Efficacies of Imipenem, Meropenem, Cefepime, and Ceftazidime in Rats with Experimental Pneumonia Due to a Carbapenem-Hydrolyzing β-Lactamase-Producing Strain of Enterobacter cloacae

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The antibacterial activities of imipenem-cilastatin, meropenem-cilastatin, cefepime and ceftazidime against Enterobacter cloacae NOR-1, which produces the carbapenem-hydrolyzing β-lactamase NmcA and a cephalosporinase, and against one of its in vitro-obtained ceftazidime-resistant mutant were compared by using an experimental model of pneumonia with immunocompetent rats. The MICs of the β-lactams with an inoculum of 5 log10 CFU/ml were as follows for E. cloacae NOR-1 and its ceftazidime-resistant mutant, respectively: imipenem, 16 and 128 μg/ml, meropenem, 4 and 32 μg/ml, cefepime, <0.03 and 1 μg/ml, and ceftazidime, 1 and 512 μg/ml. The chromosomally located cephalosporinase and carbapenem-hydrolyzing β-lactamase NmcA were inducible by cefoxitin and meropenem in E. cloacae NOR-1, and both were stably overproduced in the ceftazidime-resistant mutant. Renal impairment was induced (uranyl nitrate, 1 mg/kg of body weight) in rats to simulate the human pharmacokinetic parameters for the β-lactams studied. Animals were intratracheally inoculated with 8.5 log10 CFU of E. cloacae, and therapy was initiated 3 h later. At that time, animal lungs showed bilateral pneumonia containing more than 6 log10 CFU of E. cloacae per g of tissue. Despite the relative low MIC of meropenem for E. cloacae NOR-1, the carbapenem-treated rats had no decrease in bacterial counts in their lungs 60 h after therapy onset compared to the counts for the controls, regardless of whether E. cloacae NOR-1 or its ceftazidime-resistant mutant was inoculated. A significant decrease in bacterial titers was observed for the ceftazidime-treated rats infected with E. cloacae NOR-1 only. Cefepime was the only β-lactam tested effective as treatment against infections due to E. cloacae NOR-1 or its ceftazidime-resistant mutant.

Although the carbapenems imipenem and meropenem have the broadest antimicrobial activity among the β-lactams, acquired resistance to these antibiotics has been reported in gram-negative rods (17, 28, 33). Mechanisms of resistance to carbapenems in members of the family Enterobacteriaceae include modified penicillin-binding protein affinity, decreases in the levels of uptake of these β-lactams, or overproduction of naturally occurring β-lactamases (mostly cephalosporinases) with low levels of hydrolytic activity against carbapenems combined with a decrease in outer membrane permeability (3). However, carbapenem-hydrolyzing β-lactamases that hydrolyze several β-lactam classes including carbapenems have been reported recently in several strains of the family Enterobacteriaceae (17). Since carbapenems are used more frequently, a larger number of these enzymes may be selected in vivo, as has already been observed (23). Thus, the impacts of such enzymes on the efficacy of β-lactam therapy may be of critical importance.

Enterobacter spp. are now among the five most common nosocomial pathogens isolated from patients in U.S. and European hospitals and account for about 10% of lower respiratory tract infections in intensive care units (31, 35). Since Enterobacter sp. strains are intrinsically resistant to aminopenicillins and narrow-spectrum cephalosporins due to their chromosomally encoded inducible cephalosporinase, they may acquire resistance to extended-spectrum cephalosporins during therapy by selecting mutants that constitutively overproduce cephalosporinases (5). These resistant strains, often referred to as “stably derepressed mutants,” produce enough β-lactamase to inactivate all currently available β-lactams except carbapenems and cefepime (14, 18, 30). Carbapenem-hydrolyzing β-lactamases have been detected in several enterobacterial species in Japan, Europe, and the United States, including Enterobacter cloacae and Serratia marcescens (19, 21, 30). The metalloenzyme IMP-1, which has a broad-spectrum hydrolytic substrate profile that includes extended-spectrum cephalosporinases and carbapenems, has been reported to be epidemic among Japanese isolates (1, 24). The IMP-1 gene is located on plasmids and integrons (1). An IMP-1-like producing strain has very recently been described in Italy, indicating that an IMP-1-like β-lactamase has reached Europe (6). Among the penicillinase group (Bush functional group 2f [4]), the carbapenem-hydrolyzing β-lactamases NmcA, IMI-1, and Sme-1 have been reported from several E. cloacae and S. marcescens isolates (19, 21, 23, 29). These enzymes significantly hydrolyze imipenem, hydrolyze meropenem less so, and do not hydrolyze extended-spectrum cephalosporins. Their activities are partially inhibited by clavulanic acid. Their genes are chromosomally located and are regulated by a regulatory Lys-R type protein, the gene for which is located immediately upstream of the β-lactamase gene. These divergently expressed
β-lactamase and regulatory protein genes have common pro- 

