

## Detection of Extended-Spectrum $\beta$ -Lactamase (ESBL)-Producing Strains by the Etest ESBL Screen

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**Resistance to contemporary broad-spectrum  $\beta$ -lactams, mediated by extended-spectrum  $\beta$ -lactamase (ESBL) enzymes, is an increasing problem worldwide. The Etest (AB Biodisk, Solna, Sweden) ESBL screen uses stable gradient technology to evaluate the MIC of ceftazidime alone compared with the MIC of ceftazidime with clavulanic acid (2  $\mu$ g/ml) to facilitate the recognition of strains expressing inhibitable enzymes. In the present study, ESBL-producing strains (17 *Escherichia coli* transconjugants) were studied to define "sensitive" interpretive criteria for the Etest ESBL screen. These criteria (reduction of the ceftazidime MIC by  $>2 \log_2$  dilution steps in the presence of clavulanic acid) defined a group of 92 probable ESBL-positive organisms among the 225 tested strains of *Klebsiella* species and *E. coli* having suspicious antibiogram phenotypes. With a subset of 82 clinical strains, the Etest ESBL screen was more sensitive (100%) than the disk approximation test (87%) and was more convenient. The MICs of ciprofloxacin, gentamicin, and tobramycin at which 50% of isolates are inhibited were 16- to 128-fold higher (coresistance) for the ESBL screen-positive group of strains than for the ESBL screen-negative group of strains. Some strains for which cephalosporin MICs were elevated and which were Etest ESBL screen negative were also cefoxitin resistant, i.e., consistent with a chromosomally mediated AmpC resistance phenotype. The Etest ESBL screen test with the ceftazidime substrate appears to be a useful method for detecting or validating the presence of enteric bacilli potentially producing this type of  $\beta$ -lactamase.**

Many of the emerging antimicrobial resistance problems of this decade have been characterized by difficulty in the recognition of resistance in the laboratory, particularly by rapid susceptibility test methods. The plasmid-encoded extended-spectrum  $\beta$ -lactamase (ESBL) enzymes represent such a resistance phenomenon that is difficult to recognize (8, 9, 13, 17, 22, 23, 27, 30).

Plasmid-borne  $\beta$ -lactamases capable of hydrolyzing penicillins provide the most common mechanism of resistance to  $\beta$ -lactam antimicrobial agents among gram-negative bacteria (26), and their existence was the principal stimulus to the development of the compounds that inhibit  $\beta$ -lactamases, e.g., clavulanic acid, sulbactam, and tazobactam. These previously characterized enzymes of the TEM (TEM-1 and -2) and SHV (SHV-1) categories (Bush group 2b) were not capable of conferring resistance to the newer extended-spectrum cephalosporins (3). In the mid-1980s, plasmid-borne  $\beta$ -lactamases conferring resistance to the extended-spectrum cephalosporins were first recognized (5, 21-23). This event has been particularly associated with resistances among *Klebsiella* species and *Escherichia coli* strains, but other species of the family *Enterobacteriaceae* also express ESBL activity (4, 6, 15, 18, 22, 23). It has become apparent that changes in the substrate affinity of the enzyme were related to relatively minor changes in the sequences of the genes encoding the TEM-1, TEM-2, and SHV-1 enzymes (5, 22). These novel enzymes are defined as group 2be (hydrolysis of ceftazidime, cefotaxime, or aztreonam at rates  $>10\%$  of the rate for benzylpenicillin and subject to inhibition by  $\beta$ -lactamase inhibitors) in the classification of Bush et al. (3). A more recent phenomenon has been the evolution of TEM- and SHV-derived  $\beta$ -lactamases with reduced affinities

for  $\beta$ -lactamase inhibitors (Bush group 2br; "inhibitor-resistant TEMs") (3, 5, 22).

