Identification of Plasmid-Mediated AmpC \( \beta \)-Lactamases in *Escherichia coli*, *Klebsiella* spp., and *Proteus* Species Can Potentially Improve Reporting of Cephalosporin Susceptibility Testing Results

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The goal of this study was to determine if the interpretations of extended-spectrum and advanced-spectrum cephalosporins (ESCs and ASCs, respectively) for isolates of Enterobacteriaceae would be impacted by the results of aminophenylboronic acid (APBA) testing. Fifty-three isolates of *Escherichia coli*, 21 *Klebsiella* species, and 6 *Proteus* species that were resistant to at least one ESC were tested by disk diffusion with ceftazidime and cefotetan disks with and without APBA. Ceftazidime disks with and without clavulanic acid (CLAV) were also tested to confirm extended-spectrum \( \beta \)-lactamase (ESBL) carriage. Twenty-nine (36.3%) isolates were only APBA test positive, 27 were only CLAV test positive, 2 were positive with both substrates, and 22 were negative with both substrates. Thirteen (41.9%) of the 31 APBA-test-positive isolates (all *E. coli*) tested susceptible to cefotaxime, ceftriaxone, or ceftazidime. Since clinical data suggest that AmpC-producing isolates should be reported as resistant to all ESCs, APBA testing can be helpful in identifying such organisms. Screening for AmpC-producing organisms using nonsusceptibility to cefoxitin and amoxicillin-clavulanate was less specific than APBA testing; it identified ESBL as well as AmpC-producing organisms. Only 18 of 31 APBA-positive isolates were positive by PCR for an AmpC \( \beta \)-lactamase gene. Thus, testing with APBA could improve the accuracy of reporting ESCs, especially for *E. coli*. However, results of APBA and CLAV testing did not correlate well for isolates containing both AmpC \( \beta \)-lactamases and ESBLs. Thus, additional data are needed before formal recommendations can be made on changing the reporting of ASC test results.

\( \beta \)-Lactamase-mediated resistance to penicillins and cephalosporins is a significant problem among gram-negative bacteria worldwide (2, 4, 17). \( \beta \)-Lactamases can be divided into four major classes (A, B, C, and D) based on substrate profiles and amino acid sequences (3, 5). Class A \( \beta \)-lactamases include enzymes that are active against penicillins and cephalosporins. Mutations in key positions in class A \( \beta \)-lactamase genes can produce extended-spectrum \( \beta \)-lactamases (ESBLs) that mediate resistance to extended-spectrum cephalosporins (ESCs), such as cefotaxime (CTX), ceftriaxone, and ceftazidime (CAZ), and, in some cases, the advanced-spectrum cephalosporins (ASCs) cefepime and cefpirome (2, 13, 14). Class C \( \beta \)-lactamases, such as the AmpC enzymes, also mediate resistance to ESCs but often remain susceptible to the ASCs, which could be important information for clinicians considering therapy for gram-negative infections.

Studies by Coudron (9), Jacoby et al. (12), and Yagi et al. (33) have validated the use of boronic acid derivatives to detect AmpC \( \beta \)-lactamases in gram-negative bacilli. Coudron’s study (9) included a recommendation that for isolates positive by the boronic acid test all cephalosporin test results be reported as resistant, similar to the Clinical and Laboratory Standards Institute (CLSI) ESBL reporting algorithm. It is not clear if Coudron’s recommendation included changing the results with regard to ASCs. The other two studies did not comment on the issue of how to interpret cephalosporin results. CLSI has yet to establish a testing and reporting algorithm specifically for organisms containing AmpC \( \beta \)-lactamases.

