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Can Results Obtained with Commercially Available MicroScan Microdilution Panels Serve as an Indicator of β-Lactamase Production among *Escherichia coli* and *Klebsiella* Isolates with Hidden Resistance to Expanded-Spectrum Cephalosporins and Aztreonam?

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Among clinical isolates of *Escherichia coli*, *Klebsiella pneumoniae*, and *Klebsiella oxytoca*, there is an ever-increasing prevalence of β-lactamases that may confer resistance to newer β-lactam antibiotics that is not detectable by conventional procedures. Therefore, 75 isolates of these species producing well-characterized β-lactamases were studied using two MicroScan conventional microdilution panels, Gram Negative Urine MIC 7 (NU7) and Gram Negative MIC Plus 2 (N+2), to determine if results could be utilized to provide an accurate indication of β-lactamase production in the absence of frank resistance to expanded-spectrum cephalosporins and aztreonam. The enzymes studied included Bush groups 1 (AmpC), 2b (TEM-1, TEM-2, and SHV-1), 2be (extended spectrum β-lactamases [ESBLs] and K1), and 2br, alone and in various combinations. In tests with *E. coli* and *K. pneumoniae* and the NU7 panel, cefpodoxime MICs of ≥2 μg/ml were obtained only for isolates producing ESBLs or AmpC β-lactamases. Cefoxitin MICs of >16 μg/ml were obtained for all strains producing AmpC β-lactamase and only 1 of 33 strains producing ESBLs. For the N+2 panel, ceftazidime MICs of ≥4 μg/ml correctly identified 90% of ESBL producers and 100% of AmpC producers among isolates of *E. coli* and *K. pneumoniae*. Cefotetan MICs of ≥ 8 μg/ml were obtained for seven of eight producers of AmpC β-lactamase and no ESBL producers. For tests performed with either panel and isolates of *K. oxytoca*, MICs of ceftazidime, cefotaxime, and ceftizoxime were elevated for strains producing ESBLs, while ceftriaxone and aztreonam MICs separated low-level K1 from high-level K1 producers within this species. These results suggest that microdilution panels can be used by clinical laboratories as an indicator of certain β-lactamases that may produce hidden but clinically significant resistance among isolates of *E. coli*, *K. pneumoniae*, and *K. oxytoca*. Although it may not always be possible to differentiate between strains that produce ESBLs and those that produce AmpC, this differentiation is not critical since therapeutic options for patients infected with such organisms are similarly limited.

Resistance to β-lactam antibiotics among clinical isolates of gram-negative bacilli is most often due to the production of β-lactamases (26, 28). Until recently, β-lactamase-mediated resistance was readily detected by a variety of methods used routinely by the clinical laboratory to ascertain antimicrobial susceptibility. However, numerous changes in β-lactamases of gram-negative bacteria have been occurring over the last decade (4, 11, 13, 32). Some of these have produced new forms of older enzymes such as the extended-spectrum β-lactamases (ESBLs), derivatives of the older TEM and SHV enzymes that now can hydrolyze newer cephalosporins and aztreonam (4, 32). Other changes have involved the moving of the *ampC* gene, characteristically a chromosomal gene responsible for inducible β-lactamase production in genera like *Enterobacter*, *Serratia*, and *Pseudomonas*, onto plasmids that are now being found in strains of *Escherichia coli* and *Klebsiella pneumoniae* (4, 32).

Unfortunately, resistance to the expanded-spectrum cephalosporins and aztreonam of many strains producing ESBLs and plasmid derivatives of AmpC β-lactamases is not readily apparent in routine susceptibility tests that utilize the current National Committee for Clinical Laboratory Standards (NCCLS) breakpoints (10). This is especially true for isolates of *E. coli* and *Klebsiella* (32). Thus, the inability to detect clinically relevant resistance in these organisms has been responsible for the appearance and spread of such strains in numerous hospitals without any suspicion by the laboratory or physicians of their presence (14, 32).