Detection of ESBL expression has proved to be difficult for many laboratories, because in vitro testing may not reveal intermediate susceptibility or resistance to cefotaxime or ceftriaxone (widely used and tested) at the National Committee for Clinical Laboratory Standards interpretive breakpoint for susceptibility (13, 27, 30). The optimal substrate for the members of this enzyme group varies markedly. Ceftazidime is recognized as a good substrate for the group, although other cephalosporins including cefpodoxime and cefuroxime may be used (2, 8, 13). Even with the commonly tested compounds ceftazidime or cefuroxime, in vitro testing may result in a susceptible (MIC,  $\leq 8 \mu$ g/ml) or intermediate (MIC, 16  $\mu$ g/ml) categorization rather than a frankly resistant result (MIC,  $\geq 32 \mu$ g/ml) (13, 27, 30). Because these cephalosporins are also substrates for hydrolysis by chromosomal and chromosomally derived  $\beta$ -lactamases (Bush group 1, AmpC, etc.) (3, 11, 24), demonstration of substrate inhibition of hydrolysis by a  $\beta$ -lactamase inhibitor remains necessary to establish that the organism is a producer of a Bush group 2be enzyme (3).

One approach to the detection of ESBLs is to perform disk approximation testing with strains of *E. coli* or *Klebsiella* spp. for which the MICs of cefuroxime, ceftazidime, or related compounds are outside of the susceptible range (intermediate or resistant by National Committee for Clinical Laboratory Standards criteria). Disk approximation testing functions via the placement of cefuroxime and ceftazidime disks close (20 or 30 mm) to an amoxicillin-clavulanate disk on a plate inoculated with the test organism. Enhancement of the zone of inhibition or a so-called ghost zone between either of the cephalosporin disks and the clavulanate-containing disk (amoxicillin-clavulanic acid) indicates the presence of a Bush group 2be enzyme (10, 17, 27, 30). Precise placement of the disks, correct storage of the clavulanate-containing disks, and performance of appropriate control tests are critical to the sensitivity of the disk

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TABLE 1. Detection of ESBL production by *E. coli* C600 transconjugants

Enzyme	Ceftazidime MIC (μg/ml)		Log <sub>2</sub> reduction in MIC	Etest MIC (μg/ml)						
	Alone	With clavulanate		Cefuroxime	Cefoxitin	Amoxicillin-clavulanate	Imipenem	Ciprofloxacin	Gentamicin	Tobramycin
Non-ESBL										
TEM-1	<0.5–0.5	0.125–0.38	0–2 <sup>b</sup>	8	4	8/2	0.25	0.016	256	8
TEM-2	0.75–1.5	0.75–1.5	0–0.5 <sup>b</sup>	12	6	24/2	0.25	0.016	0.38	0.75
SHV-1	2–4	0.75–1.0	1.5–2.0 <sup>b</sup>	12	8	16/2	0.5	0.016	0.75	2
ESBL										
TEM-3	>32	0.75	6.5	>256	6	8/2	0.5	0.023	1.5	24
TEM-3	32	0.38	6.5	128	6	8/2	0.38	0.019	1	16
TEM-4	>32	0.5	7.0 <sup>b</sup>	>256	6	6/2	0.25	0.023	0.38	0.5
TEM-5	>32	0.75	6.5	64	6	8/2	0.25	0.016	0.5	1
TEM-6	>32	1.0	6.0	16	4	16/2	0.25	0.016	2.0	1
TEM-6	>32	0.19	8.5	4	2	4/2	0.5	0.023	>256	256
TEM-7	>32	0.75	6.5	12	6	16/2	0.5	0.016	>256	256
TEM-8	>32	0.75	6.5	16	6	8/2	0.25	0.016	1.5	6
TEM-9	>32	1.5	5.5	16	6	12/2	0.38	0.016	256	24
TEM-9	>32	1.0	6.0	16	4	8/2	0.5	0.016	>256	0.38
SHV-2	>32	0.5–1	6.5–7.0 <sup>b</sup>	256	2	16/2	0.5	0.016	0.5	0.38
SHV-3	16	0.38	5.5	256	6	8/2	0.38	0.023	4	16
SHV-4	>32	0.5	7.0	256	6	8/2	0.25	0.016	4	32
SHV-5	>32	0.5	7.0	256	6	8/2	0.38	0.016	32	0.75

<sup>a</sup> Results from Etest ESBL screen strip.