CLSI’s algorithm to detect and confirm the presence of ESBLs is limited to isolates of *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Escherichia coli*, and *Proteus mirabilis* (6–8). Using the algorithm is critical for accurate test reporting since ESBL-producing organisms often test susceptible to one or more ESCs even though these drugs are not clinically active against infections caused by ESBL-producing organisms (20, 21). Thus, confirmation of the presence of an ESBL in an isolate of *E. coli*, *P. mirabilis*, or a *Klebsiella* species indicates that all penicillin and cephalosporin results should be reported as indicating resistance. However, resistance to ESCs in *E. coli*, *Enterobacter* species, and several other species of *Enterobacteriaceae* can also be the result of mutations that upregulate production of chromosomal AmpC \( \beta \)-lactamases or can be the result of acquisition of plasmid-encoded AmpC enzymes among organisms that do not contain a chromosomal AmpC \( \beta \)-lactamase gene (24, 32). Pai and colleagues (19) have indicated that organisms that produce AmpC \( \beta \)-lactamases, similar to those that produce ESBLs, may test as susceptible to ESCs,
thus producing misleading clinical information. This suggests that the identification of AmpC β-lactamases among *E. coli*, *P. mirabilis*, or a *Klebsiella* species could also increase the accuracy of antimicrobial susceptibility test reports for ESCs if the results were used to modify the interpretations of cephalosporin results.

As noted above, in contrast to bacterial isolates containing ESBLs, isolates containing AmpC β-lactamases typically remain susceptible to ASCs (13, 14, 26, 27, 31). Although the occurrence is rare, AmpC β-lactamases, such as CMY-19, show modest cephalosporin hydrolyzing activity (31) even though the organisms carrying this β-lactamase remain fully susceptible to both cephalosporin and cefpirome in vitro. Thus, it may be clinically relevant to distinguishing between bacterial isolates containing ESBLs, for which ASC results should be reported as resistant, and those containing AmpC β-lactamases, for which ESC results would be reported as resistant but the ASC results would be reported as they test.

The major goals of this study were to determine (i) whether testing 3-aminophenylboronic acid hemisulfate (APBA) in conjunction with CAZ and cefotetan (CTT) disks could improve the reporting of results for ESCs and ASCs by indicating the combination of resistance to amoxicillin-clavulanate and cefoxitin as a surrogate had equivalent sensitivity and specificity to the APBA assay for identifying AmpC β-lactamase-producing organisms, (iii) whether organisms containing both ESBLs and AmpC β-lactamases would be consistently detected with both clavulanic acid (CLAV) and APBA, and (iv) whether a disk diffusion test using APBA dissolved in water gave comparable results to dissolving the free acid compound in dimethyl sulfoxide (DMSO). With regard to the last goal, one of the drawbacks to using boronic acid is the requirement to dissolve the free acid in DMSO, a toxic chemical not often used in microbiology laboratories. Thus, we hypothesized that a boronic acid compound that did not have to be dissolved in DMSO could improve the general acceptance of the test in clinical laboratories.

**MATERIALS AND METHODS**

### Bacterial strains

A total of 80 clinical isolates of *E. coli*, *Klebsiella* species, and *Proteus* species collected during a 5-year period from 15 hospitals in the United States that participate in Project ICARE (Intensive Care Antimicrobial Resistance Epidemiology) (11) were tested (Table 1). Sixty-one isolates were previously characterized for ESBL production (18, 28, 30). All isolates were resistant to at least one ESC (i.e., CTX, CAZ, ceftriaxone, or ceftizoxime). The study was conducted in three phases. In phase I, all 80 isolates were tested. In phase II, a subset of 24 isolates from phase I (21 *E. coli*, 1 *K. oxytoca*, and 2 *K. pneumoniae* isolates), which had been collected prospectively at the University of Wisconsin because they were nonsusceptible to both cefoxitin (FOX) and amoxicillin-clavulanate, were further examined (Table 2). In phase III, an additional 11 isolates (8 *E. coli* and 2 *K. pneumoniae* isolates and 1 *P. mirabilis* isolate) containing multiple β-lactamases from the Wyeth Research collection were tested (Table 3). Isolates from phase I were identified using Vitek 2 (bioMérieux, Durham, NC) or standard biochemical methods (10). Isolates from phase II were identified using Vitek, conventional biochemical tests, and phenotypic tests, and isolates in phase III were identified using API-20E strips (bioMérieux). All isolates were stored at −70°C prior to testing. Each isolate was subcultured on Trypticase soy agar supplemented with 5% sheep blood (BD Biosciences, Sparks, MD) twice before testing. Control strains were kindly provided by the following individuals: *E. coli* TG1 containing the β-lactamase FON-1 was from George Jacoby (Lahey Clinic, Boston, MA); *E. coli* isolates containing the DHA-1 and ACC-1 β-lactamases were obtained from Nancy Hanson (Creighton University School of Medicine, Omaha, NB); *K. pneumoniae* isolates containing the CMY-8 was from Jing-Jou Yan (National Cheng Kung University Medical College, Taiwan); and *Salmonella enterica* AM03430 containing a CMY-2 β-lactamase was obtained from Jean Whichard (Centers for Disease Control and Prevention, Atlanta, GA).