The increasing incidence of ESBLs and other new β-lactamases (11) in strains of the family *Enterobacteriaceae* isolated from patients has stimulated the need for new methods to detect the β-lactamases that are responsible for clinically relevant resistance that is not apparent in routine susceptibility tests (2, 3, 5, 6, 8, 14, 17, 21, 23, 24, 30, 34). There are a number of approaches currently under consideration for the detection of ESBLs (7, 10, 18, 27, 31, 33, 35). However, until these become available, there must be some way in which the clinical laboratory can become suspicious of strains potentially possessing these enzymes. Several approaches to screen for the presence of ESBLs have been suggested (9, 15, 16, 32, 33). One possibility includes the use of modified breakpoints for standard methods of susceptibility testing as suggested by the NCCLS (15, 16). With these modifications, the NCCLS has suggested that strains of *E. coli* and *Klebsiella* spp. be screened for production of ESBLs by utilizing new interpretive criteria

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TABLE 1. β-Lactamases present in the test strains

Species (no. of strains)	No. of strains by β-lactamase group			
	ESBL[a]	AmpC[b]	High K1[c]	Other[d]
E. coli (31)	15	5		11
K. pneumoniae (31)[e]	18	3		10
K. oxytoca (13)	3	0	4	6
Total	36	8	4	27

[a] ESBLs belonging to Bush group 2be included TEM-3, TEM-4, TEM-5, TEM-6, TEM-7, TEM-8, TEM-9, TEM-10, TEM-12, SHV-2, SHV-4, SHV-5, and four other TEM- or SHV-derived ESBLs.

[b] Plasmid derivatives of AmpC β-lactamase (Bush group 1) included LAT-1, LAT-2, MIR-1, MOX-1, and FOX-1.

[c] High-level producers of the chromosomally encoded K1 β-lactamase (Bush group 2be) of *K. oxytoca*.

[d] Other β-lactamases were low-level K1 producers (Bush group 2be) in wild-type strains of *K. oxytoca*; TEM-1 (low and high level expression), TEM-2, SHV-1 (low and high level expression), and PSE-1 (Bush group 2); and TRC-1 (Bush group 2br).

[e] One strain of *K. pneumoniae* produced both an AmpC β-lactamase and an ESBL.

TABLE 2. β-Lactam antibiotics on microdilution panels examined for their ability to discriminate β-lactamases

β-Lactam antibiotic	Range of concn (μg/ml) for:	
	N + 2 panel	NU 7 panel
Aztreonam	1–32	—[a]
Cefamandole	4–32	—
Cefonicid	2–16	—
Cefoperazone	4–32	4–32
Cefotaxime	2–64	—
Cefotetan	4–32	—
Cefoxitin	—	2–16
Cefpodoxime	—	0.5–4
Ceftazidime	1–32	2–16
Ceftizoxime	2–32	—
Ceftriaxone	2–64	4–32
Cefuroxime	—	2–16
Cephalothin	—	2–16

[a] —, not included on panel.

for MIC or disk diffusion testing with ceftazidime, cefotaxime, ceftriaxone, cefpodoxime, and aztreonam (15, 16). Somewhat similar criteria were suggested earlier by Thomson et al. (32).

To date, there has been no systematic study to assess the ability of several proposed approaches to detect the presence of ESBLs or other β-lactamases capable of producing hidden resistance to expanded-spectrum cephalosporins and aztreonam in *E. coli* and *Klebsiella*. Therefore, a study was designed using a commercial broth microdilution test procedure available for use in the routine clinical laboratory to determine the best method for indicating the presence of such enzymes. Strains of *E. coli* and *Klebsiella* spp. for this study were chosen either because they produced β-lactamases known to cause hidden resistance to expanded-spectrum cephalosporins and aztreonam or because they produced other types of β-lactamases that might give false-positive results in nonspecific tests for the former enzymes.