<sup>b</sup> Four MICs were determined for each transconjugant.

approximation method (17). ESBL-mediated resistance may also be recognized by a modified three-dimensional susceptibility test method (29). This method has a reported sensitivity of 95% for ESBL detection compared with a sensitivity of 79% for the disk approximation test (30).

Recently, two commercially available products for ESBL detection have become available, the Vitek (bioMérieux Vitek, Hazelwood, Mo.) ESBL test (28) and an ESBL screening Etest (AB Biodisk, Solna, Sweden) strip. Both tests are based on recognition of a reduction in ceftazidime MICs in the presence of a fixed concentration (2 μg/ml) of clavulanic acid. The Etest ESBL strip is a plastic drug-impregnated strip, one side of which generates a stable concentration gradient for ceftazidime and the remaining side of which generates a gradient of ceftazidime and clavulanate (MIC test range, 32 to 0.12 μg/ml).

In the study described here we used *E. coli* isolates transfected with plasmids encoding ESBL resistance (control strains) and clinical strains to define interpretive criteria for the Etest ESBL screen test. The sensitivities of detection of ESBLs by the disk approximation method and the Etest ESBL screen were also compared for a group of 82 strains. In addition, the Etest ESBL screen was used to define a large population of ESBL-producing or -nonproducing clinical strains of enteric bacilli and to compare the activities of a series of β-lactams and other antimicrobial agents against these two populations of organisms.

#### MATERIALS AND METHODS

**Bacterial strains.** A collection of 17 *E. coli* strains transfected with plasmids encoding β-lactamases (with or without other resistance determinants) were kindly provided principally by George Jacoby (Boston, Mass.). The β-lactamases expressed were TEM-1 to TEM-9 and SHV-1 to SHV-5 (see Table 1). A second collection of 225 recent clinical strains of *E. coli* or *Klebsiella* spp. from the United States, Mexico, and several European countries including the United Kingdom, Austria, Germany, France, and Italy were studied. These isolates were selected because of resistance phenotypes or MIC patterns that were suspicious for greater resistance to various β-lactams and other classes of antimicrobial agents. The phenotypes of the strains included strains for which the MICs of

extended-spectrum cephalosporins (cefotaxime, ceftriaxone, ceftizoxime, and ceftazidime) or aztreonam were elevated (>2 μg/ml) or which demonstrated resistance to a fluoroquinolone (ciprofloxacin or ofloxacin) or an aminoglycoside. Susceptibility patterns to β-lactamase inhibitor combinations, cephamycin, or carbapenems were not used as defining criteria for testing.

A subset (82 organisms) of the 225 strains listed above was tested by the disk approximation method (10), and the results obtained by that method were compared with the Etest ESBL screen results. Strains for which the results between the methods were discordant were tested again, and only the reproducible data were tabulated.

**Antimicrobial agents.** Ceftazidime, ceftazidime-clavulanate, cefuroxime, cefoxitin, imipenem, amoxicillin-clavulanate, ciprofloxacin, gentamicin, and tobramycin Etest strips and the Etest ESBL screen were obtained from AB Biodisk. Disks containing ceftazidime (30 μg), cefuroxime (30 μg), and amoxicillin-clavulanate (20/10 μg) were obtained from Becton Dickinson Microbiology Systems (Cockeysville, Md.).

**Susceptibility tests.** Susceptibility testing was performed on Mueller-Hinton agar by the Etest methodology in accordance with the manufacturer's instructions. Briefly, after overnight growth on brain heart infusion agar, the organisms were suspended in saline to a turbidity equal to that of a 0.5 McFarland turbidity standard. The suspension was used to inoculate Mueller-Hinton agar plates by swabbing them with a cotton swab. After drying for 15 min, the Etest strips were placed on the plates and the plates were incubated for 18 h in air at 35°C. The MIC was interpreted as the point of intersection of the inhibition ellipse with the Etest strip edge. Agar dilution MICs were determined by the NCCLS methodology of the National Committee for Clinical Laboratory Standards (19) for ceftazidime alone and for ceftazidime with clavulanate (2 μg/ml) for a subset of 35 isolates. These isolates were those strains for which the ceftazidime MICs were reduced 1 to 4 dilution steps with the Etest ESBL strip (16 strains) and strains randomly selected from among those strains for which no reduction in the ceftazidime MICs were shown (8 strains) and those for which the MICs were reduced by >4 dilution steps (11 strains).

