Clinical Characteristics and Molecular Epidemiology Associated with Imipenem-Resistant Klebsiella pneumoniae

Muhammad Ahmad, Carl Urban, Noriel Mariano, Patricia A. Bradford, Ellen Calceagni, Steven J. Projan, Karen Bush, and James J. Rahal

Eight patients were infected or colonized with imipenem-resistant Klebsiella pneumoniae (IRKP) from December 1994 to November 1995. Initial Klebsiella isolates were susceptible to imipenem but resistant to all cephalosporins, aminoglycosides, and β-lactam inhibitor combinations. All patients had been in the surgical intensive care unit and had undergone abdominal surgery or tracheostomy during hospitalization. The average age of the patients was 71 years (range, 41–81 years). All patients were treated with imipenem for 5 to 36 days, and IRKP was recovered from each during or after therapy. Pulsed-field gel electrophoresis (PFGE) of the IRKP isolates revealed three distinct clonal patterns. Paired sequential isolates of imipenem-susceptible K. pneumoniae and IRKP from two patients had identical PFGE patterns, suggesting the development of clonal stepwise resistance to imipenem during therapy. Thus, imipenem resistance in Klebsiella may occur when this agent is used for treatment of infection due to ceftazidime- and aminoglycoside-resistant strains.

For the past 8 years, our hospital has coped with Klebsiella pneumoniae isolates that are resistant to multiple classes of antibiotics, including third-generation cephalosporins but not imipenem [1, 2]. Therefore, imipenem has been used therapeutically for patients infected with these organisms. We reported the emergence and control of imipenem-resistant Acinetobacter baumannii secondary to use of imipenem, although the mechanism of imipenem resistance in these isolates was unclear [3]. Subsequently, we demonstrated the addition of an AMP-C-type enzyme, designated ACT-1, onto plasmids in K. pneumoniae and Escherichia coli that contained genes encoding extended-spectrum β-lactamases [4]. K. pneumoniae isolates possessing these enzymes were uniformly resistant to β-lactam-β-lactamase inhibitor combinations, cephalosporins, and cephemycins. They remained susceptible to imipenem with the exception of several isolates that became deficient in a specific 42-kDa porin protein, resulting in resistance to imipenem as well as all other available antibiotics [4]. In this report, we present the molecular epidemiology and clinical characteristics of eight patients infected or colonized with imipenem-resistant K. pneumoniae (IRKP) and document the development of imipenem resistance during therapy with this agent.

Methods

Microbiology surveillance. All clinical isolates resistant to third-generation cephalosporins or imipenem were routinely reported to infection control personnel by the clinical microbiology laboratory. IRKP isolates were first identified by the clinical microbiology laboratory in December 1994 by using the Kirby-Bauer disk diffusion method. A zone diameter of ≥13 mm was used to detect imipenem resistance. Eight IRKP isolates were recovered and subjected to broth macrodilution methods by the infectious disease research laboratory according to guidelines of the National Committee for Clinical Laboratory Standards [5]. Imipenem resistance was defined by an MIC of ≥8 μg/mL.

Patient surveillance. Corresponding patient medical records were reviewed by physicians or infection control personnel. Patients from whom IRKP isolates were recovered from any site were classified as infected if their medical conditions satisfied the Centers for Disease Control and Prevention’s definitions for nosocomial infection [6].

Environmental and personnel surveillance. Epidemiological surveys were initiated to identify environmental and personnel reservoirs when IRKP isolates were recovered from three patients in September 1995. Environmental swabs were taken from faucets, sinks, medical charts, doorknobs, bed rails, countertops, tabletops, ventilator and monitor touch keypads, feeding pump touch keypads, and patient charts and were placed into sterile tubes with 5 mL of brain-heart infusion broth. Hand specimens for culture were taken from randomly selected personnel in the medical intensive care unit (ICU) and surgical ICU. Individuals were instructed to immerse their hands into 100 mL of trypticase soy broth in large plastic bags and scrub from hands to wrists. Brain-heart infusion broth and trypticase soy broth cultures were incubated for 24 hours at 37°C. Bags with growth were then subcultured onto MacConkey agar and incubated for an additional 24 hours. Identification of K. pneumoniae was made by using standard microbiological methods.
**Infection control measures.** Culture reports for IRKP were surveyed daily, and contact precautions were used for all infected or colonized patients. To enforce these measures, infection control personnel monitored proper glove use, hand washing, and gown use by physicians, nurses, and other personnel.

