

Detection of Extended-Spectrum- β -Lactamase-Producing Members of the Family *Enterobacteriaceae* with the Vitek ESBL Test

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A three-phase analysis of the Vitek ESBL test and a double-disk (2-disk) test was performed to assess their ability to detect extended-spectrum β -lactamases (ESBLs) in members of the family *Enterobacteriaceae*. In the first two phases involving detection of ESBLs in 157 strains possessing well-characterized β -lactamases, sensitivity and specificity were found to be 99.5 and 100%, respectively, for the Vitek ESBL test and 98.1 and 99.4%, respectively, for the 2-disk test. In the third phase, in which the ability of each test to detect ESBLs in 295 clinical isolates was assessed, there was only one false positive (Vitek ESBL test). Across all three phases, the Vitek ESBL test was found to be much easier to perform than the 2-disk test. The latter also involved subjective interpretation of results. There were a total of 176 *Escherichia coli* and 157 *Klebsiella pneumoniae* isolates and less than 40 isolates of each of 14 other species evaluated. In a supplemental study of *Klebsiella oxytoca*, an organism possessing a chromosomal β -lactamase similar to an ESBL, the Vitek ESBL test was found to be capable of detecting hyperproduction of this enzyme in strains of this species as well. These data indicate that the Vitek ESBL test is reliable for the detection of ESBLs in *E. coli* and *K. pneumoniae*, the two species in which ESBLs are most common, and of hyperproduction of the *K. oxytoca* β -lactamase, a situation which engenders a level of resistance to this species similar to that seen with ESBLs.

After the widespread use of the expanded-spectrum cephalosporins, strains of *Klebsiella pneumoniae* that were resistant to these new drugs began to appear (9, 18, 20). Outbreaks of infections due to these unusually resistant strains also began to appear (9, 18, 20). The resistance spread to some strains of *Escherichia coli* and, in rare instances, to other gram-negative organisms as well. Investigation into the mechanisms responsible for this resistance revealed the presence of new forms of the older plasmid-mediated TEM and SHV β -lactamases. Mutations within the structural genes encoding the older enzymes had occurred, giving rise to derivatives that possessed an extended substrate profile compared with that of the parental enzymes (4, 9, 18). Thus, these new enzymes were given the name extended-spectrum β -lactamases (ESBLs) to reflect the fact that they were derivatives of older enzymes and had a new capability to hydrolyze a broader spectrum of β -lactam drugs. To date, there are over 25 derivatives of TEM-1 and TEM-2 β -lactamases that are ESBLs (TEM-3, TEM-4, TEM-5, etc.) and there are more than 5 derivatives of SHV-1 β -lactamase (SHV-2, SHV-3, SHV-4, etc.) (1, 2, 4, 9, 18). Since the parental enzymes are sensitive to β -lactamase inhibitors, like clavulanic acid, the ESBLs are also sensitive to these inhibitors (4).

The most difficult task facing clinical microbiologists, infection control practitioners, and infectious disease specialists today is the reliable detection of ESBL-producing organisms. Many ESBL-producing *E. coli* and *K. pneumoniae* strains do not appear resistant to newer cephalosporins or aztreonam in routine susceptibility tests (12, 14, 22). In disk diffusion tests, between 29 and 75% of strains appear susceptible to cefo-

taxime or ceftazidime, while in dilution tests, one-half of strains may appear susceptible (22). However, it is clear that these drugs are rarely successful in treating infections due to ESBL-producing members of the family *Enterobacteriaceae* unless the infection is confined to the urinary tract (5, 11, 15, 25). Absence of a reliable test for ESBL-producing *Enterobacteriaceae* has also allowed dissemination within and between hospitals to go undetected for prolonged periods of time (5, 14, 15, 20, 23, 25). In one outbreak, dissemination of ESBL-producing *K. pneumoniae* had been occurring for a year before it was detected (15). Only the recognition of therapeutic failures with newer cephalosporins during treatment of apparently susceptible *Enterobacteriaceae* led to the recognition of the presence of ESBL-producing *Enterobacteriaceae* in an institution (15, 25).