**Data analysis.** For isolates for which ceftazidime MICs were >32 μg/ml, the MIC was taken as 64 μg/ml and the reduction in the ceftazidime MIC was calculated from that value. Results off scale (either high or low) by the two methods were regarded as being in agreement.

#### RESULTS

The ceftazidime MICs for the 17 *E. coli* transconjugants expressing a β-lactamase enzyme resistance plasmid are presented in Table 1, together with the reductions in the ceftazidime MICs in the presence of 2 μg of clavulanic acid per ml. For the TEM-1, TEM-2, TEM-4, SHV-1, and SHV-2 transcon-

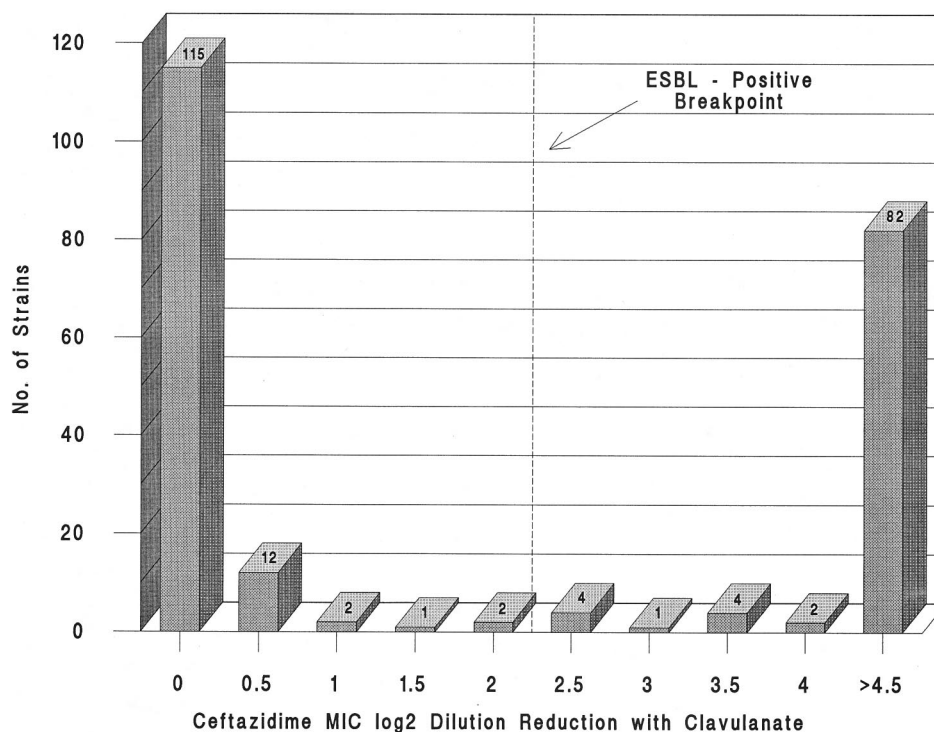


FIG. 1. Reduction (log<sub>2</sub>) of ceftazidime MICs in the presence of clavulanate (2 µg/ml) for 225 clinical isolates of *E. coli* and *Klebsiella* species.

jugants, the Etest ESBL screen test was performed on four occasions. For TEM-1 and TEM-2 transconjugants the reduction in the ceftazidime MIC due to clavulanic acid was routinely nil. For SHV-1 transconjugants the reduction was consistently 1.5 to 2 log<sub>2</sub> dilution steps. For TEM-4 and SHV-2 transconjugants the reduction in the ceftazidime MIC was consistently greater than 5 log<sub>2</sub> dilution steps. For ESBL transconjugants a >5 log<sub>2</sub> dilution lower ceftazidime MIC was found when ceftazidime was combined with 2 µg of clavulanic acid per ml, and for all non-ESBL strains a ≤2 log<sub>2</sub> dilution change was found.

