin (Pfizer, New York, USA), piperacillin and tazobactam (Wyeth–Ayerst, Philadelphia, PA, USA), cefotetan and meropenem (Zeneca, Wilmington, DE, USA), imipenem (Merck, Rahway, NJ, USA), ceftazidime (Glaxo Wellcome, Research Triangle Park, NC, USA), amikacin, cefepime, gatifloxacin (Bristol–Myers Squibb, Plainsboro, NJ, USA), cefpodoxime (Pharmacia and Upjohn, Kalamazoo, MI, USA), ciprofloxacin (Bayer, West Haven, CT, USA), clavulanic acid (Smith–Kline Beecham, Collegeville, PA, USA). The MIC of ceftazidime was determined alone and in the presence of clavulanic acid or tazobactam, each at a fixed concentration of 4 mg/L. Isolates with cefpodoxime MICs ≥ 2 mg/L were presumed to possess an ESBL.3 ATCC isolates E. coli 25922, E. coli 35218 and Pseudomonas aeruginosa 27853 were used as controls. Pulsed field gel electrophoresis (PFGE) was performed on selected ceftazidime-resistant isolates of K. pneumoniae and E. coli using a modification of a previously described method.4

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Antibiotic usage data, provided by 11 of 15 participating hospitals, were expressed as the average number of defined daily doses per 1000 patient days. When available, the actual number of units of each antibiotic dispensed during November 1997 was used. In some cases, only the average number of units purchased during the 6 months ending in November 1997 was available. The relationship between antibiotic usage and the rate of isolation of ESBL-producing *K. pneumoniae* at each hospital was evaluated by multiple linear regression analysis (True Epistat, Richardson, TX, USA). The average daily census and length of stay for each hospital were included as independent variables, along with the following antibiotic groups: anti-pseudomonal penicillins, β-lactamase inhibitor combinations, cephalosporins plus aztreonam, carbapenems, quinolones and aminoglycosides.

### Results

#### *K. pneumoniae*

A total of 409 isolates of *K. pneumoniae* were collected (17% of all Gram-negative bacteria). Only 62% were susceptible to ceftazidime (Table). The addition of clavulanate or tazobactam substantially increased the susceptibility to ceftazidime. Of note, six isolates from three hospitals had reduced susceptibility to both carbapenems (MIC 8–32 mg/L). Two isolates were not susceptible to any of the antibiotics tested. Overall, 44% of isolates were presumed to contain ESBLs. Although ESBL-containing isolates were obtained at all hospitals, the rate varied from 5 to 71%. Most ESBL-containing isolates remained susceptible to meropenem and cefepime; however, the MIC of cefepime for most isolates was in the 1–8 mg/L range (Table). Approximately half of the ESBL-containing *K. pneumoniae* were susceptible to amikacin, ceftriaxone, piperacillin–tazobactam or quinolones. The addition of clavulanate or tazobactam decreased the MIC of ceftazidime by eight-fold or more for 56 and 50% of these isolates, respectively.

PFGE was performed on 31 ceftazidime-resistant *K. pneumoniae* isolates from 15 hospitals (Figure). Twenty-three unrelated isolates were identified. Both related isolates and unique isolates were often found within a single hospital. There were five occasions when hospitals were found to share related isolates. Three of four carbapenem-resistant isolates were unrelated; one of these was closely related to a carbapenem-susceptible isolate at the same hospital.

Multiple linear regression analysis revealed an association between cephalosporin plus aztreonam usage and the isolation rate of ESBL-containing *K. pneumoniae* at each hospital (*P* = 0.055). No relationship was found between the usage of any other antibiotic group and the ESBL rate. Of note, the hospital with four carbapenem-resistant isolates had the highest rate of imipenem usage of all the hospitals.

#### *E. coli*

A total of 771 isolates of *E. coli* were collected (32% of all Gram-negative bacteria). The rates of resistance to ampicillin, co-trimoxazole and quinolones were 46, 23 and 5%, respectively. More than 95% were susceptible to the aminoglycosides, cephalosporins and meropenem. Fourteen isolates were not susceptible to any of the antibiotics tested. Overall, 44% of isolates were presumed to contain ESBLs. Although ESBL-containing isolates were obtained at all hospitals, the rate varied from 5 to 71%. Most ESBL-containing isolates remained susceptible to meropenem and cefepime; however, the MIC of cefepime for most isolates was in the 1–8 mg/L range (Table). Approximately half of the ESBL-containing *K. pneumoniae* were susceptible to amikacin, ceftriaxone, piperacillin–tazobactam or quinolones. The addition of clavulanate or tazobactam decreased the MIC of ceftazidime by eight-fold or more for 56 and 50% of these isolates, respectively.