MATERIALS AND METHODS

Strains. Tests were performed with 75 isolates of *E. coli* ($n = 31$), *K. pneumoniae* ($n = 31$), and *Klebsiella oxytoca* ($n = 13$). Forty-four of these isolates were chosen because they possessed a β-lactamase (ESBL and/or AmpC β-lactamase) that should confer clinically relevant resistance to expanded-spectrum cephalosporins and aztreonam but the MICs obtained in routine broth microdilution tests were ≤16 μg/ml with ceftazidime or aztreonam or ≤32 μg/ml with ceftriaxone, ceftizoxime, or cefotaxime. Thus, these strains were defined as having hidden resistance to expanded-spectrum cephalosporins and aztreonam. The other 31 strains were chosen because they were known to produce other β-lactamases some of which, such as the *K. oxytoca* K1 enzyme, were biochemically very similar to ESBLs (4). These strains were collected from multiple centers across the United States and Europe. The strains were stored at −70°C in a mixture of horse serum and brain heart infusion broth. These isolates were subcultured only once, and the presence of the known β-lactamase was confirmed. All of the 75 isolates were obtained from clinical sources except for 13 laboratory strains of *E. coli*. For the purposes of this study, the organisms were divided into groups according to the type of β-lactamase produced. These groups included (i) ESBLs, (ii) AmpC, (iii) high-level K1 producers, and (iv) other β-lactamases (Table 1). Within the AmpC group, there were strains of *E. coli* that hyperproduced their chromosomal enzyme as well as those that had acquired a plasmid-derived AmpC β-lactamase from another species. A number of organisms produced multiple β-lactamases, and levels of β-lactamase expression varied as well. One strain produced two different ESBLs and a plasmid derivative AmpC β-lactamase. Since preliminary studies with this strain indicated that results of susceptibility tests reflected the activity of the broader-spectrum AmpC β-lactamase, this strain was considered in the AmpC group. Other organisms with combinations of β-lactamases were assigned to the group representing the broader-spectrum enzymes (e.g., organisms possessing ESBL and TEM-1 were assigned to the ESBL group), or if no dominant enzymes were present, the strain would be assigned to the other β-lactamases group (e.g., low-level K1, TEM-1, SHV-1, etc.). All β-lactamase identifications were confirmed in the laboratory by appropriate biochemical or molecular procedures including isoelectric focusing substrate profile, inhibitor profile, plasmid isolation, recombinant DNA techniques, and transformations (1, 12, 19, 25, 29, 35). The quality control strain utilized in this study was *E. coli* ATCC 25922 (15).

Susceptibility tests. Antibiotic susceptibilities were determined according to the manufacturer's recommendations by overnight microdilution method with commercial dehydrated panels provided by Dade Behring MicroScan (Sacramento, Calif.) that were read by the Walkaway 40 and interpreted according to NCCLS criteria (15). The two panels studied were the Gram Negative Urine MIC 7 (NU7) and the Gram Negative MIC Plus 2 (N+2). They were selected on the basis of the concentrations and types of β-lactam drugs in the panel from among a number of panels available to the routine clinical laboratory (Table 2). Since previous studies had indicated that cefpodoxime was the single best indicator of the presence of ESBLs (33) and that ceftazidime, cefotaxime, ceftriaxone, or aztreonam may also be used to indicate the presence of ESBLs (15, 16), the two commercially available panels containing as many of these drugs as possible with concentrations as low as 2 μg/ml were chosen for study. These also contained at least one cephamycin which had the potential to help discriminate ESBLs from AmpC β-lactamases. The antibiotics listed in Table 2 are those that were potentially useful for the detection and differentiation of the β-lactamases present in these strains. Although there were additional β-lactams and other classes of antibiotics on these panels that are not listed in Table 2, these were not useful for the current study and will not be considered further.