**Disk diffusion testing.** BBL Sensi-Disc (6 mm) disks containing 30 μg of either CTT, 30 μg of CAZ, or 30 μg of CAZ plus 10 μg of CLAV were purchased from BD Biosciences (Sparks, MD). APBA hemisulfate (120 mg) was dissolved in 6 ml of distilled H₂O, and 100 mg of APBA was dissolved in DMSO; 30 μl of either solution was added to CAZ and CTT disks. Disks were dried for 30 min prior to use or stored at 4°C for up to 1 month without loss of activity. Organisms were suspended in Mueller-Hinton broth to the turbidity of a 0.5 McFarland standard and inoculated onto 150-mm Mueller-Hinton agar plates (BD Biosciences) as for disk diffusion (7). Disks containing 30 μg of CTT, 30 μg of CAZ, 30 μg of CTT plus 600 μg of APBA, 30 μg of CAZ plus 600 μg of APBA, and 30 μg of CAZ plus 10 μg of CLAV were placed on the plate. (All isolates had previously been

### Table 1. Results of APBA, CLAV, and AmpC PCR tests for all study isolates

<table>
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<tr>
<th>Organism</th>
<th>No. of isolates tested</th>
<th>No. of isolates positive by APBA test by substrate (family)</th>
<th>No. of isolates positive with CLAV and APBA (family)</th>
<th>No. of isolates positive with CLAV only</th>
<th>No. of isolates negative with both APBA and CLAV</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td>53</td>
<td>7 (2 CIT, 5 NEG)</td>
<td>17 (4 CIT, 1 DHA, 1 FOX, 5 MOX, 6 NEG)</td>
<td>2 (1 EBC, 1 NEG)</td>
<td>11 (4 CIT, 1 DHA, 1 FOX, 5 MOX, 6 NEG)</td>
</tr>
<tr>
<td><em>Klebsiella oxytoca</em></td>
<td>7</td>
<td>1 (CIT)</td>
<td>6</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td>14</td>
<td>3 (2 FOX, 1 MOX)</td>
<td>10</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td><em>Proteus mirabilis</em></td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td><em>Proteus penneri</em></td>
<td>4</td>
<td></td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>80</td>
<td>7</td>
<td>21</td>
<td>2</td>
<td>27</td>
</tr>
</tbody>
</table>

* Multiplex PCR was performed as described by Perez-Perez and Hanson (23) only on isolates that were positive in the APBA disk diffusion test. NEG, negative PCR assay. PCR results represent families of AmPC genes, not individual determinants (See Materials and Methods).

* Organisms were tested previously with CAZ and CTT with and without CLAV per CLSI criteria (7). Results of ESBL production were consistent with previous results.

* pI values of β-lactamases determined by aIEF of five PCR negative isolates: (i) 8.5; (ii) 8.9, 8.1, 7.7, 7.0, 6.5; (iii) 9.0; (iv) 9.0; (v) 9.0, 8.8, 5.4.

* aIEF of six PCR negative isolates: (i) 8.4, 5.4; (ii) 8.4, 5.4; (iii) 8.9; (iv) 8.4, 5.3; (v) 9.0, 8.4, 7.6, 7.0, 6.4; (vi) 9.2, 8.0, 6.8, 5.4.

