In Vitro Activity of Fosfomycin against Extended-Spectrum-β-Lactamase-Producing *Escherichia coli* and *Klebsiella pneumoniae*: Comparison of Susceptibility Testing Procedures

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The agar dilution, broth microdilution, and disk diffusion methods were compared to determine the in vitro susceptibility of 428 extended-spectrum-β-lactamase (ESBL)-producing *Escherichia coli* and *Klebsiella pneumoniae* to fosfomycin. Fosfomycin showed very high activity against all ESBL-producing strains. Excellent agreement between the three susceptibility methods was found for *E. coli*, whereas marked discrepancies were observed for *K. pneumoniae*.

Fosfomycin tromethamine is a stable salt of fosfomycin which is licensed for the single-dose treatment of acute uncomplicated urinary tract infections (UTIs) caused by susceptible organisms (8, 12). After many years of fosfomycin use, both in Spain and worldwide, fosfomycin continues to be active against the most common uropathogens, and there is a very low incidence of resistant strains in *Escherichia coli* (about 2%) (2, 12, 14).

Isolation of extended-spectrum-β-lactamase (ESBL)-producing *E. coli* and *Klebsiella pneumoniae* strains is increasing in Spain, and a high proportion of these isolates are recovered from outpatients with uncomplicated UTIs (4, 7, 11). It is common to find that the same plasmid coding for ESBL also contains genes conferring resistance to several groups of antimicrobial agents, such as aminoglycosides and cotrimoxazole (7, 10). The concurrence of quinolone resistance, particularly in ESBL-producing *K. pneumoniae*, is frequent (6, 10, 13), there being few alternatives for the appropriate oral treatment of uncomplicated UTIs caused by ESBL-producing microorganisms.

The present study was designed to assess the in vitro activity of fosfomycin by three different methods against 428 ESBL-producing strains, made up of 290 (68%) *E. coli* and 138 (32%) *K. pneumoniae* isolates.

The strains were collected in two Spanish studies: the first, developed in 2000, included 40 hospitals representing different regions of Spain, and the second was carried out in Seville, Spain, at the University Hospital of Virgen Macarena (UHVM), using strains recovered between 1995 and 2001 (7, 11). In both studies ESBL production was determined by broth microdilution according to CLSI guidelines (5).

The strains were collected in the multicenter study included 170 *E. coli* and 70 *K. pneumoniae* strains, while those obtained from the UHVM included 120 *E. coli* and 68 *K. pneumoniae* strains. One-hundred thirty-two out of 290 (45.5%) *E. coli* and 16 out of 138 (11.6%) *K. pneumoniae* strains were isolated from outpatients with community-acquired infections, and among these isolates found in the community, 66 (22.7%) *E. coli* and 9 (6.5%) *K. pneumoniae* strains were isolated from urine samples of women with uncomplicated UTIs.

The MICs of fosfomycin (Zambon, Milan, Italy) were determined by the agar dilution and broth microdilution methods in cation adjusted Mueller-Hinton medium, supplemented with 25 mg/liter of G-6-P (glucose-6-phosphate; Sigma Chemical Co.). A twofold dilution across a range of 0.25 to 512 mg of fosfomycin per liter was used. The inocula were prepared to achieve 1 × 10⁶ CFU per spot (agar dilution) or 5 × 10⁵ CFU/ml (broth microdilution) according to CLSI recommendations (5). At the same time, disks containing 200 μg fosfomycin and 50 μg G-6-P (Oxoid) were tested following the method outlined by CLSI (9).

Fosfomycin breakpoints for the interpretative criteria for *E. coli* were used according to CLSI guidelines (5, 9). Because of the absence of accepted breakpoints for *K. pneumoniae*, those proposed by CLSI for *E. coli* have been used (5, 9).

Susceptibility results of the broth microdilution and disk diffusion methods were compared with those obtained from the agar dilution method used as the reference method. Agreement and discrepancies between the evaluated and reference method were classified as complete agreements, very major errors, major errors, and minor errors. Differences in proportions among paired categorical data were calculated by the McNemar test and for independent categorical data by the McNemar test.
chi-square test. The Wilcoxon test was used for comparing MICs. Statistical significance was established as $P < 0.05$, and very significant was established as $P < 0.01$.