RESULTS

***E. coli* and *K. pneumoniae*.** For tests performed with the N+2 panel, no single drug at any one concentration accurately differentiated between strains producing ESBLs, AmpC, or other β-lactamases (Table 3). Although the breakpoint of ≥2 μg/ml of aztreonam or ceftazidime that is currently recommended by NCCLS did correctly identify most ESBL producers (82 to

TABLE 3. Detection of β-lactamases among *E. coli* and *K. pneumoniae* isolates by various interpretive criteria applied to results obtained in tests with the N + 2 panel

Interpretive criterion[a]	No. of strains fulfilling interpretive criterion based upon type of β-lactamase		
	ESBL ($n = 33$)	AmpC ($n = 8$)	Other ($n = 21$)
Aztreonam ≥ 2	27	7	0
Ceftazidime ≥ 2	30	8	2
Ceftazidime ≥ 4	29	8	0
Cefotaxime ≥ 4	23	5	0
Ceftriaxone ≥ 4	24	5	0
Ceftizoxime ≥ 4	21	7	0
Cefotetan ≥ 8	0	7	0

[a] Listed as MIC (in micrograms per milliliter) of antibiotic given.

TABLE 4. Detection of β-lactamases among *E. coli* and *K. pneumoniae* isolates by various interpretive criteria applied to results obtained in tests with the NU7 panel

Interpretive criterion[a]	No. of strains fulfilling interpretive criterion based upon type of β-lactamase		
	ESBL (n = 33)	AmpC (n = 8)	Other (n = 21)
Cefpodoxime ≥ 2	33	8	0
Ceftazidime ≥ 4	29	7	0
Ceftriaxone ≥ 8	22	4	0
Cefoxitin > 16	1	8	0

[a] Listed as MIC (in micrograms per milliliter) of antibiotic given.

91%), it also included most strains producing AmpC β-lactamase. For ceftazidime, MICs of ≥2μg/ml were obtained for two of three strains of *K. pneumoniae* producing high levels of SHV-1 β-lactamase, giving a false-positive rate of 10% for producers of β-lactamases other than ESBLs and AmpC (Table 3). These false positives could be eliminated by raising the ceftazidime breakpoint to ≥4 μg/ml, just slightly reducing the number of ESBL producers identified at this breakpoint from 30 to 29. The four ESBL producers for which ceftazidime MICs were <4 μg/ml included three *E. coli* isolates and one *K. pneumoniae* isolate with SHV-derived ESBLs. The MIC of ceftriaxone was ≥4 μg/ml for one of these three strains, while the MIC of cefotaxime was ≥4 μg/ml for another. MICs of aztreonam and ceftizoxime were not elevated for the two remaining strains. Thus, 94% of ESBL producers and 100% of AmpC producers were indicated by ceftazidime, ceftriaxone, or cefotaxime MICs of ≥4 μg/ml. The lower percentage of strains with ESBLs or AmpC β-lactamases identified by cefotaxime, ceftriaxone, or ceftizoxime MICs was most likely related to the absence of concentrations below 2 μg/ml on the panel. Cefotetan did not completely discriminate between producers of AmpC and ESBLs, although MICs of ≥ 8 μg/ml were obtained in tests with all but one AmpC producer (Table 3).

In tests with the NU7 panel, cefpodoxime clearly was the best single antibiotic in its ability to discriminate the producers of ESBLs or AmpC β-lactamases from other types of β-lactamases (Table 4). Cefpodoxime MICs of ≥2 μg/ml were obtained in tests with all of the ESBL or AmpC producers, while MICs in tests with producers of other β-lactamases were ≤1 μg/ml. In fact, MICs of cefpodoxime were ≥4 μg/ml in tests with all of the ESBL or AmpC producers. Cefoxitin differentiated somewhat between producers of AmpC and ESBLs (Table 4). MICs of cefoxitin were >16 μg/ml for all strains producing AmpC β-lactamase and for only one strain producing an ESBL, which was a *K. pneumoniae* isolate (Table 4).

