Urine Bactericidal Activity against *Escherichia coli* Isolates Exhibiting Different Resistance Phenotypes/Genotypes in an In Vitro Pharmacodynamic Model Simulating Urine Concentrations Obtained after Oral Administration of a 400-Milligram Single Dose of Cefditoren-Pivoxil

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Activity of simulated cefditoren urinary concentrations was determined against seven *Escherichia coli* isolates. Bactericidal activity was obtained from 4 to 24 h against TEM-1 (penicillinase production/hyperproduction), TEM-34 (IRT-6), and TEM-116 (extended-spectrum beta-lactamase [ESBL]) and from 6 to 8 h against SHV/TEM-116 (ESBL) but never against SHV/TEM-1 (ESBL). Extension of bactericidal activity depended on the resistance genotype/phenotype tested.

Resistance rates to amoxicillin-clavulanic acid in *Escherichia coli* have remained over time around 10% in Spain (3) (although some reports increased this rate up to 25%) (1) due to the prevalence of TEM-1 β-lactamase hyperproduction and the low frequency of inhibitor-resistant TEM β-lactamase (IRT) derivatives (0.9%) (3) or extended-spectrum beta-lactamas (ESBLs) (0.7%) in isolates from community-acquired urinary tract infections (3). This low frequency of ESBLs (TEM derivatives and SHV being some of the most frequent) (12, 16), together with the mentioned β-lactam resistance phenotypes and the 1% per-annum increase in quinolone resistance (9), may be the rationale for exploring the activity of new compounds.

A pharmacokinetic-pharmacodynamic approach studying the concentration-effect relationship along time has been advocated as a preclinical tool in drug development (11) to explore antibiotic activity against strains harboring specific resistance phenotypes, thus saving time and avoiding inadequate clinical trials (11). Cefditoren is an oral expanded-spectrum cephalosporin with mean serum values of 3.7 g/ml for the maximum concentration of drug in serum, 12.5 g · h/ml for the area under the concentration-time curve, and 1.54 h for the half-life after an oral 400-mg single dose (13). The amount of drug excreted in urine in 24 h is 18.2% (with mean concentrations in urine of 186.5 µg/ml at 2 to 4 h and 12.7 µg/ml at 8 to 12 h) (13), and the drug exhibits good activity against *E. coli* (7, 14), with a MIC of 0.5 µg/ml (8, 10).

The aim of this study was to evaluate the cefditoren urine bactericidal activity against *E. coli* isolates exhibiting different resistance phenotypes/genotypes in an in vitro pharmacodynamic model.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Phenotype</th>
<th>Genotype</th>
<th>MICa (µg/ml) of:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>CIP</td>
</tr>
<tr>
<td>S1</td>
<td>Penicillinase</td>
<td>TEM-1</td>
<td>0.007</td>
</tr>
<tr>
<td>S2</td>
<td>Penicillinase</td>
<td>TEM-1</td>
<td>&gt;32</td>
</tr>
<tr>
<td>S3</td>
<td>Penicillinase hyperproduction</td>
<td>TEM-1</td>
<td>0.015</td>
</tr>
<tr>
<td>S4</td>
<td>IRT</td>
<td>TEM-34 (IRT-6)</td>
<td>0.03</td>
</tr>
<tr>
<td>S5</td>
<td>ESBL</td>
<td>TEM-116</td>
<td>0.5</td>
</tr>
<tr>
<td>S6</td>
<td>ESBL</td>
<td>TEM-116 + SHV-12 (5-2a)</td>
<td>16</td>
</tr>
<tr>
<td>S7</td>
<td>ESBL</td>
<td>TEM-1 + SHV-12 (5-2a)</td>
<td>&gt;32</td>
</tr>
</tbody>
</table>

*a MICs were determined by following CLSI recommendations (5). CIP, ciprofloxacin; AMC, amoxicillin-clavulanic acid; CXM, cefuroxime; CDN, cefditoren.

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Seven *E. coli* isolates from urinary tract infections were used. Table 1 shows β-lactamase profiles and in vitro susceptibilities of the strains. Genotypic characterization was performed through amplification of TEM, SHV, and CTX-M β-lactamase genes by PCR and direct sequencing (12).