There is currently no reliable method designed specifically for the detection of ESBLs in isolates of gram-negative bacteria that can be easily performed in a clinical laboratory. A double-disk (2-disk) test has been developed by French investigators to assess the prevalence of ESBL-producing organisms (10). However, the accuracy of the 2-disk test has never been assessed under conditions practical for use in the clinical laboratory. Therefore, a three-phase trial of both the 2-disk method and the Vitek ESBL test was undertaken. In the first two phases, the accuracy of each method was compared with a standard consisting of a biochemical and/or molecular characterization of the β -lactamase expressed by the test strain. In the third phase, the two methods were compared with each other. This three-phase approach allowed assessment of any deficiencies in the two tests prior to the time they were compared with each other. Since *Klebsiella oxytoca* possesses a chromosomal β -lactamase similar to an ESBL (4), an enzyme therefore classified in the same group as that of ESBLs (group 2be, according to the classification system of Bush et al. [4], hereinafter termed Bush group 2be), it was of interest to assess

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whether the Vitek ESBL test and the 2-disk method would detect this enzyme as well. Therefore, a supplemental study was performed with *K. oxytoca*.

MATERIALS AND METHODS

This study was performed at three sites: the Center for Research in Anti-Infectives and Biotechnology (CRAB), Creighton University School of Medicine, Omaha, Nebr.; the Cleveland Clinic Foundation (CCF), Cleveland, Ohio; and the Clinical Microbiology Institute (CMI), Tualatin, Oreg. The ability of the 2-disk test and the Vitek ESBL test to detect ESBLs in *Enterobacteriaceae* was assessed in a three-phase study at each site.

ESBL tests. The 2-disk test was performed as a standardized disk diffusion assay (17) in which an amoxicillin-clavulanic acid disk was placed in the center of a plate and cefotaxime, ceftazidime, aztreonam, and ceftriaxone disks were placed 30 mm (center to center) from the amoxicillin-clavulanic acid disk (10). Enhancement of the zone of inhibition in the area between the amoxicillin-clavulanic acid disk and any one of the four drug disks in comparison with the zone of inhibition on the far side of the drug disk was interpreted as indicative of the presence of an ESBL in the test strain.

The Vitek ESBL test utilized cefotaxime and ceftazidime alone (at 0.5 µg/ml) and in combination with clavulanic acid (at 4 µg/ml) for the detection of ESBLs that are susceptible to inhibition by this β-lactamase inhibitor. After inoculation of cards containing the four wells of the Vitek ESBL test, analysis of all wells was performed automatically once the growth control well reached the set threshold (4 to 15 h of incubation). A predetermined reduction in growth of the cefotaxime or ceftazidime well containing clavulanic acid, when compared with the level of growth in the well with drug alone indicated the presence of an ESBL that was inhibited by clavulanic acid. Results were generated as either ESBL positive or ESBL negative. For *K. pneumoniae* and *K. oxytoca*, the cefotaxime wells had to indicate the presence of an ESBL for the results to be considered positive. This was due to the fact that certain strains with non-ESBL β-lactamases appeared positive with ceftazidime but not cefotaxime. For all other organisms, results were considered ESBL positive if either the cefotaxime- or ceftazidime-containing wells indicated the presence of an ESBL.

Appropriate quality control strains were included in each day's testing. In each phase of testing, both the Vitek ESBL test and the 2-disk test were repeated once if discrepant results occurred.

β-Lactamases. Strains containing well-characterized β-lactamases were obtained from collections on hand at CRAB or bioMérieux Vitek. The presence of the enzymes in the actual strains used at the three sites in phases 1 and 2 was confirmed by isoelectric focusing and by determination of inhibitor and substrate profiles in tests performed at CRAB (19, 21). For studies with *K. oxytoca*, β-lactamases were characterized similarly. For the purposes of this study, β-lactamases were considered to be ESBLs (derivatives of TEM and SHV that hydrolyzed newer cephalosporins and/or aztreonam), non-ESBL Bush group 2be enzymes (the *K. oxytoca* β-lactamase), or non-ESBLs (all other enzymes [4, 9, 18]). The specific enzymes assessed in phases 1 and 2 included ESBLs (TEM-3, TEM-4, TEM-5, TEM-6, TEM-7, TEM-8, TEM-10, TEM-12, TEM-16, TEM-24, SHV-2, SHV-3, SHV-4, and SHV-5) and non-ESBLs (TEM-1, TEM-2, TRC-1, SHV-1, OXA-1, OXA-2, OXA-3, OXA-4, OXA-5, OXA-6, OXA-7, PSE-1, PSE-2, PSE-3, PSE-4, CARB-4, HMS-1, LCR-1, OHIO-1, SAR-1, Bush group 1 β-lactamases, Bush group 2c β-lactamases, and MIR-1).