***K. oxytoca*.** In tests with the N+2 panel, ceftazidime MICs were ≥2 μg/ml, cefotaxime MICs were ≥4 μg/ml, and ceftizoxime MICs were ≥4 μg/ml only for ESBL producers (Table 5). In fact, MICs of ceftazidime and ceftizoxime were ≥16 μg/ml for ESBL producers. Ceftriaxone MICs of ≥4 μg/ml or aztreonam MICs of ≥2 μg/ml in the absence of elevated MICs for ceftazidime, cefotaxime, or ceftizoxime indicated high-level producers of the K1 β-lactamase. MICs of none of these drugs were elevated in tests with low-level producers of K1 with or without other β-lactamases. In tests with the NU7 panel, ceftazidime MICs of >16 μg/ml were obtained only in tests with ESBL producers, while ceftriaxone MICs but not ceftazidime MICs were elevated for high-level producers of K1 β-lactamase (Table 5).

DISCUSSION

The results of this study indicate that broth microdilution panels currently available to the clinical laboratory can provide a vehicle for the detection of β-lactamases capable of producing hidden resistance to expanded-spectrum cephalosporins and aztreonam in isolates of *E. coli*, *K. pneumoniae*, and *K. oxytoca*. Expanding the current study with additional strains that produce ESBLs and other sorts of β-lactamases might be useful for fine-tuning the conclusions presented here. A panel containing cefpodoxime was the most useful in identifying strains possessing ESBLs or AmpC β-lactamases, although this drug could not be used to distinguish between these two types of β-lactamases. However, this should not be considered a liability since the therapeutic options for patients infected with strains of these species possessing ESBLs or AmpC β-lactamases are limited and usually involve a carbapenem as the drug of choice (32). For panels not containing cefpodoxime, maximal identification of strains possessing ESBLs or AmpC β-lactamases was obtained in tests with ceftazidime.

The use of a cephamycin to discriminate between producers of AmpC and ESBLs was not completely reliable as one strain with AmpC β-lactamase was susceptible to cefotetan (MIC, <8 μg/ml) and one strain without AmpC appeared to be resistant to cefoxitin (MIC, >16 μg/ml). The cefoxitin resistance was most probably due to the fact that a porin mutation in strains of *K. pneumoniae* expressing an ESBL often leads to resistance to the cephamycins (20, 22). Although precise separation of AmpC from ESBL producers was not possible in single-drug tests like those available on conventional microdilution panels, it can be concluded from the results of this study that cephamycin-susceptible strains of *E. coli*, *K. pneumoniae*, and *K. oxytoca* are highly likely to be producers of ESBLs and not AmpC β-lactamases.

The results of this study also suggest that the recommendations of the NCCLS (15) need some modification. First, the recommendations should be modified to indicate that they apply to both ESBLs and AmpC β-lactamases that may produce hidden resistance to expanded-spectrum cephalosporins and aztreonam. Second, if ceftazidime is to be used as a screen for ESBLs and AmpC β-lactamases, a concentration of ≥4 μg/ml is preferred to a concentration of ≥2 μg/ml. Third, recommendations for screening for *K. oxytoca* must be different from those for *E. coli* and *K. pneumoniae*. For *K. oxytoca*, cefodoxime, ceftriaxone, and aztreonam are not adequate drugs for screening for ESBLs since MICs for high-level producers of

TABLE 5. Detection of β-lactamases among *K. oxytoca* by various interpretive criteria

Interpretive criterion[a]	No. of strains fulfilling interpretive criterion based upon type of β-lactamase		
	ESBL (n = 3)	High K1 (n = 4)	Other (n = 6)
N+2 panel			
≥ 2 Aztreonam	3	4	0
≥ 2 Ceftazidime	3	0	0
≥ 4 Cefotaxime	3	0	0
≥ 4 Ceftriaxone	3	4	0
≥ 4 Ceftizoxime	3	0	0
NU7 panel			
≥ 2 Cefpodoxime	3	2	0
> 16 Ceftazidime	3	0	0
≥ 8 Ceftriaxone	2	4	0

[a] Listed as MIC (in micrograms per milliliter) of antibiotic given.