By the agar dilution method, 417 (97.4%) strains were susceptible to fosfomycin, with MICs of $\leq 64 \mu g/mL$. The fosfomycin 50% minimum inhibitory concentration (MIC$_{50}$) was 2 $\mu g/mL$, and the MIC$_{90}$ was 32 $\mu g/mL$ (range, 0.5 to 512 $\mu g/mL$). Most isolates with intermediate susceptibility or resistance to fosfomycin (2.6%) were $K$. pneumoniae isolates: four resistant strains (MIC, $\geq 256 \mu g/mL$) and six intermediate strains (MIC, 128 $\mu g/mL$). Only one strain of $E$. coli of all 290 tested showed intermediate susceptibility to fosfomycin (MIC, 128 $\mu g/mL$). $K$. pneumoniae isolates have the highest MICs for fosfomycin (MIC$_{50}$ and MIC$_{90}$ of 16 to 64 $\mu g/mL$) but are still within the susceptible range, whereas more than 90% of $E$. coli isolates yielded very low MICs ($\leq 4 \mu g/mL$) (Fig. 1). These results are similar to those described in previous reports of non-ESBL-producing isolates (2, 8, 14), confirming that fosfomycin retains its activity against ESBL-producing isolates, and cross-resistance with other classes of antimicrobial agents is not a problem at present.

Important discrepancies have been reported between broth and agar dilution MICs for fosfomycin, and, so far, agar dilution is the only approved fosfomycin MIC susceptibility testing method (5). The use of disks containing 200 $\mu g$ of fosfomycin and 50 $\mu g$ of G-6-P in Mueller-Hinton medium is only indicated for testing with $E$. coli (9).

However, in common with others (1, 3, 6) who have used Mueller-Hinton broth supplemented with G-6-P, we observed only minor differences (average, $\pm 1.93$ dilutions; range, 0 to 7). In 241 (56.4%) strains the MIC broth values were higher than those obtained with agar ($P < 0.001$). The complete percentage agreement between the broth and disk dilution method and agar dilution results was higher for $E$. coli (98.6%) than for $K$. pneumoniae strains (72.5%) ($P < 0.001$). Table 1 compares the categories obtained by the microdilution broth and disk diffusion methods with those obtained by the agar dilution method used as reference. For both evaluated methods, the error rate observed for $K$. pneumoniae was significantly higher than that for $E$. coli ($P < 0.001$).

Our study confirms that the result of fosfomycin susceptibility testing is dependent on the method used and the microorganisms tested. Significant differences were not found between the two evaluated methods for clinical categories for $E$. coli (broth, $P = 0.625$; disk, $P = 1.00$). However, the results of susceptibility testing of $K$. pneumoniae showed poor correlation with the broth microdilution ($P < 0.001$) and disk diffusion methods ($P = 0.001$) reporting greater resistance to fosfomycin. Besides, for $K$. pneumoniae, the error rate obtained with the broth microdilution was significantly higher than that with the disk method ($P = 0.01$).

When broth microdilution or disk diffusion methods are used to determine the susceptibility to fosfomycin of $K$. pneumoniae, the results for resistance and intermediate susceptibility should be confirmed by the reference method. Because most automated systems for antimicrobial susceptibility testing are microdilution-based methods, resistance to fosfomycin may be overestimated in laboratories employing such systems, especially for isolates other than $E$. coli.

Our data suggest an excellent in vitro activity of fosfomycin against ESBL-producing $E$. coli and $K$. pneumoniae strains. Since fosfomycin is only approved in the United States for $E$. coli UTI treatment, further clinical studies are required to assess the clinical efficacy of fosfomycin for the treatment of UTIs caused by ESBL-producing $K$. pneumoniae.

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### REFERENCES


### TABLE 1. Correlation of broth microdilution and disk diffusion methods with the reference agar dilution method

<table>
<thead>
<tr>
<th>ESBL-producing strain</th>
<th>No.</th>
<th>Method</th>
<th>Error rate, % (no. of strains)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Very major</td>
</tr>
<tr>
<td>$E$. coli</td>
<td>290</td>
<td>Broth microdilution</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Disk diffusion</td>
<td>0</td>
</tr>
<tr>
<td>$K$. pneumoniae</td>
<td>138</td>
<td>Broth microdilution</td>
<td>1.4 (2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Disk diffusion</td>
<td>0.7 (1)</td>
</tr>
<tr>
<td>Total</td>
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<td>Broth microdilution</td>
<td>0.46 (2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Disk diffusion</td>
<td>0.23 (1)</td>
</tr>
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