c-moter regions, as found for the cephalosporinase \( \text{ampC} \) gene of \( E. \text{cloaca} \), which is also regulated at least by an \( \text{Lys-R} \) type 

protein, \( \text{AmpR} \) (11). Both carbapenem-hydrolyzing β-lactama- 

escases and cephalosporinases are inducible upon the addition of 

strong inducers such as carbapenems or ceftoxitin (28).

Taking into account the similar hydrolytic properties of the 

carbapenem-hydrolyzing β-lactamases of the penicillinase 

group, the aim of the present study was to compare the bac- 

terial efficacies in vivo of human regimens of imipenem, 

meropenem, meropenem-cilastatin, or ceftazidime against the \( \text{NMCa} \)-pro- 

ducing \( E. \text{cloa} \) NOR-1 and one of its in vitro-obtained 

ceftazidime-resistant mutants by using a model of pneumonia in nonneutropenic rats developed previously (18).

**MATERIALS AND METHODS**

**Organisms tested.** The infectious organisms were either \( E. \text{cloaa} \) NOR-1 or its in vitro-obtained ceftazidime-resistant mutant. The original strain was isolated from a patient hospitalized in France and treated intravenously with 500 mg of 

imipenem; this strain produced an identified carbapenem-hydrolyzing β-lacta- 

mase, \( \text{NmcA} \) (23). An isogenic ceftazidime-resistant mutant was obtained by 

plating \( 9 \log_{10} \) CFU of \( E. \text{cloa} \) NOR-1 onto a Trypticase soy (TS) agar plate 

containing 32 μg of ceftazidime per ml. Resistant strains were obtained at a 

frequency of \( 10^{-5} \). One of them was retained for further analysis.

The stability of the ceftazidime resistance phenotype of the mutant was checked by 

the stability of the plating strain onto either antibiotic-free or ceftazidime-containing plates.

The same number of bacteria were obtained. To ensure pathogenicity, \( E. \text{cloaa} \) NOR-1 and its ceftazidime-resistant mutant were submitted to two subsequent 

passages in mice inoculated intraperitoneally and infected for 24 h. Then, 

the strains were stored at \( -70°C \) in Mueller-Hinton broth (bioMérieux, Marcy- 

l'Etoile, France) supplemented with 10% glycerol. Fresh inocula were prepared 

for each experiment from cultures grown for 24 h in 10 ml of TS broth (bio- 

myces) and were then rinsed twice and suspended in normal saline prior to 

their use.

**Antimicrobial agents.** Imipenem-clastatin, cefotaxin and cefoxitin were from Merck Sharp & Dohme-Chibret (Paris, France), meropenem was from Zeneca Pharma (Cergy, France), cefepine was from Bristol-Myers Squibb (Paris, France), cefazolin was from GlaxoWellcome (Evreux, France) and cephalo- 

thin was from Roche (Neuilly-sur-Seine, France). Antibiotic powders were 

freshly diluted with saline before each experiment with animals, according to 

the manufacturers’ instructions.

**Susceptibility testing.** MICs were determined in duplicate in Mueller-Hinton broth (bioMérieux) by means of a tube macrodilution method with geometric 

twofold concentrations of equal inoculum of \( 5,7 \log_{10} \) CFU/ml. All plates were 

incubated at 37°C for 18 h prior to determination of the MICs of imipenem, 

meropenem, cefepine, and ceftazidime (22).

