Detection of Favorable Oral Cephalosporin-Clavulanate Interactions by In Vitro Disk Approximation Susceptibility Testing of Extended-Spectrum-Beta-Lactamase-Producing Members of the Enterobacteriaceae

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Extended-spectrum-beta-lactamase (ESBL)-producing members of the Enterobacteriaceae are often resistant to multiple drug classes, making therapy of urinary infections with oral antibiotics difficult. Previously it was shown that amoxicillin-clavulanate can provide clavulanate inhibition of ESBLs and protect an oral cephalosporin present in combination when tested by broth microdilution. This study has shown that disk approximation testing could detect favorable cephalosporin-clavulanate interactions among a group of 101 previously characterized members of the Enterobacteriaceae with CTX-M, SHV, or TEM ESBLs.

Community onset infections, specifically urinary tract infections due to extended-spectrum-beta-lactamase (ESBL)-producing members of the Enterobacteriaceae, have become an area of increasing concern with regard to evolving antibiotic resistance (4, 9, 10). Genes encoding ESBLs often reside on plasmids that frequently harbor other resistance determinants for a variety of other antibiotic classes (10). This multidrug resistance leaves providers with few if any oral treatment options for these strains. Many ESBLs and other class A beta-lactamases are inhibited by the beta-lactamase inhibitors clavulanate, sulbactam, and tazobactam, with inhibition of third-generation cephalosporin hydrolysis by clavulanate used by clinical laboratories to confirm the presence of ESBLs in members of the Enterobacteriaceae (2, 10). Clavulanate is the only beta-lactamase inhibitor available in an oral formulation, coformulated with amoxicillin (5). Recent literature suggests a high cure rate (93%) for cystitis using amoxicillin-clavulanate for susceptible ESBL-producing Escherichia coli (14). However, many ESBL-producing isolates are resistant to amoxicillin-clavulanate (14).

Two orally administered cephalosporins, cefpodoxime and cefdinir, have been used successfully for the treatment of cystitis involving susceptible, non-ESBL-producing members of the Enterobacteriaceae (13, 15, 17). However, the activities of these agents are compromised by amoxicillin-clavulanate can provide clavulanate inhibition of ESBLs and protect cefdinir from hydrolysis when tested in combination using specially prepared broth microdilution susceptibility testing panels (11). Adding amoxicillin-clavulanate increased the susceptibility rate for cefdinir to 89.1% against the strain collection studied. Other authors have published similar findings, demonstrating increased susceptibility when 10 μg clavulanate was added to ce-fixime or cefpodoxime dilutions in susceptibility testing panels (8, 12). The broth microdilution susceptibility testing methodology utilized in our previous report, utilizing custom-prepared panels, is not practical for routine use in clinical laboratories. Therefore, the objective of the present study was to determine the feasibility of utilizing disk approximation testing to evaluate the susceptibilities of ESBL-producing members of the Enterobacteriaceae to cefdinir or cefpodoxime when combined with amoxicillin-clavulanate.

Isolates tested in this study were 101 previously characterized members of the Enterobacteriaceae harboring CTX-M, SHV, or TEM ESBLs recovered from 2001 to 2008 (4, 11). In addition to cefpodoxime and penicillin resistance, most isolates were also resistant to trimethoprim-sulfamethoxazole (~86%), fluoroquinolones (76.5%), and doxycycline (81.5%). Isolates were tested utilizing CLSI disk diffusion methodology and commercially available (BBL) cefdinir (5-μg), cefpodoxime (10-μg), and amoxicillin-clavulanate (20/10-μg) disks alone and then by placing cefdinir and cefpodoxime disks 15 mm from an amoxicillin-clavulanate disk (3). MICs of cefdinir, cefpodoxime, and amoxicillin-clavulanate were determined utilizing CLSI broth microdilution methodology (2). MICs and zones of inhibition were determined for each individual agent and interpreted according to current CLSI standards (3). They were also tested as combinations of cefpodoxime plus amoxicillin-clavulanate and cefdinir plus amoxicillin-clavulanate. The combination tests were performed by preparing 2-fold dilutions of cefpodoxime and cefdinir in a fixed combination of 8 μg/ml amoxicillin and 4 μg/ml clavulanate (11). All isolates were tested with cefdinir, and a subset of 49 of the isolates was tested with cefpodoxime.