* aIEF of PCR negative isolate, 7.3.

* All ESBL screen test positive.
screened for ESBL production by disk diffusion with both CAZ and CTX, with and without CLAV. Thus, in this study we screened only with disks containing CAZ and CAZ plus CLAV to ensure that the ESBL type was consistent with previous results. All ESBL results were consistent). Plates were incubated for 18 to 20 h in ambient air at 35°C. Zone diameter differences of ≤5 mm between the CTT or CAZ disks without APBA and those supplemented with APBA were considered positive for AmpC β-lactamases, as previously described (9, 12, 33). Positive control strains for APBA testing included the genes within the following families: ACC, CIT (including LAT-1 to LAT-5, LAT-7, LAT-9 to LAT-11), DHA (DHA-1 and DHA-2), EBC (including MIR-1T and ACT-1), FOX (including FOX-1 to FOX-5b), and MOX (including MOX-1, MOX-2, CMY-1, and CMY-8 to CMY-11). Positive- and negative-control organisms were included in each assay.

Organisms were tested using the disk diffusion method. The five AmpC control organisms and the ESBL-positive control (K. pneumoniae ATCC 700603) and an AmpC β-lactamase-negative E. coli isolate were tested in parallel with disks containing CAZ and CTT in combination with either APBA dissolved in DMSO or the APBA hemisulfate salt dissolved in water. The zone diameters around the corresponding pairs of disks containing APBA dissolved in either water or DMSO were within ±1 mm for all organisms, and all of the interpretations (positive or negative for AmpC β-lacta-

### Table 2. Isolates presumptively positive for AmpC β-lactamases using cefoxitin and amoxicillin-clavulanic acid screening

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<th>APBA-positive result with CAZ/CTX</th>
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<tr>
<td>E. coli</td>
<td>1</td>
<td>MOX</td>
<td>R</td>
<td>CAZ/CTT</td>
</tr>
<tr>
<td>E. coli</td>
<td>2</td>
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<td>CIT</td>
<td>R</td>
<td>CAZ, CTT</td>
</tr>
<tr>
<td>E. coli</td>
<td>1</td>
<td>Negative</td>
<td>I</td>
<td>CAZ</td>
</tr>
<tr>
<td>K. pneumoniae</td>
<td>1</td>
<td>MOX</td>
<td>I</td>
<td>CAZ</td>
</tr>
<tr>
<td>K. pneumoniae</td>
<td>1</td>
<td>FOX</td>
<td>I</td>
<td>CAZ</td>
</tr>
<tr>
<td>K. oxytoca</td>
<td>1</td>
<td>CIT</td>
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</tr>
</tbody>
</table>

a The AmpC gene family is indicated for positive results.

b Positive for SHV-type β-lactamase by PCR assay, but DNA sequence was not determined.

c Organisms were tested using the disk diffusion method.

d ESBL positive.

### Table 3. Test results for isolates containing both ESBL and AmpC β-lactamases

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<td>Yes</td>
<td>No</td>
<td>R/R</td>
</tr>
<tr>
<td>E. coli</td>
<td>CTX-M-2, ACT-1</td>
<td>No</td>
<td>No</td>
<td>R/R</td>
</tr>
<tr>
<td>E. coli</td>
<td>CTX-M-5, ACT</td>
<td>Yes</td>
<td>No</td>
<td>R/R</td>
</tr>
<tr>
<td>K. pneumoniae</td>
<td>SHV-type, CTX-M-2, DHA</td>
<td>No</td>
<td>Yes</td>
<td>S/S</td>
</tr>
<tr>
<td>E. coli</td>
<td>TEM-1, SHV-7, CMY-2</td>
<td>Yes</td>
<td>Yes</td>
<td>R/S</td>
</tr>
<tr>
<td>K. pneumoniae</td>
<td>TEM-1, TEM-10, SHV-7, ACT-1</td>
<td>No</td>
<td>Yes</td>
<td>S/S</td>
</tr>
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a R, resistant; I, intermediate; S, susceptible.

b Positive for SHV-type β-lactamase by PCR assay, but DNA sequence was not determined.

c Ceftiraxone MIC of 4 μg/ml.