**β-Lactamase assays.** β-Lactamase activities were determined in triplicate with 

or without 10 μg/ml of cefoxitin or 0.25 μg/ml of the inhibitor. 

Overnight cultures of \( E. \text{cloa} \) NOR-1 or its ceftazidime-resistant mutant were 

diluted 1:10 into 10 ml of TS broth. Then, the cultures were grown for an 

additional 2 h with or without inducer. Bacterial suspensions were centrifuged 

for 15 min at \( 1,000 \times g \), and the pellets were suspended in 10 

ml of phosphate buffer (pH 7.0) and were disrupted by sonication (twice for 30 s 

for 10 min at \( 4°C \) to separate the plasma. Plasma samples were stored at \( -70°C \) and 

were assayed within 7 days. Saline (600 μl) was injected intra-arterially (via 

the catheter) after each blood sampling to restore the blood volume. Individual 

antibiotic pharmacokinetic parameters were determined by using a noncompart- 

mental model (Siphar software package; Simed, Créteil, France).

The potential binding of the β-lactams studied to the plasma proteins of rats was 

assessed by exposing several concentrations of drugs to plasma. To obtain 

conditions comparable to those in our animals, proteins isolated from 

renally impaired rats was used; antibiotic solutions were added to obtain 

final concentrations that corresponded to peak and middle-interval antibiotic 

concentrations observed in animals. A final antibiotic concentration of 4 μg/ml, 

corresponding to the French cutoff for determination of susceptibility, for each 

β-lactam studied, was also obtained. The free antibiotic fractions in these 

preparations were determined in triplicate. Total and free antibiotic levels were 

determined after equilibration of the drug in plasma for 1 h at \( 37°C \). The 

free drug concentrations were determined by ultrafiltration, using the Microsep 3 K 

Microporaction System (Filtron Technology Corporation, Polylabo, Strasbourg, 

France).

Imipenem, meropenem, cefepine, and ceftazidime concentrations in rat 

plasma and ultrafiltrate were determined by a modified version of the high- 

pressure liquid chromatography assays described elsewhere (2, 8, 9, 12).

The lower detection limits of the assays were 0.5, 0.5, 1, and 5 μg/ml for imipenem, 

meropenem, cefepine, and ceftazidime, respectively.

For each noninfected rat with uranil nitrate-induced renal impairment, we 

determined the time that the free antibiotic concentration in plasma exceeded the 

MIC (\( T_{\text{MIC}} \)) for each strain, using MICs obtained with inoculum sizes of 

5, 10, 50, and 1,000 CFU/ml.

**Pneumonia model.** The animal model used was adapted from one previously 

developed in our laboratory (18). Briefly, male Wistar rats (weight, 280 to 300 g) 

were rendered renally insufficient by subcutaneous administration of 1 mg of 

urea/kg per kg and were intraperitoneally administered 3 mg/kg of 

phenobarbital (60 mg/kg), and each rat trachea was exposed by a vertical midline 

incision. A total of 0.5 ml of a bacterial suspension containing 8.5 \( \log_{10} \) CFU of 

\( E. \text{cloa} \) was injected intra-arterially with a syringe with a 25-gauge needle.

Following inoculation, the animals were gently shaken for 15 s to equally dis- 

tribute the bacterial inoculum in the lungs. Previous studies had shown that 3 h 

after bacterial inoculation, all animals develop bilateral pneumonia with bacte- 

rial densities of \( \geq 7 \log_{10} \) CFU/g of tissue in both lungs and an intense inflam- 

matory reaction.

**Treatment regimens.** Each strain used to induce pneumonia was studied sep- 

erately. Among the 200 animals included in this study, 92 and 83 of them infected 

with \( E. \text{cloa} \) NOR-1 or its ceftazidime-resistant mutant, respectively, were still 

alive 3 h after bacterial inoculation. At this time, 10 rats from each study group 

were killed to document that pneumonia had been established. The remaining 

rats were randomly assigned to one control group (i.e., rats not treated with 

antibiotic) and four treatment groups. Treatment groups received intraperito- 

eal injections of imipenem-clastatin (30 mg/kg/h each), meropenem-clastatin 

(30 mg/kg/8 h each), cefepine (60 mg/kg/12 h), or ceftazidime (60 mg/kg/8 h).