**DNA analysis.** All IRKP isolates were cut with the low-frequency restriction endonuclease XbaI and subjected to pulsed-field gel electrophoresis (PFGE) with use of the Gene Path System (Bio-Rad, Hercules, CA) and preset program 2. Two observers visually inspected the PFGE banding patterns and assigned them to categories according to the interpretation schemes specified by Tenover et al. [7]. The Molecular Analyst Fingerprinting and Fingerprinting Plus software programs (Bio-Rad) were also used to compare PFGE patterns. These programs use unweighted pair group arithmetic averages clustering techniques and Dice correlation with 1.9% band tolerance to generate dendrograms.

### Results

**Microbiology.** For the IRKP isolates, the MICs of imipenem and meropenem ranged from 8 to 32 μg/mL by the macrodilution assay, and the MICs of cefepime ranged from 16 to 64 μg/mL by broth macrodilution susceptibility methodology (table 1). Isolates were resistant to cefotetan at MICs of ≥128 μg/mL [4].

**Clinical findings, epidemiology, and therapy.** IRKP was isolated from eight patients between December 1994 and November 1995. The mechanism of imipenem resistance in three of the eight isolates was previously described and shown to be due to a combination of a plasmid-mediated AMP-C-type β-lactamase and the loss of a major outer membrane protein [4]. The five additional isolates had the same antibiograms, supporting a similar mechanism of resistance to imipenem. The source of each isolate, PFGE pattern, and clinical characteristics associated with infection or colonization by IRKP are listed in table 2.

### Discussion

Bacterial resistance to antibiotics is a worldwide problem that threatens the successful treatment of a growing number of infections caused by nosocomial isolates. This escalating scenario has necessitated the use of broad-spectrum carbapenems as adequate therapy for many infections due to multidrug-resistant gram-negative bacteria. Use of imipenem in this setting has resulted in the emergence of resistant mutants, notably during therapy for *P. aeruginosa* and *Enterobacter* infections [8–10]. A variety of mechanisms of resistance to carbapenems exist in these species [11]. Most commonly, specific porin mutations prevent penetration of the agent and access to its target. Synergistic resistance may occur because of porin mutations in conjunction with chromosomally encoded cephalo-
sporinases [8–12]. Ominously, imipenem resistance due to plasmid-encoded carbapenemases in P. aeruginosa and Serratia marcescens strains has been reported in Japan [13, 14].

Because no new classes of antibacterial agents effective against multidrug-resistant gram-negative bacteria are likely to become available within the next few years, the carbapenems will continue to be used with increasing frequency. More species are therefore likely to develop varying mechanisms of resistance to this class of antibacterial agents.

Several reports documented induced resistance to cephalosporins that was due to various porin protein deletions in K. pneumoniae [15, 16]. We reported previously that K. pneumoniae possessing a plasmid-encoded AMP-C-type β-lactam-hydrolyzing enzyme can develop carbapenem resistance by the deletion of specific outer membrane porins. A recent report also documented the development of carbapenem resistance in K. pneumoniae that was due to increased amounts of simian herpes virus–type extended-spectrum β-lactamase and the loss of an outer membrane protein [17]. In this report, we document the development of imipenem resistance among strains of ceftazidime-resistant K. pneumoniae (CRKP) during therapy with this agent, a phenomenon consistent with a stepwise decrease in imipenem penetration.

The eradication of IRKP isolates from our institution was probably due to a number of factors. The outbreak occurred in late 1994 and 1995 before institution of class restriction of cephalosporins in 1996 [18]. It is noteworthy that two IRKP isolates evolved in patients with documented infection with CRKP, suggesting that extended-spectrum β-lactamase production in combination with imipenem use predisposes to the emergence of IRKP. Thus, the marked reduction in the incidence of CRKP in the surgical ICU (88%) in 1996 may have contributed substantially to the disappearance of IRKP despite

![Figure 1](https://academic.oup.com/cid/article-abstract/29/2/352/274273)

**Figure 1.** Temporal distribution of eight imipenem-resistant Klebsiella pneumoniae isolates recovered from patients in a surgical intensive care unit between December 1994 and November 1995 and the pulsed-field gel electrophoresis patterns of these isolates: white bar = a; horizontal line bar = b; diagonal line bar = b’; black bar = c; and dotted bar = c’.