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<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>MIC&lt;sub&gt;50&lt;/sub&gt;/MIC&lt;sub&gt;90&lt;/sub&gt;</th>
<th>susceptible (%)</th>
<th>MIC&lt;sub&gt;50&lt;/sub&gt;/MIC&lt;sub&gt;90&lt;/sub&gt;</th>
<th>susceptible (%)</th>
<th>breakpoint MIC</th>
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<tr>
<td>Ampicillin–sulbactam</td>
<td>16/32</td>
<td>45</td>
<td>&gt;64/64</td>
<td>8</td>
<td>≤8/4</td>
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<tr>
<td>Piperacillin</td>
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<td>46</td>
<td>&gt;128/128</td>
<td>5</td>
<td>≤16</td>
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<tr>
<td>Piperacillin–tazobactam</td>
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<td>75</td>
<td>32/128</td>
<td>46</td>
<td>≤16/4</td>
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<td>Cefotetan</td>
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<td>2/64</td>
<td>70</td>
<td>≤8</td>
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<tr>
<td>Ceftriaxone</td>
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<td>16/64</td>
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<td>&gt;64/64</td>
<td>14</td>
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<td>81</td>
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<td>58</td>
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<tr>
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<td>0.06/0.5</td>
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<tr>
<td>Gentamicin</td>
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<td>&gt;16/16</td>
<td>22</td>
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<tr>
<td>Amikacin</td>
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<td>≤16</td>
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<tr>
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<td>&gt;4/4</td>
<td>28</td>
<td>≤2/38</td>
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<tr>
<td>Ciprofloxacin</td>
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<td>50</td>
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<tr>
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<td>78</td>
<td>2/8</td>
<td>54</td>
<td>≤2</td>
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<tr>
<td>Gatifloxacin</td>
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<td>82</td>
<td>1/8</td>
<td>62</td>
<td>≤2</td>
</tr>
</tbody>
</table>
of 15 hospitals submitted at least one ESBL-containing isolate (range 0–11.5%). Overall, the percentage of isolates presumed to contain ESBLs was 4.7%. PFGE performed on 18 of these isolates from six hospitals revealed nine unique isolates (Figure). Four of the hospitals had more than one unique isolate. One related isolate was shared by three hospitals, and related isolates were recovered more than once within two hospitals. No relationship was found between antibiotic usage and the isolation rate of ESBL-containing E. coli.

**P. mirabilis**

A total of 168 isolates of *P. mirabilis* were collected (7% of all Gram-negative bacteria). Surprisingly, 11% were resistant to the fluoroquinolones. Only 72% were susceptible to ampicillin, and 85% to gentamicin. At least 90% of isolates were susceptible to the remaining antibiotics. The MIC of ceftazidime for 16 of the 168 isolates (9.5%) was ≥2 mg/L. All but two of these isolates were susceptible to ampicillin–sulbactam, suggesting the presence of an ESBL.
Discussion

This paper presents a snapshot of the citywide prevalence and epidemiology of ESBL-producing Enterobacteriaceae in Brooklyn. The percentage of *K. pneumoniae* that produce ESBLs (44%) appears to be considerably higher than in previous studies. The ceftazidime resistance rate among ICU *K. pneumoniae* isolates submitted to the National Nosocomial Infection Surveillance System in the USA was 10.7%.5

Jones et al.6 reported a ceftazidime susceptibility rate of 74% of isolates collected from hospitals throughout the USA. However, these isolates were selected by the hospitals because they were considered to be problems at their institutions.

Nearly one-third of the ESBL-containing *K. pneumoniae* isolates were not susceptible to cefotetan and this resistance was not abolished by the addition of clavulanate. These data suggest a high prevalence of isolates that may contain AmpC type β-lactamases and/or outer membrane porin defects.7 The proliferation of isolates with these features is particularly worrying in view of the ability of such organisms to develop resistance to carbapenems.7 The potential for widespread proliferation of carbapenem-resistant *K. pneumoniae* should be a serious concern in this region.

There are few published reports of inter-hospital spread of ESBL-containing Enterobacteriaceae.8 This study provides evidence for considerable intra- and inter-hospital spread of ESBL-containing isolates in Brooklyn. Resistance plasmids can be transferred between unrelated isolates and species, and so these data may have underestimated the degree of sharing of ESBLs.

This study is the first to demonstrate an association between cephaparin use and ESBL-producing *K. pneumoniae* in multiple hospitals throughout a city. Unlike other reports, an inverse relationship between the use of the β-lactamase inhibitor antibiotics and resistant *K. pneumoniae* was not demonstrated. The relatively high percentage of inhibitor-resistant isolates in this region may account for these findings. Alternatively, a reduction of cephaparin use may be the lone factor in limiting the prevalence of ESBL-producing Enterobacteriaceae.

The city-wide ESBL-producing rate for *E. coli* remains fairly low at 4.7%. However, these isolates were present in all but one hospital and were shared among hospitals. These findings, along with the presence of ESBLs in up to 10% of isolates of *P. mirabilis*, highlight the fact that this problem is expanding and not confined to *K. pneumoniae*.

Multidrug-resistant ESBL-containing Enterobacteriaceae, particularly *K. pneumoniae*, have become endemic in Brooklyn, are spread between hospitals, and their presence is associated with cephaparin and aztreonam usage. Control of this problem is likely to require a regional effort aimed at increasing surveillance and infection control, identification of colonized patients and coordinating antibiotic use strategies including the limitation of unnecessary antibiotic use.

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References


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