These dosages were retained to achieve concentrations in serum close to those 

observed in humans. Therapy began 3 h after bacterial inoculation and was con- 

tinued for 2.5 days.

**Evaluation of antibiotic treatments.** At 2.5 days, animals were killed approx- 

imately 5 to 7 h after administration of the last antibiotic dose. Blood was 

obtained by aortic puncture and placed in a heparin-containing tube, the tube 

was then placed in a refrigerator, and the plasma was extracted after two alco- 

holic precipitation of antibiotic concentrations and creatinine levels. The imipen- 

em-containing plasma was immediately mixed after sampling (1:1) with a stabil- 

izing buffer containing equal volumes of 1 M morpholinoethane sulfonate and 

ethylene glycol before freezing. Creatinine levels in plasma were determined to 

document that renal impairment was established (18). The lungs were aseptically 

removed, gently blotted with sterile absorbent paper to remove blood, weighed, 

placed in 25 ml of ice-cold saline, and homogenized with a homogenizer (Ul- 

traturax, Staufen, Germany). The homogenate was quantitatively cultured after 

serial dilution (up to \( 5 \times 10^{-5} \) ) on Drigalski agar (bioMérieux) with a Spiral 

System plate (Interscience, Saint-Nom-La-Bretèche, France). After overnight 

incubation at 37°C, the viable bacteria were counted and the counts were ex- 

pressed as \( \log_{10} \) CFU per gram of lungs. When no bacterial growth was noted, 

the value of the detection limit for the specific animal was entered for statistical 

analysis.

**Statistical analysis.** Results are expressed as medians and their ranges. Bac- 

terial counts in the lungs of the control and treatment groups were compared by 

one-way nonparametric analysis of variance (Kruskal-Wallis test); when the 

value of this test was statistically significant, the value for each treatment group 

was compared to those for the control group and each of the other treatment 

groups using the Mann-Whitney U test. For all tests, a \( P \) value of <0.05 was 

considered significant.

**RESULTS**

**Susceptibility testing.** The susceptibilities of \( E. \text{cloa} \) NOR-1 and its ceftazidime-resistant mutant are given in Table
TABLE 1. In vitro susceptibilities of an *E. cloacae* NOR-1 strain and its in vitro-obtained ceftazidime-resistant mutant to the β-lactams studied for various inoculum sizes

<table>
<thead>
<tr>
<th>β-Lactam agent</th>
<th><em>E. cloacae</em> NOR-1</th>
<th><em>E. cloacae</em> NOR-1 mutant</th>
</tr>
</thead>
<tbody>
<tr>
<td>MIC (µg/ml) with the indicated inoculum size (log₁₀ CFU/ml):</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Imipenem</td>
<td>8</td>
<td>16</td>
</tr>
<tr>
<td>Meropenem</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Cefepime</td>
<td>&lt;0.03</td>
<td>1</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>0.03</td>
<td>1</td>
</tr>
</tbody>
</table>

For various initial bacterial concentrations, *E. cloacae* NOR-1 was susceptible to cefepime and ceftazidime, was moderately susceptible to meropenem, and was resistant to imipenem. The ceftazidime-resistant mutant remained susceptible to cefepime but was resistant to imipenem, meropenem, and ceftazidime. As expected, an inoculum effect proportional to the bacterial titer was observed with the four β-lactams tested against the two strains but was less pronounced with cefepime against *E. cloacae* NOR-1.

**Pharmacokinetic-pharmacodynamic analyses.** The values of the pharmacokinetic parameters for each antibiotic given to renally insufficient rats were similar to those observed when a 1-g imipenem or meropenem dose or a 2-g cefepime or ceftazidime dose was given intravenously to healthy humans (Table 3). In particular, the level of binding of each β-lactam to the plasma proteins of rats was relatively low and was linear over the range of concentrations tested; we secondarily calculated the free concentrations of each antibiotic given to renally impaired rats as the product of multiplying its free fraction by the concentration of total antibiotic. The percentages of the dosing interval that the free drug concentrations exceeded the MICs for the two strains, by using the MIC obtained for various inoculum sizes, are given in Table 4.