RESULTS

Phase 1. The first phase of testing was designed to identify any areas in which the Vitek ESBL test or 2-disk test might have inherent problems. Seventy-two bacterial strains containing well-characterized β-lactamases were sent to the three test sites by bioMérieux Vitek. Thirty-three of the strains produced ESBLs, and the enzymes in 27 of the 72 isolates had been introduced by genetic manipulation (i.e., the strains were not clinical isolates). The Vitek ESBL test and 2-disk test were performed on the isolates, and both tests were repeated if any discrepancies occurred between the two. Upon initial testing in phase 1, there were 3 false negatives and 1 false positive by the Vitek ESBL test and 5 false negatives and 11 false positives by the 2-disk test. Following repeat testing, there was only a single false-positive result with the 2-disk test at one site (*E. coli*, OXA-7). No other discrepant results occurred at any other site following repeat testing. The total number of discrepant results for each site varied from six to eight per site.

Phase 2. The second phase of testing was designed to evaluate the reliability of the Vitek ESBL test and 2-disk test in detecting ESBLs in clinical isolates containing well-character-

TABLE 1. Overall agreement of the Vitek ESBL test and the 2-disk test with characterizations of β-lactamases in strains evaluated in phase 1 and phase 2

Parameter	Result by indicated test compared with standard result ^a	
	Vitek ESBL test	2-Disk test
No. of strains with positive standard and test result	205	202
No. of strains with negative standard and test result	159	157
No. of strains with positive standard and negative test result	1	4
No. of strains with negative standard and positive test result	0	1
Agreement (%)	99.73	98.63
Sensitivity (%)	99.51	98.06
Specificity (%)	100	99.37

^a The standard in these comparisons was the identity of the β-lactamase that had been established by standard biochemical and molecular methods (see Materials and Methods). A total of 365 tests were performed with the Vitek ESBL test, while 364 were performed with the 2-disk test. One test result by the 2-disk test could not be interpreted at one site.

ized β-lactamases, and it was conducted in two parts. In the first part, 32 clinical isolates were sent to the three test sites by bioMérieux Vitek for testing by all three sites. Thirty of these isolates produced ESBLs. There were no false-positive or false-negative results in this portion of the study. In the second part, clinical isolates originating at each of the three trial sites were tested at their own site. There were a total of 53 trial site organisms tested, 17 of which produced ESBLs. Upon initial testing, there was one false negative by the Vitek ESBL test and there were four false negatives and two false positives by the 2-disk test. Following repeat testing, there was one false-negative result by the Vitek ESBL test (*E. coli*, TEM-6) and there were four false-negative results by the 2-disk test (*K. pneumoniae*, two with SHV-2, one with TEM-12, and one with TEM-16). Overall for phase 2, there were three to six discrepant results at each of the three test sites.

For the final evaluation of phases 1 and 2, data were pooled to assess overall agreement of the two tests with the known characterizations of the β-lactamases in the strains evaluated in these two phases. As shown in Table 1, the overall agreement, sensitivities, and specificities of the two tests were high.

Phase 3. In the third phase of testing, clinical isolates of *Enterobacteriaceae* (most of which were ampicillin resistant) were collected by each trial site and tested by the Vitek ESBL test and the 2-disk test. Both tests were repeated if results were discrepant, and strains giving discrepant results after repeat testing were sent to one site (CRAB) for analysis of the β-lactamase produced. Ninety-five isolates were tested at CCF, 101 isolates were tested at CMI, and 99 isolates were tested at CRAB. There were two discrepant results between the two tests at each test site, only one of which remained discrepant upon retesting. Among the 295 total isolates tested, only one produced an ESBL (as indicated by positive Vitek ESBL test and 2-disk test results and subsequent characterization). There was only one false-positive result by the Vitek ESBL test (*Enterobacter cloacae*, Bush group 1 β-lactamase); there were no false positives by the 2-disk test.