For the 225 clinical strains studied, the frequency distributions of the reductions in the ceftazidime MICs are presented in Fig. 1. For the five strains for which the ceftazidime MIC was reduced 2.5 to 3 log<sub>2</sub> dilution steps, the Etest ESBL screen was repeated in triplicate. In four cases the reduction in the MIC resolved to a higher value (3 to 3.5 log<sub>2</sub> dilution steps), and in one case it resolved to a lower value (2 log<sub>2</sub> dilution steps). If a reduction of >2 log<sub>2</sub> dilution steps was taken as the breakpoint criterion for recognition of a Bush group 2be enzyme-producing strain, then the result for one of these five strains could be regarded as an initial false-positive result.

The Etest ESBL screen results compared with the results of a disk approximation test with a subset of 82 clinical isolates are summarized in Table 2. The Etest ESBL screen was more sensitive than the disk approximation method (in which the disks were 25 mm apart). The ceftazidime MICs determined with the Etest ESBL strip are compared in Table 3 with those determined with the routine ceftazidime Etest strip (15 log<sub>2</sub> dilution steps), and no significant differences were observed. The agreement between ceftazidime MICs, ceftazidime MICs with clavulanate (2 µg/ml), and the degree of reduction of the ceftazidime MIC in the presence of clavulanate determined with the Etest ESBL strip and by the reference agar dilution method are summarized in Table 4. The MICs at which 50%

and 90% of strains tested are inhibited (MIC<sub>50</sub>s and MIC<sub>90</sub>s, respectively) were compared by using Etest products for the ESBL screen-positive population (92 strains) and the ESBL screen-negative population (133 strains). The MIC<sub>50</sub>s and MIC<sub>90</sub>s of a variety of β-lactams, ciprofloxacin, gentamicin, and tobramycin are presented in Table 5. A tendency toward increased resistance to aminoglycosides and fluoroquinolones for ESBL-producing strains of the family *Enterobacteriaceae* was reflected in the higher MIC<sub>50</sub>s for those strains compared with those for the ESBL screen-negative organisms.

Of the 92 Etest ESBL screen-positive clinical strains, ceftazidime MICs were in the range 4 to 8 µg/ml for 11 (12%) strains and were in the range of 12 to 24 µg/ml for 8 (9.1%) strains. Therefore, 12 and 9% of ESBL screen-positive strains were considered susceptible and intermediate to ceftazidime, respectively (19). A total of 89 clinical isolates resistant to ceftazidime (MIC, ≥32 µg/ml) were identified, of which 16 were negative for ESBL enzyme expression. All of these isolates were also resistant to ceftazidime (MICs, >256 µg/ml for

TABLE 2. Agreement between disk approximation test and Etest ESBL screen test results for 82 clinical strains of the family *Enterobacteriaceae*

Disk approximation test result	No. of strains with the indicated Etest screen result	
	Positive (39) <sup>a</sup>	Negative (43)
Positive	34	0
Negative	5 <sup>b</sup>	43

<sup>a</sup> The ceftazidime MIC was reduced by ≥3.5 log<sub>2</sub> dilution steps in all Etest ESBL screen tests.

<sup>b</sup> Corestrictances were found for cefuroxime (five of five strains), ciprofloxacin (four of five strains), and aminoglycosides (five of five strains).

TABLE 3. Agreement of ceftazidime MICs determined by routine Etest and with Etest ESBL screen strips for 17 *E. coli* transconjugants and 225 clinical strains of the family *Enterobacteriaceae*

Method	Cumulative no. of isolates inhibited at ceftazidime MIC ( $\mu\text{g/ml}$ ) of:													
	$\leq 0.5$	0.75	1.0	1.5	2.0	3.0	4.0	6.0	8.0	12	16	24	32	$>32$
Routine Etest	70	82	94	102	104	112	118	124	132	135	139	144	153	242
Etest ESBL screen	83	93	99	102	111	116	121	125	131	134	138	140	144	242

12 isolates), a finding consistent with AmpC-mediated Bush group 1 resistance (3).