**Efficacy of therapy.** The 10 animals from each study group killed at the start of therapy presented with bilateral pneumonia, with median counts of 7.0 log₁₀ CFU/g of lung (range, 6.1 to 7.6 log₁₀ CFU/g of lung) and 6.3 log₁₀ CFU/g of lung (range, 6.0 to 7.2 CFU/g of lung) for *E. cloacae* NOR-1 and its ceftazidime-resistant mutant, respectively. Twenty-one of 25 infected animals died during the antibiotic treatment period; these animals received no antibiotic (*n* = 3), imipenem-cilastatin (*n* = 4), meropenem-cilastatin (*n* = 6), cefepime (*n* = 4), or ceftazidime (*n* = 4).

At the time of killing (i.e., 60 h after starting therapy and 5 to 7 h after administration of the last antibiotic dose), creatinine levels in plasma were not statistically different between the study groups, indicating that renal impairment was identical regardless of the treatment received (Table 5). At that time, β-lactam concentrations in plasma did not differ significantly when β-lactams were administered to animals infected with either *E. cloacae* NOR-1 or its ceftazidime-resistant mutant and were then pooled to simplify the presentation. As indicated in Table 5, these β-lactam levels were broadly similar to those usually reported in human plasma. At the end of the period of study (2.5 days), the bacterial counts in untreated animals were 6.2 log₁₀ CFU/g of lung (range, 4.9 to 7.0 log₁₀ CFU/g of lung) and 5.2 log₁₀ CFU/g of lung (range, 3.9 to 6.6 CFU/g of lung) for *E. cloacae* NOR-1 and its ceftazidime-resistant mutant, respectively. At 60 h after the onset of therapy the carbapenem-treated rats had bacterial counts in their lungs similar to those in the lungs of untreated animals, regardless of whether *E. cloacae* NOR-1 or its ceftazidime-resistant mutant was inoculated (Fig. 1). Ceftazidime treatment led to a significant decrease in the bacterial titers in the lungs only for the carbapenem-treated rats. As expected for an *E. cloacae* NOR-1 or its ceftazidime-resistant mutant, the bacterial counts in the lungs of meropenem-treated rats did not differ significantly from those of untreated animals.

**TABLE 2. β-Lactamase activities of an *E. cloacae* NOR-1 strain and its in vitro-obtained ceftazidime-resistant mutant with or without inducers**

<table>
<thead>
<tr>
<th>Strain and growth condition</th>
<th>β-Lactamase activity&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Imipenem substrate</td>
</tr>
<tr>
<td><em>E. cloacae</em> NOR-1</td>
<td>5</td>
</tr>
<tr>
<td><em>E. cloacae</em> NOR-1 mutant</td>
<td>1,250</td>
</tr>
<tr>
<td><em>E. cloacae</em> NOR-1 mutant + cefoxitin&lt;sup&gt;b&lt;/sup&gt;</td>
<td>30</td>
</tr>
<tr>
<td><em>E. cloacae</em> NOR-1 mutant + meropenem&lt;sup&gt;b&lt;/sup&gt;</td>
<td>33</td>
</tr>
<tr>
<td><em>E. cloacae</em> NOR-1 mutant + ceftazidime</td>
<td>1,370</td>
</tr>
</tbody>
</table>

<sup>a</sup>β-Lactamase activity is expressed as units of specific activity. One unit of specific activity was defined as the enzymatic activity which hydrolyzed 1 µmol of cephalothin or imipenem per min per g of protein. Results are geometric means of three independent measures. Standard deviations were within 10% of the geometric mean in all cases.

<sup>b</sup>The final concentrations added to the cultures were 10 and 0.25 µg/ml for cefoxitin and meropenem, respectively.
DISCUSSION

The aim of our work was to study the therapeutic potential of β-lactam antibiotics for the treatment of infections due to an E. cloacae strain that produces a cephalosporinase and a carbapenem-hydrolyzing β-lactamase. The initial mortality rate was relatively low (25 of 200 animals) and was mainly the result of trauma from the operation or overwhelming sepsis. Since the mortality rate during the treatment period was near zero and the rate of spontaneous clearance of bacteria was low (~1 log₁₀ CFU of E. cloacae/g of tissue), the level of clearance of bacteria from the lungs was used to compare treatment groups.