Cefditoren urine concentrations after an oral 400-mg single dose (13) were simulated over 24 h in a previously described two-compartment system (2) (volume of distribution ($V_o$) of 450 ml), with an additional compartment ($V_o$ of 71 ml [calculated with the equation $V_o = V_C/K_e$]) and $C_0$ of 1.672 μg/ml to simulate the accumulation of cefditoren in urine. Exponential decay of urine concentrations was estimated using the mean concentration of each documented interval in a phase I study (13) by using the expression $x_{n+1} = x_n - \sqrt{2}$, where $x$ is the concentration and $t_n$ the time point. The resulting polyphasic clearance from urine was obtained in the central compartment by a continuous dilution-elimination process using computerized peristaltic pumps (Masterflex; Cole-Parmer Instrument Co., Chicago, IL) at 0.86 ml/min (period from 0 to 3 h), 2.88 ml/min (period from 3 to 5 h), 3.04 ml/min (period from 5 to 12 h), and 0.86 ml/min (period from 12 to 24 h). Rates were synchronized using Win Lin software (Cole-Parmer Instrument Co., Chicago, IL).

Sixty milliliters of an inoculum of $5 \times 10^7$ CFU/ml was introduced into the peripheral compartment. Samples (0.5 ml) were collected at 0, 1, 2, 3, 4, 6, 8, 10, 12, and 24 h for colony counting (detection limit of $5 \times 10^5$ CFU/ml) and from the central compartment for bioassay measurement of experimental concentrations (15). Pharmacokinetic analysis was performed based on a noncompartmental approach (WinNonlin; Pharsight, Mountain View, CA).

Figure 1 shows target and experimental concentrations of cefditoren. Figure 2 shows colony counts over 24 h in control and cefditoren simulations. No regrowth was observed with the TEM-1 (penicillinase production or hyperproduction) or IRT strains (Fig. 2, top row). The ESBL TEM-116 strain showed regrowth from 12 h on but maintained low colony counts at this time point (24 h). The other two ESBL strains (S6 and S7) that produced SHV in addition to TEM-116 (S6) or TEM-1 (S7) showed regrowth from 8 h on, with high colony counts at 12 h and 24 h, close to those in antibiotic-free simulations in the case of S7.

At 8 h, bactericidal activity ($\geq 3$ log$_{10}$ reduction) was obtained against all strains but strain S7. At 12 h and 24 h, bactericidal activity was obtained against all strains except those harboring the SHV β-lactamase gene (S6 and S7). MICs prior to and after exposition were identical.

In antimicrobial assessment for uncomplicated cystitis, urine bactericidal activity is a relevant endpoint that is better assessed with antibiotic concentrations changing over time because subinhibitory concentrations may allow bacterial regrowth, resulting in therapeutic failure (4). In the present simulation, the system used was closed (bacteria were not eliminated with alterations in the flow rate); thus, changes in bacterial counts are attributable only to the action of the antimicrobial, as in a previous study (2). Therefore, it should be noted that we evaluated the antibiotic activity under conditions...
more adverse than the in vivo situation, where bladder emptying helps in clearing the microorganism. Since the medium used in the simulation was broth and not urine, it should be noted that a previous publication concluded that no major differences in the in vitro activities of cefditoren were found by using different media or pH values or by the presence of magnesium or calcium ions (6). Under these conditions, cefditoren exhibited bactericidal activity (>4 log_{10} reduction) against TEM-1 (penicillinase production or hyperproduction) and TEM-34 derivative (IRT-6) isolates from 4 to 24 h, suggesting that concentrations achieved in urine after once-daily 400-mg dosing may be enough to eradicate *E. coli* strains harboring these β-lactamase genetic determinants.

When considering ESBL strains, the situation is different for the TEM-116 strain and the strains harboring the SHV determinant. Against the strains harboring the SHV determinant in addition to TEM-116 and TEM-1, bactericidal activity was achieved only in the 6- to 8-h period in the first case (strain S6) and never over 24 h in the second case (strain S7). The results of this study show that the magnitudes and extensions of cefditoren’s urine bactericidal activity varied depending on the resistance genotype/phenotype of the *E. coli* isolates tested. Dosing intervals to be used in possible future clinical trials to explore the potential of cefditoren in the treatment of cystitis would depend on the prevalence of these resistance phenotypes in the community.

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REFERENCES