### RESULTS

**APBA testing.** The five AmpC control organisms and the ESBL-positive control (K. pneumoniae ATCC 700603) and an AmpC β-lactamase-negative E. coli isolate were tested in parallel with disks containing CAZ and CTT in combination with either APBA dissolved in DMSO or the APBA hemisulfate salt dissolved in water. The zone diameters around the corresponding pairs of disks containing APBA dissolved in either water or DMSO were within ±1 mm for all organisms, and all of the interpretations (positive or negative for AmpC β-lacta-

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</tr>
<tr>
<td>E. coli</td>
<td>1</td>
<td>Negative</td>
<td>I</td>
<td>CAZ</td>
</tr>
<tr>
<td>K. pneumoniae</td>
<td>1</td>
<td>MOX</td>
<td>I</td>
<td>CAZ</td>
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<td>1</td>
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<td>1</td>
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b Positive for SHV-type β-lactamase by PCR assay, but DNA sequence was not determined.

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</tr>
<tr>
<td>K. pneumoniae</td>
<td>TEM-1, TEM-10, SHV-7, ACT-1</td>
<td>No</td>
<td>Yes</td>
<td>S/S</td>
</tr>
</tbody>
</table>

a R, resistant; I, intermediate; S, susceptible.

b Positive for SHV-type β-lactamase by PCR assay, but DNA sequence was not determined.

c Ceftiraxone MIC of 4 μg/ml.

### RESULTS

**APBA testing.** The five AmpC control organisms and the ESBL-positive control (K. pneumoniae ATCC 700603) and an AmpC β-lactamase-negative E. coli isolate were tested in parallel with disks containing CAZ and CTT in combination with either APBA dissolved in DMSO or the APBA hemisulfate salt dissolved in water. The zone diameters around the corresponding pairs of disks containing APBA dissolved in either water or DMSO were within ±1 mm for all organisms, and all of the interpretations (positive or negative for AmpC β-lacta-
mas genes) were the same for each pair. Thus, the results for the APBA hemisulfate salt dissolved in water were comparable to the results of the free acid dissolved in DMSO (data not shown). The remainder of the testing was performed using the APBA hemisulfate salt dissolved in water.

In phase I, a total of 80 clinical isolates of \textit{E. coli}, \textit{Klebsiella} species, and \textit{Proteus} species that were resistant to one or more ESCs were tested by disk diffusion with CAZ and CTT disks and with and without APBA (an increase in zone diameter of \( \approx \)5 mm with either CAZ only [7 isolates], CTT only [1 isolate], or both drugs [21 isolates]), 27 were positive only with CLAV (indicating ESBL production), 2 were positive with both substrates, and 22 were negative with both substrates. The CLAV-positive results for the 27 isolates were consistent with those of our previous studies on ESBL production (18, 28, 30). Of the 31 APBA-positive isolates (including the two that were also CLAV positive), 13 (41.9\%) all \textit{E. coli} tested as susceptible to at least one ESC. The other 18 isolates were intermediate or resistant to CTX, CAZ, ceftiraxone, and cefepoxide. Twenty-eight of the 29 (96.5\%) APBA-positive isolates that were CLAV negative tested susceptible to the ASC cephalosporin (MIC \( \leq 8 \) \( \mu \)g/ml) (data not shown). Surprisingly, only 18 of the 31 APBA-positive isolates were positive for an AmpC gene product by PCR (Table 1) although all 31 had an IEF product (Table 1). The genes present encoded \( \beta \)-lactamases included in each family.