<table>
<thead>
<tr>
<th>Patient no., age (y)/sex</th>
<th>Diagnosis</th>
<th>Procedure(s)</th>
<th>Source(s) of isolate</th>
<th>Surgical ICU bed no.</th>
<th>PFGE pattern</th>
<th>Date</th>
<th>Duration of imipenem therapy (d)</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1, 80/F</td>
<td>Gastric cancer</td>
<td>Gastroctomy, splenectomy</td>
<td>Sputum</td>
<td>7</td>
<td>a</td>
<td>12/7/94</td>
<td>5</td>
<td>Recovered</td>
</tr>
<tr>
<td>2, 60/M</td>
<td>Pancreatic abscess</td>
<td>Laparotomy</td>
<td>Sputum</td>
<td>2 and 3</td>
<td>b</td>
<td>7/12/95</td>
<td>12</td>
<td>Died</td>
</tr>
<tr>
<td>3, 67/F</td>
<td>Axillary artery thrombosis</td>
<td>Tracheostomy, laparotomy</td>
<td>Peritoneum</td>
<td>2 and 3</td>
<td>b</td>
<td>9/2/95</td>
<td>5</td>
<td>Died</td>
</tr>
<tr>
<td>4, 75/M</td>
<td>Abdominal abscess</td>
<td>Hemicolecotomy</td>
<td>Peritoneum</td>
<td>2</td>
<td>b</td>
<td>9/10/95</td>
<td>26</td>
<td>Died</td>
</tr>
<tr>
<td>5, 80/F</td>
<td>Gastric cancer</td>
<td>Gastroctomy, pancreatectomy</td>
<td>Blood, peritoneum</td>
<td>2</td>
<td>b</td>
<td>9/18/95</td>
<td>5</td>
<td>Died</td>
</tr>
<tr>
<td>6, 80/F</td>
<td>Perforated diverticulum</td>
<td>Hemicolecotomy</td>
<td>Sputum, peritoneum</td>
<td>8 and 2</td>
<td>b’</td>
<td>10/11/95</td>
<td>21</td>
<td>Died</td>
</tr>
<tr>
<td>7, 81/M</td>
<td>Colon cancer</td>
<td>Sigmoid resection</td>
<td>Sputum</td>
<td>7</td>
<td>c</td>
<td>11/24/95</td>
<td>36</td>
<td>Died</td>
</tr>
<tr>
<td>8, 41/M</td>
<td>Alcoholism</td>
<td>Tracheostomy</td>
<td>Sputum</td>
<td>2</td>
<td>c’</td>
<td>11/27/95</td>
<td>14</td>
<td>Recovered</td>
</tr>
</tbody>
</table>

**Table 2.** Clinical characteristics of eight ICU patients from whom imipenem-resistant Klebsiella pneumoniae isolates were recovered.

NOTE. ICU = intensive care unit; PFGE = pulsed-field gel electrophoresis.

![Figure 2](https://academic.oup.com/cid/article-abstract/29/2/352/274273)

**Figure 2.** Pulsed-field gel electrophoresis patterns of XbaI-digested DNA from imipenem-resistant Klebsiella pneumoniae (IRKP) and imipenem-susceptible K. pneumoniae (ISKP) isolates recovered from eight surgical intensive care unit patients. Lane 1, λ ladder standards; lanes 2 and 13, Saccharomyces cerevisiae standards; lane 3, ISKP from patient 2; lane 4, IRKP from patient 2; lane 5, ISKP from patient 7; lane 6, IRKP from patient 7; lane 7, IRKP from patient 6; lane 8, IRKP from patient 1; lane 9, IRKP from patient 3; lane 10, IRKP from patient 5; lane 11, IRKP from patient 4; lane 12, IRKP from patient 8. kb = kilobase.
increased use of imipenem in 1996 [18]. A total of 948 g of imipenem was used in the surgical ICU during 1995 when most of the IRKP isolates were identified compared with 1,351 g in 1996. Reinforcement of infection control, environmental disinfection by housekeeping personnel, and increased awareness of health care personnel because of hand specimen cultures may have contributed further to eradication of IRKP.

We previously reported a limited outbreak of imipenem-resistant Acinetobacter in the same surgical ICU during 1991–1992 that responded to infection control efforts, cultures of environmental and personnel hand specimens, and application of a polymyxin B solution to colonized wounds [3]. The fact that imipenem resistance in both Acinetobacter and Klebsiella cannot be attributed to a plasmid-mediated mechanism favors clonal or oligoclonal epidemiology, which is amenable to strict infection control procedures. In contrast, our polyclonal CRKP outbreak required the addition of class restriction of cephalosporins before a significant reduction in incidence was achieved [18].

A recent report describing the molecular epidemiology of an outbreak of multidrug-resistant Enterobacter aerogenes also documented the development of imipenem resistance in two of 34 isolates during imipenem therapy [10]. The two isolates were also resistant to meropenem and cefepime, and both patients infected with these strains died. These investigators were able to reduce the incidence of multidrug-resistant bacteria only after all colonized patients had been discharged from the hospital [10].

K. pneumoniae has now been added to those bacterial species that can become resistant to all β-lactam agents by escalating selection pressure of antibiotics. New strategies for antibiotic utilization and infection control, as well as investigations into porin protein regulation and novel antibacterial targets, are critical to the preservation of effective therapy against gram-negative pathogens [19, 20].

References