## DISCUSSION

The emergence of plasmid-encoded ESBLs is a significant evolution in antimicrobial resistance. Outbreaks due to the dissemination of ESBL-producing bacterial strains and to the dissemination of ESBL-encoding plasmids among different species of the family *Enterobacteriaceae* have been described in hospitals and other health care facilities (16, 18, 20, 23, 25). The spread of ESBL-producing clones between hospitals has also been reported (1, 27). Precise data on the prevalence of ESBL-producing strains in the United States are not available. This is related at least in part to difficulty in recognizing these strains by clinical microbiology laboratories. It is clear, however, that ESBL-producing strains are present in many hospitals throughout the United States (27), and greater than 7% of *Klebsiella* species are intermediate or resistant to ceftazidime, suggesting that ESBL-mediated resistance is not rare (12). It is likely, from previous experience with the TEM-1 and TEM-2 enzymes, that this resistance mechanism will become a major problem in the future. Knowledge of the extent of ESBL-mediated resistance appears limited by the inability of many commercially available systems or standardized methods of susceptibility testing to detect this resistance (13, 27, 30). Supplemental disk approximation testing has proved to be a useful detection method; however, suboptimal placement of the disks leaves the method with limited sensitivity but acceptable specificity (17, 30).

Ceftazidime is an excellent substrate for most ESBL enzymes that have been described (2, 13), and Bush group 2be enzymes can be differentiated from other  $\beta$ -lactamase enzymes (e.g., chromosomal or chromosomally derived AmpC enzymes) by the reduction in the ceftazidime MIC in the presence of some clinically useful  $\beta$ -lactamase inhibitors such as clavulanic acid (10, 13, 17). Two commercially available products (Vitek and Etest) which have been developed for the detection of ESBLs rely on this alteration in susceptibility to only one cephalosporin substrate. One method uses an automated growth-monitoring system (Vitek) (28), and the other (Etest) applies the stable gradient technology to an agar diffusion test. Our results and those of Sanders et al. (28) suggest that both methods are more sensitive than the disk approximation test for the detection of Bush group 2be resistance.

Our data derived from the studies with *E. coli* transconjugants demonstrate a clear separation between strains expressing the conventional plasmid-borne group 2b  $\beta$ -lactamases (TEM-1, TEM-2, and SHV-1), for which the reduction in the ceftazidime MIC was  $\leq 2 \log_2$  dilution steps, and the Bush group 2be-expressing strains, for which the reduction in the ceftazidime MIC was generally  $\geq 5.5$  to  $7 \log_2$  dilution steps. Since high sensitivity is appropriate for a screening test, we defined any reduction in the ceftazidime MIC of  $>2 \log_2$  dilution steps as indicating the potential presence of a Bush group 2be  $\beta$ -lactamase. A distinct polarization of the impact of

clavulanate on ceftazidime resistance was observed (Fig. 1) among the 225 clinical strains studied, and only a small number (3.6%) of strains produced results close ( $\pm 1 \log_2$  dilution) to the proposed interpretive breakpoint criterion. Most of these tests either gave a reproducible reduction in the ceftazidime MIC or resolved toward a greater reduction of the substrate MIC when the screening test was repeated. Similar results were obtained by the reference agar dilution method. Further studies of these strains by molecular techniques may be required to characterize definitively the nature of the resistance mechanisms present (1, 5, 7, 14).

In the series of 82 clinical isolates screened for ESBL production by the Etest and the disk approximation methods, the five discrepancies could be interpreted as false-positive results by the Etest or as false-negative results by the disk approximation method. For these five strains, reductions in ceftazidime MICs of  $\geq 4 \log_2$  dilution steps were demonstrated in the presence of clavulanate, and this together with previous publications indicating that the disk approximation test had less than optimal sensitivity (30) tends to support the conclusion that the strains represent disk approximation test failures. In addition, four of these strains were resistant to cefuroxime and one was intermediate, and all strains were resistant to one or more of the compounds ciprofloxacin, gentamicin, or tobramycin. Coresistance to aminoglycosides and fluoroquinolones has previously been described as a marker for ESBL-producing strains (23). This feature of ESBL-producing organisms was confirmed with our series of clinical isolates, in which the MIC<sub>50s</sub> of ciprofloxacin, gentamicin, and tobramycin were much higher for the ESBL screen-positive group compared with those for the other strains (Table 5). This is not a specific marker, however, because resistance to these agents was observed by strains in both groups (ESBL screen positive and negative).