Screening for AmpC-containing organisms using cefoxitin and amoxicillin-clavulanate results. In the second phase of the study, we evaluated whether an alternate strategy to APBA testing, i.e., using nonsusceptibility to both cefoxitin and amoxicillin-clavulanate, was an accurate indicator of AmpC \( \beta \)-lactamase-producing organisms. Twenty-four isolates, all of which had been collected prospectively at a single institution, were evaluated. The organisms included 21 \textit{E. coli} and 2 \textit{K. pneumoniae} isolates and 1 \textit{K. oxytoca} isolate that were intermediate or resistant to cefoxitin and amoxicillin-clavulanate and intermediate or resistant to at least one ESC by Vitek testing (Table 2). Two \textit{E. coli} isolates that were intermediate to either cefoxitin or amoxicillin-clavulanate were positive for ESBLs by CLAV testing and negative with APBA. Four additional \textit{E. coli} isolates, although resistant to cefoxitin and amoxicillin-clavulanate, were susceptible to CAZ, ceftiraxone, CTX, and cefepime by broth microdilution reference testing and gave negative test results with APBA. The remaining 18 isolates, which included the three \textit{Klebsiella} species isolates, were resistant to both cefoxitin and amoxicillin-clavulanate and intermediate or resistant to at least one ESC; all gave a positive APBA test (Table 2), indicating AmpC \( \beta \)-lactamase carriage. There were seven different cephalosporin resistance phenotypes among the 15 \textit{E. coli} isolates. While all of the APBA-positive \textit{E. coli} isolates were either intermediate or resistant to CAZ, four remained susceptible to CTX, ceftiraxone, or both; however, all were susceptible to cefepime (MIC of \( \leq 8 \) \( \mu \)g/ml). Twelve of 18 APBA-positive isolates (which include the three \textit{Klebsiella} species isolates) were APBA positive with both CAZ and CTT disks. 5 \textit{E. coli} isolates were APBA positive with only the CAZ disk, and 1 \textit{E. coli} isolate was APBA positive with only the CTT disk. Among the 18 isolates that were APBA positive, 7 contained a MOX family \( \beta \)-lactamase gene (6 \textit{E. coli} isolates and 1 \textit{K. pneumoniae} isolate), 1 \textit{K. oxytoca} and 1 \textit{E. coli} isolate each contained a CIT family \( \beta \)-lactamase gene, and 1 \textit{K. pneumoniae} isolate contained a FOX family gene.

**Isolates harboring multiple \( \beta \)-lactamases.** In addition to the original 80 isolates tested, eight \textit{E. coli}, one \textit{P. mirabilis}, and three \textit{K. pneumoniae} isolates that had previously been shown to express both ESBLs and AmpC \( \beta \)-lactamases were tested using both APBA and CLAV (Table 3). One \textit{E. coli} isolate containing a known ESBL (CTX-M-3) and an AmpC \( \beta \)-lactamase (ACT-1) gave false-negative results for both APBA and CLAV. A second \textit{E. coli} isolate containing TEM-1, SHV-7, and CMY-2 gave true-positive results for both APBA and CLAV. Four additional \textit{E. coli} isolates, each containing a combination of a CTX-M and ACT-1 \( \beta \)-lactamases, gave true-positive results for the CLAV test but were falsely negative with APBA. All four also tested resistant to cefepime by MIC and disk diffusion. On the other hand, two \textit{K. pneumoniae} isolates and an \textit{E. coli} isolate that each contained an SHV and AmpC \( \beta \)-lactamase in conjunction with either a CTX-M or TEM \( \beta \)-lactamase gave false-negative CLAV results but true-positive APBA results (Table 3). All three organisms were cefepime susceptible by both MIC and disk diffusion testing. Thus, the results of APBA and CLAV testing rarely correlated with isolates containing both AmpC \( \beta \)-lactamases and ESBLs.