As expected, E. cloacae NOR-1 produces basal levels of cephalosporinase. Since ceftazidime is a weak cephalosporinase inducer, it was logical that it was active for the treatment of rats infected with E. cloacae NOR-1. In the case of infection with the stably derepressed E. cloacae NOR-1 mutant, only cefepime was active since ceftazidime is hydrolyzed by overproduction of the chromosomally mediated cephalosporinase. On the contrary, cefepime retained good activity against infection due to the ceftazidime-resistant mutant. It is known that cefepime is active against such strains because of a combination of factors, including faster penetration through the outer membranes of gram-negative bacteria and a low affinity for enterobacterial cephalosporinases (13, 14, 26). The cephalosporinase activity in the present study agrees with the results obtained in experimental models in the present study were recently supported by a clinical study in which 15 of 17 infections due to ceftazidime-resistant and cefepime-susceptible Enterobacter sp. strains were successfully treated with cefepime. In particular, cefepime was successful as treatment for chronic infections that had responded poorly to repeated therapy (30).

Since the carbapenem-hydrolyzing β-lactamase NmcA does not hydrolyze ceftazidime or cefepime, even when it is produced at a high level, it was logical that NmcA, whatever its in vivo level, did not play any role in the results obtained for cefepime and ceftazidime when they were used as treatments for infections due to E. cloacae NOR-1 or its ceftazidime-resistant mutant. Our results indicated that imipenem and meropenem are equally ineffective for the treatment of infections due to E. cloacae NOR-1. Although these results could have been predicted by the high MIC of imipenem, they are more surprising for meropenem, which has a relatively low MIC. At least two hypotheses may explain the inefficacy of meropenem. An inoculum effect may provide large amounts of the carbapenem-hydrolyzing β-lactamase NmcA in the lungs of animals and may lead to in vivo resistance to meropenem, as indicated by our in vitro studies. Interestingly, meropenem (as imipenem [23]) or cefoxitin significantly induced the cephalosporinase activity and the carbapenem-hydrolyzing β-lactamase activity, both of which are found in E. cloacae NOR-1. The cephalosporinase induction by carbapenems is not of clinical relevance since carbapenems are not hydrolyzed significantly by enterobacterial cephalosporinases. On the contrary, the induction of the carbapenem-hydrolyzing β-lactamase NmcA of E. cloacae NOR-1 by meropenem may also explain the in vivo inefficacy of meropenem. In this regard, it has recently been shown that clavulanate, a potent inducer of cephalosporinase from Pseudomonas aeruginosa, may antagonize the antibacterial activity of ticarcillin in a ticarcillin-clavulanate combination even when MICs of ticarcillin-clavulanate are below the breakpoint for resistance (16). The inefficacy of meropenem for the treatment of E. cloacae NOR-1 infection was not due to meropenem hydrolysis by rat lung dehydropeptidase since cilastatin addition permitted the retrieval of levels in plasma close to those obtained with the regimens used for humans.

Interestingly, and for reasons that are not yet known, the ceftazidime-resistant E. cloacae NOR-1 mutant produced not only high levels of cephalosporinase but also high levels of the β-lactamases were no longer inducible. This result implies that the ceftazidime-resistant E. cloacae NOR-1

<table>
<thead>
<tr>
<th>β-Lactam agent</th>
<th>No. of animals</th>
<th>Dose (mg/kg)</th>
<th>Median (range) antibiotic level (µg/ml) in plasma</th>
<th>Protein binding (%)</th>
<th>Half-life (h)</th>
<th>Area under curve (µg · h/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Peak (30 min after dosing)</td>
<td>Trough (8 h after dosing)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Imipenem</td>
<td>5</td>
<td>30</td>
<td>83 (66–104)</td>
<td>&lt;0.5 (&lt;0.5–1)</td>
<td>37 (30–53)</td>
<td>10 (0.7–1.3)</td>
</tr>
<tr>
<td>Meropenem</td>
<td>6</td>
<td>30</td>
<td>76 (61–96)</td>
<td>&lt;0.5 (&lt;0.5–0.5)</td>
<td>48 (37–57)</td>
<td>0.9 (0.6–1.4)</td>
</tr>
<tr>
<td>Cefepime</td>
<td>6</td>
<td>60</td>
<td>160 (125–220)</td>
<td>12 (6–28)</td>
<td>17 (12–24)</td>
<td>2.0 (1.4–2.8)</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>4</td>
<td>60</td>
<td>197 (139–214)</td>
<td>15 (5–24)</td>
<td>20 (9–22)</td>
<td>2.1 (1.9–2.6)</td>
</tr>
</tbody>
</table>

* Median, with range in parentheses.