The combinations of cefpodoxime and amoxicillin-clavulanate in broth microdilution achieved a favorable interaction if the combination lowered the MIC of the cephalosporin from the resistant cat-
category into the susceptible range for that agent, i.e., \( \leq 1 \mu g/ml \) for cefdinir and \( \leq 2 \mu g/ml \) for cefpodoxime (3). Disk approximation test results were evaluated and agreed upon by three investigators (Jennifer D. Campbell, James S. Lewis II, and James H. Jorgensen) and were defined as follows: favorable potentiation, a distortion of the cefdinir or cefpodoxime zones by amoxicillin-clavulanate to create an area of enhanced inhibition between the disks, with the zone reaching the cefdinir or cefpodoxime disk; partial potentiation, distortion of the cefdinir or cefpodoxime zones by amoxicillin-clavulanate to create an area of enhanced inhibition between the disks but without the zone of inhibition reaching the cefdinir or cefpodoxime disk; and no potentiation, defined as minimal or no distortion of the cefdinir or cefpodoxime zones by amoxicillin-clavulanate (see Fig. 1 for examples).

Based upon broth microdilution testing, 79 of 101 (78.2\%) isolates in this collection were susceptible to the combination of cefdinir plus amoxicillin-clavulanate by broth microdilution (Table 1); 60 of these 79 (75.9\%) demonstrated full potentiation, and 16 (20.2\%) demonstrated partial potentiation by disk testing. Three organisms that were susceptible to cefdinir plus amoxicillin-clavulanate in broth did not reveal potentiation by disk testing. The MICs for these three isolates were 0.06, 0.25, and 0.5 \( \mu g/ml \) of the cefdinir/amoxicillin-clavulanate combination. A total of 38 out of 49 (77.6\%) tested isolates were susceptible to cefpodoxime plus amoxicillin-clavulanate (Table 2); of these 38 isolates, 34 (89.5\%) demonstrated full potentiation by disk testing and the other 4 (10.5\%) isolates demonstrated partial potentiation. None of the isolates that were resistant to cefdinir and cefpodoxime plus amoxicillin-clavulanate by broth microdilution demonstrated zone potentiation by disk diffusion. The favorable clavulanate effect on the activity of the companion cephalosporin did not differ based upon the species tested, with the exception of one Morganella morganii isolate and five Enterobacter spp. isolates that were refractory to the clavulanate effect, most likely due to their intrinsic AmpC \( \beta \)-lactamase. In addition, there were some E. coli and Klebsiella spp. isolates known to produce ESBLs that were
SHV-containing strains were more likely to also contain an AmpC bored a SHV ESBL. This is most likely due to the fact that the lanate potentiation of the cephalosporin than strains that harbor TEM ESBL, it is not included in the tables. It should be noted that according to the class of ESBL. Since only one isolate produced a activity by broth microdilution and disk approximation data ac-

not susceptible to the cephalosporin plus amoxicillin-clavulanate combinations. Ten such isolates were examined for possible AmpC production by testing ceftazidime and cefoxitin disks alone and with 300 μg alpha-phenyl boronic acid added (18). Five of the 10 isolates showed a 5 mm or greater increase in zone di-

Among this isolate collection, 26/101 (25.7%) and 21/101 (20.8%) of the isolates were susceptible to amoxicillin-clavulanate by disk and broth microdilution testing, respectively. Eight isolates were susceptible to amoxicillin-clavulanate by disk testing but not by broth and four isolates that were not susceptible by disk testing were susceptible using broth microdilution.