The compression of the ceftazidime gradient required by the design of the Etest ESBL screen test does not compromise the accuracies of the ceftazidime MICs for those strains (Table 3), especially when evaluation values were in range for the gradient used. Because this scale encompasses the most clinically significant MIC range, the Etest ESBL screen strip was an adequate tool for testing susceptibility to ceftazidime alone as well as to its use as an ESBL screen test.

The ESBLs provide a relatively new therapeutic dilemma

TABLE 4. Reference agar dilution ceftazidime MIC, ceftazidime with clavulanate MIC, and reduction of ceftazidime MIC with the inhibitor compared with Etest ESBL strip results (35 strains)

Parameter	Percent agreement for the following dilution steps:			
	$\pm 0.5$	$\pm 1$	$\pm 1.5$	$\pm 2$
Ceftazidime MIC	51	71	89	91
Ceftazidime-clavulanate MIC	34	63	86	91
Reduction of ceftazidime MIC	83	94	97	97

TABLE 5. Antimicrobial activities (MIC<sub>50</sub>s and MIC<sub>90</sub>s) of cefuroxime, cefoxitin, amoxicillin-clavulanate, imipenem, ciprofloxacin, gentamicin, and tobramycin for ESBL-producing and ESBL-nonproducing clinical strains of the family *Enterobacteriaceae*

Enzyme group (no. of isolates)	Cefuroxime		Cefoxitin		Amoxicillin-clavulanate		Imipenem		Ciprofloxacin		Gentamicin		Tobramycin	
	MIC <sub>50</sub> (μg/ml)	MIC <sub>90</sub> (μg/ml)	MIC <sub>50</sub> (μg/ml)	MIC <sub>90</sub> (μg/ml)	MIC <sub>50</sub> (μg/ml)	MIC <sub>90</sub> (μg/ml)	MIC <sub>50</sub> (μg/ml)	MIC <sub>90</sub> (μg/ml)	MIC <sub>50</sub> (μg/ml)	MIC <sub>90</sub> (μg/ml)	MIC <sub>50</sub> (μg/ml)	MIC <sub>90</sub> (μg/ml)	MIC <sub>50</sub> (μg/ml)	MIC <sub>90</sub> (μg/ml)
ESBL (92)	32	>256	6	32	8 <sup>a</sup>	16 <sup>a</sup>	0.25	0.38	4 <sup>a</sup>	>32 <sup>a</sup>	32 <sup>a</sup>	>256 <sup>a</sup>	32 <sup>a</sup>	192 <sup>a</sup>
Not ESBL (133)	12	>256	6	>256	12 <sup>b</sup>	48 <sup>b</sup>	0.25	0.50	0.038 <sup>b</sup>	>32 <sup>b</sup>	0.75 <sup>b</sup>	128 <sup>b</sup>	1 <sup>b</sup>	64 <sup>b</sup>

<sup>a</sup> On the basis of the results for 56 randomly selected isolates.

<sup>b</sup> On the basis of the results for 57 randomly selected isolates.

with potentially serious clinical implications for β-lactam chemotherapy and for the epidemiology of nosocomial infections (16, 18, 20, 23, 25). It is likely that the true extent of this problem is underrecognized. Among the reasons for this poor detection has been the lack of a convenient and sensitive method for recognizing these strains (13, 27, 30). Both the Etest ESBL screen and the Vitek (28) ESBL detection systems appear to be acceptable for clinical use, and each was more sensitive and convenient than the alternative disk approximation method. The use of these tests may contribute to a wider recognition and more careful monitoring of this emerging resistance problem among some *Enterobacteriaceae* (*E. coli* and *Klebsiella* spp.). Furthermore, it is likely that these tests will also prove to be useful for selecting strains for more detailed molecular analysis.

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