**DISCUSSION**

Pai et al. (19) have shown that AmpC \( \beta \)-lactamase-producing \textit{K. pneumoniae} isolates did not respond clinically to ESCs even when in vitro test results indicated susceptibility to those agents. Our study of a convenience sample of 80 selected isolates of \textit{E. coli}, \textit{Klebsiella} species, and \textit{Proteus} species indicated that 41.9\% of APBA test-positive, CLAV test-negative isolates remained susceptible to at least one ESC, indicating the potential for misclassifying isolates of these three species as susceptible to ESCs to which they may not respond clinically. Using the boronic acid test, the accuracy of which has been validated by multiple laboratories (9, 12, 33), to confirm the presence of an AmpC \( \beta \)-lactamase could improve the accuracy of test results for the ESCs by reporting these cephalosporin results as resistant. This would be consistent with the CLSI algorithm for ESBL testing and reporting (8). Thus, laboratories should consider performing APBA testing in conjunction with ESBL testing on \textit{E. coli}, \textit{Klebsiella} species, and \textit{Proteus} species to improve the accuracy of susceptibility testing results for all ESCs.

An important difference between ESBL- and AmpC-containing organisms is that those containing only AmpC \( \beta \)-lactamases often remain susceptible to ASCs, such as cefepime and cefpirome (13, 15, 26, 27). CLSI recommends changing ASC results, such as for cefepime, from susceptible to resistant for organisms in which an ESBL has been identified by CLAV testing (6, 7). We presumed that a positive APBA test for a cefepime-susceptible isolate of \textit{E. coli}, \textit{Klebsiella} species, and \textit{Proteus} species would indicate that the results for ASCs should not be changed to resistant since ASCs usually retain activity
against AmpC-producing isolates. Reporting test results of “susceptible” to ASCs for strains of *E. coli*, *Klebsiella* species, and *Proteus* species could decrease unnecessary use of carbapenems. However, the data from organisms containing both ESBLs and AmpC β-lactamas, some of which tested as APBA positive but falsely CLAV negative, raise a potential concern for this strategy since an ESBL may still be present in these organisms even though they test as susceptible to cepefime. In addition, a recent report of the acquisition of a new ESBL gene by an organism that initially harbored only an AmpC β-lactamase (and thus initially appeared to be cepfime susceptible), which ultimately led a cepfime treatment failure, also has to be considered (29).

Another issue that impacts the decision regarding the reporting of ASCs is the clinical relevance of the inoculum effect (i.e., the presence of large quantities of β-lactamase-producing organisms at the site of infection) that has been observed with cepfime and other cephalosporins with isolates containing strains of *E. coli* “susceptible” to ASCs for strains of *E. coli*, *Klebsiella* species, and *Proteus* species could decrease unnecessary use of carbapenems.

Screening for AmpC β-lactamas with cepfotixin and amoxicillin-clavulanic acid, which is easier to implement in a laboratory than APBA testing, detects isolates with a variety of AmpC β-lactamas but includes ESBL-containing isolates and isolates that are susceptible to all the ESCs by broth microdilution reference testing (although these organisms were originally reported to be resistant to at least one ESC by Vitek testing). Thus, an algorithm that uses cepfotixin and amoxicillin-clavulanate to screen for AmpC-containing isolates would have to include criteria of full resistance (not intermediate results) to both of these antimicrobial agents and resistance to at least one ESC to increase the specificity of the testing algorithm. Other approaches to AmpC β-lactamase identification, such as the EDTA disk test proposed by Black et al. (1), may be more effective than testing cepfotixin and amoxicillin-clavulanate.

Only about 58% of the APBA-positive isolates in our study gave a positive result in the AmpC multiplex PCR assay although aIEF results were consistent with AmpC carriage. This suggests that the diversity of AmpC β-lactamase genes continues to expand beyond those contained in the six families of genes covered by the multiplex assay.

Finally, in this study we used the hemisulfate salt of APBA, which can be dissolved in water, rather than DMSO. This makes the use of the test more practical for a clinical laboratory since DMSO can be toxic and requires special handling in the laboratory.

In summary, APBA testing may improve the accuracy of susceptibility test results for *E. coli*, *Klebsiella* species, and *Proteus* species by indicating that ESC results should be reported as resistant. The caveats of hidden ESBL carriage among APBA-positive, CLAV-negative organisms and of potential inoculum effects require additional data before any formal recommendations can be made on changing the reporting of ASC results for these organisms.

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REFERENCES