TABLE 6. Interpretive criteria giving best identification of β-lactamases

Species	β-Lactamase group[a]	Panel	Interpretive criterion (−a)[b]	% True positive	% False positive	% False negative
E. coli and *K. pneumoniae*	AmpC/ESBL	N+2	CAZ ≥ 4	90	0	10
			CAZ, CTR, or CTX ≥ 4	95	0	5
	Other		CAZ, CTR, or CTX < 4	100	5	0
	AmpC		CAZ ≥ 4 and CTT ≥ 8	88	0	12
	ESBL		CAZ, CTR, or CTX ≥ 4 and CTT < 8	95	2	5
	AmpC/ESBL	NU7	CEPD ≥ 2	100	0	0
	Other		CEPD < 2	100	0	0
	AmpC		CEPD ≥ 2 and FOX > 16	100	2	0
	ESBL		CEPD ≥ 2 and FOX ≤ 16	97	0	3
K. oxytoca	ESBL	N+2	CAZ ≥ 2[c] and CTR ≥ 4[d]	100	0	0
	High K1		CAZ < 2 and CTR ≥ 4	100	0	0
	Other		CAZ < 2 and CTR < 4	100	0	0
	ESBL	NU7	CAZ > 16	100	0	0
	High K1		CAZ < 16 and CTR ≥ 8	100	0	0
	Other		CAZ < 16 and CTR < 8	100	0	0

[a] See Table 1 for explanation of β-lactamase groups.
[b] Listed as concentration (in micrograms per milliliter) of antibiotic given. Abbreviations: CAZ, ceftazidime; CTR, ceftriaxone; CTX, cefotaxime; CTT, cefotetan; CEPD, cefpodoxime; FOX, cefoxitin.
[c] Cefotaxime or ceftizoxime (≥4 μg/ml) can be substituted for ceftazidime.
[d] Aztreonam (≥2 μg/ml) can be substituted for ceftriaxone.

the K1 β-lactamase may be ≥2 μg/ml with these antibiotics. Only cefotaxime, ceftazidime, and ceftizoxime were reliable indicators of the presence of ESBLs in *K. oxytoca*.

The results of this study clearly show that more-specific tests are needed for the identification of AmpC β-lactamases and ESBLs in isolates of *E. coli*, *K. pneumoniae*, and *K. oxytoca*. Such tests are currently under study (7, 10, 18, 27, 31, 33, 35) and by necessity involve the use of combinations of β-lactam antibiotics with inhibitors of β-lactamases. However, until these tests become available for routine use in the clinical laboratory, it should be possible for the laboratory to gain a high degree of suspicion concerning the presence of ESBLs or AmpC β-lactamases from the results of conventional antimicrobial susceptibility tests. As summarized in Table 6, for laboratories using the N+2 or NU7 panel, an isolate of *E. coli* or *K. pneumoniae* should be suspected of harboring an ESBL or an AmpC β-lactamase if cefpodoxime MICs are ≥2 μg/ml or if MICs of ceftazidime, ceftriaxone, or cefotaxime are ≥4 μg/ml. If the strain is susceptible to the cephamycins, it is most likely to have an ESBL rather than an AmpC β-lactamase. It is interesting to note that with both of the panels tested, false positives indicating the presence of AmpC β-lactamase or ESBLs were not encountered with the interpretive criteria. Thus, no strains producing other β-lactamases appeared falsely positive for ESBLs (AmpC/ESBL) or AmpC β-lactamase. The only false positives encountered with the interpretive criteria indicating the presence of AmpC β-lactamase specifically or the presence of ESBLs specifically involved strains producing ESBLs or AmpC β-lactamase, respectively. For *K. oxytoca*, elevated MICs of ceftazidime indicate the presence of an ESBL while elevated MICs of ceftriaxone indicate a high-level producer of K1 β-lactamase. If these guidelines are followed, they should greatly enhance the ability of a laboratory to suspect the presence of ESBLs or AmpC β-lactamases among *E. coli*, *K. pneumoniae*, and *K. oxytoca* isolates.

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