<table>
<thead>
<tr>
<th>β-Lactam agent</th>
<th>% of dosing interval of:</th>
<th>E. cloacae NOR-1 at inoculum size (log₁₀ CFU/ml) of:</th>
<th>E. cloacae NOR-1 mutant at inoculum size (log₁₀ CFU/ml) of:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Imipenem</td>
<td></td>
<td>23 (7–29)</td>
<td>0 (0–0)</td>
</tr>
<tr>
<td>Meropenem</td>
<td></td>
<td>31 (23–39)</td>
<td>12 (9–16)</td>
</tr>
<tr>
<td>Cefepime</td>
<td></td>
<td>100 (100–100)</td>
<td>100 (100–100)</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td></td>
<td>100 (100–100)</td>
<td>100 (100–100)</td>
</tr>
</tbody>
</table>

* Median, with range in parentheses.
mutant is a stably derepressed mutant not only for cephalosporinase biosynthesis but also for NmcA biosynthesis. Therefore, meropenem and imipenem were not active as treatments for infections due to the ceftazidime-resistant E. cloacae NOR-1 mutant.

According to the current model for cephalosporinase regulation (11), it may be hypothesized that a mutated ampD gene in the ceftazidime-resistant E. cloacae NOR-1 mutant produced an inactive AmpD protein, thus explaining the high levels of both cephalosporinase and the carbapenem-hydrolyzing β-lactamase (15). AmpD is an amidase that cleaves peptidoglycan precursors, thus preventing their accumulation in the cytoplasm (10). In cases of an inactive AmpD, these precursors displace AmpR from its repressor binding site in the cytoplasm (10). In cases of an inactive AmpD, these precursors displace AmpR from its repressor binding site in the promoter regions, thus explaining the stable overproduction of cephalosporinase. In this regard, it should be remembered that the cephalosporinase and the carbapenem-hydrolyzing β-lactamase NmcA are at least regulated by the structurally related LysR-type proteins, AmpR and NmcR, respectively.

For β-lactam antibiotics, T>MIC is the better pharmacokinetic parameter for influencing the outcome of infection (7). Maximal killing is approached when concentrations are one to four times the MIC to 70% of the time, provided that the levels of unbound drug are used to assess the efficacy of highly protein-bound drugs. However, since the efficacies of β-lactams are affected by the inoculum size (7), T>MIC correlates better with drug efficacy when the MIC is determined with the corresponding inoculum size, as observed in our study.

In conclusion, cefepime, which is more stable than narrow-spectrum cephalosporins against the activities of the cephalosporinases and the carbapenem-hydrolyzing β-lactamase NmcA of E. cloacae NOR-1, even in cases of overproduction, was the best β-lactam for the treatment of experimental infections due to such isolates. It may also decrease in vivo the likelihood of development of cephalosporinases and the carbapenem-hydrolyzing β-lactamase NmcA due to both cephalosporinase and the carbapenem-hydrolyzing β-lactamase. Sme-1 and IMI-1, have similar hydrolytic properties and are regulated similarly to NmcA (28), it is likely that cefepime may cure infections due to Sme-1 or IMI-1-producing strains. On the contrary, the efficacy of cefepime for the treatment of infections due to enterobacteria that produce carbapenem-hydrolyzing β-lactamases of other types such as the metalloenzyme IMP-1 cannot be deduced from our experimental data. Actually, IMP-1 has a much larger β-lactam substrate profile than NmcA. Finally, further work shall be directed toward assessment of the efficacy of combined antibiotic therapy, including therapy with aminoglycosides or fluoroquinolones, which are often used for the treatment of pneumonia due to Enterobacter sp. strains.

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**REFERENCES**