ESBL-producing members of the Enterobacteriaceae are often resistant to most oral antibiotics used to treat urinary tract infec-

<table>
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<tr>
<th>ESBL group (no. of isolates)</th>
<th>MIC tested alone</th>
<th>Cefpodoxime MIC tested with Amox-Clav&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Cefdinir MIC tested with Amox-Clav&lt;sup&gt;a&lt;/sup&gt;</th>
<th>% susceptible&lt;sup&gt;b&lt;/sup&gt;</th>
<th>% detected by disk approximation&lt;sup&gt;c&lt;/sup&gt;</th>
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<tr>
<td></td>
<td>Cefpodoxime</td>
<td>Amox-Clav&lt;sup&gt;d&lt;/sup&gt;</td>
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<td>MIC&lt;sub&gt;50&lt;/sub&gt;</td>
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<td>MIC&lt;sub&gt;50&lt;/sub&gt;</td>
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<tr>
<td>CTX-M (55)&lt;sup&gt;e&lt;/sup&gt;</td>
<td>&gt;32</td>
<td>&gt;32</td>
<td>2–&gt;32</td>
<td>32</td>
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<tr>
<td>SHV (40)&lt;sup&gt;f&lt;/sup&gt;</td>
<td>&gt;16</td>
<td>&gt;16</td>
<td>0.5–&gt;16</td>
<td>32</td>
<td>&gt;32</td>
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<tr>
<td>CTX-M + SHV (6)&lt;sup&gt;g&lt;/sup&gt;</td>
<td>&gt;16</td>
<td>&gt;16</td>
<td>8–&gt;16</td>
<td>32</td>
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<sup>a</sup> Cefdinir was tested in standard 2-fold dilution series in the presence of a fixed concentration of 8 μg/ml amoxicillin and 4 μg/ml clavulanate.
<sup>b</sup> Percent of those susceptible to the drug combination by MIC that were detected by disk approximation testing.
<sup>c</sup> Activity of the amoxicillin-clavulanate combination expressed as the amoxicillin component.
<sup>d</sup> Cefpodoxime was tested in standard 2-fold dilution series in the presence of a fixed concentration of 8 μg/ml amoxicillin and 4 μg/ml clavulanate.
<sup>e</sup> Percent of those susceptible to the drug combination by MIC that were detected by disk approximation testing.
<sup>f</sup> Activity of the amoxicillin-clavulanate combination expressed as the amoxicillin component.
<sup>g</sup> Cefpodoxime was tested in standard 2-fold dilution series in the presence of a fixed concentration of 8 μg/ml amoxicillin and 4 μg/ml clavulanate.
<sup>h</sup> Percent of those susceptible to the drug combination by MIC that were detected by disk approximation testing.
<sup>i</sup> Activity of the amoxicillin-clavulanate combination expressed as the amoxicillin component.
<sup>j</sup> Cefpodoxime was tested in standard 2-fold dilution series in the presence of a fixed concentration of 8 μg/ml amoxicillin and 4 μg/ml clavulanate.
<sup>k</sup> Percent of those susceptible to the drug combination by MIC that were detected by disk approximation testing.
<sup>l</sup> Activity of the amoxicillin-clavulanate combination expressed as the amoxicillin component.
<sup>m</sup> Cefpodoxime was tested in standard 2-fold dilution series in the presence of a fixed concentration of 8 μg/ml amoxicillin and 4 μg/ml clavulanate.
<sup>n</sup> Percent of those susceptible to the drug combination by MIC that were detected by disk approximation testing.
<sup>o</sup> Activity of the amoxicillin-clavulanate combination expressed as the amoxicillin component.
<sup>p</sup> Cefpodoxime was tested in standard 2-fold dilution series in the presence of a fixed concentration of 8 μg/ml amoxicillin and 4 μg/ml clavulanate.
<sup>q</sup> Percent of those susceptible to the drug combination by MIC that were detected by disk approximation testing.
<sup>r</sup> Activity of the amoxicillin-clavulanate combination expressed as the amoxicillin component.
<sup>s</sup> Cefpodoxime was tested in standard 2-fold dilution series in the presence of a fixed concentration of 8 μg/ml amoxicillin and 4 μg/ml clavulanate.
cystitis due to ESBL-producing E. coli deserves further assessment (6, 8, 13, 15, 16, 17). However, isolates that produce an AmpC β-lactamase in addition to an ESBL do not usually demonstrate a clavulanate effect and would not be candidates for this potential treatment strategy. This study has shown that disk approximation testing with two cephalosporins and amoxicillin-clavulanate can detect favorable in vitro interactions and would be a simple approach for testing by clinical laboratories. These in vitro observations require data for patient outcomes to determine if this novel therapeutic approach has merit for therapy of urinary tract infections due to multidrug-resistant Enterobacteriaceae.

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REFERENCES