Organisms tested. The distribution of strains among the various species tested across the three phases of the study is shown in Table 2. Except for *E. coli* and *K. pneumoniae*, there

TABLE 2. Species of bacteria included in phases 1, 2, and 3 of the study

Species	No. of strains
<i>Citrobacter amalonaticus</i>	2
<i>Citrobacter diversus</i>	3
<i>Citrobacter freundii</i>	9
<i>Escherichia coli</i>	176
<i>Enterobacter aerogenes</i>	16
<i>Enterobacter cloacae</i>	35
<i>Enterobacter species</i>	1
<i>Klebsiella ozaenae</i>	3
<i>Klebsiella pneumoniae</i>	157
<i>Morganella morganii</i>	12
<i>Proteus mirabilis</i>	5
<i>Proteus vulgaris</i>	1
<i>Providencia stuartii</i>	2
<i>Pseudomonas aeruginosa</i>	3
<i>Salmonella</i> spp.	1
<i>Serratia marcescens</i>	17

were fewer than 40 isolates of each of the other species tested. Most of the ESBLs encountered in this study were in *E. coli* or *K. pneumoniae*. Therefore, little data were generated on the reliability of either test to detect ESBLs in other species of *Enterobacteriaceae*.

***K. oxytoca*.** Most strains of *K. oxytoca* produce a chromosomally mediated β -lactamase that shares many characteristics with ESBLs; i.e., it hydrolyzes expanded-spectrum cephalosporins and aztreonam and is sensitive to inhibition by clavulanic acid (4). This β -lactamase was classified by Bush et al. to be in the same group, 2be, as that of the ESBLs, and when produced at a sufficient level, it provides resistance to newer cephalosporins and aztreonam (4, 6–8). Since this species was not addressed in phases 1, 2, and 3, an additional study was performed at one test site (CRAB). In this study, 60 isolates of *K. oxytoca* were collected from diverse sources. Their β -lactamases were characterized and quantified as described in Materials and Methods, their susceptibilities to a variety of β -lactam antibiotics were determined by standard microbroth dilution tests (16), and the presence or absence of ESBLs was determined by both the Vitek ESBL test and the 2-disk test. On the basis of the results of β -lactamase characterization, the 60 strains could be divided into four distinct groups: wild type (non-ESBL Bush group 2be β -lactamase produced at low level), wild type plus Bush group 2b enzyme (most were TEM-1), wild type plus ESBL, and high-level non-ESBL Bush group 2be β -lactamase. Characteristics of each of the four groups are listed in Table 3. Only the strains producing an ESBL or high levels of the non-ESBL Bush group 2be β -lactamase showed reduced susceptibility or resistance to expanded-spectrum cephalosporins and aztreonam. All but one of the strains of these groups were ESBL positive by the Vitek ESBL test. Conversely, all wild types with or without a Bush group 2b β -lactamase were ESBL negative by the Vitek ESBL test. The 2-disk test did not differentiate well between the four groups. For the wild types with or without a Bush group 2b β -lactamase, 10 of the 41 strains appeared ESBL positive; 9 of these gave positive test results with aztreonam only, and 1 gave a positive test result with cefotaxime and aztreonam. For strains producing a high level of non-ESBL Bush group 2be β -lactamase or an ESBL, eight appeared ESBL positive by the 2-disk test and all were positive with three of the four test disks. Eight of the 13 strains producing high levels of the non-ESBL Bush group 2be β -lactamase could not be tested by the 2-disk test

because zones were too small to analyze. Interestingly, the single strain producing high levels of the non-ESBL Bush group 2be β -lactamase that appeared susceptible to expanded-spectrum cephalosporins and aztreonam, appeared ESBL positive by the 2-disk test but not by the Vitek ESBL test.

Reproducibility testing. Eleven strains from phase 1 were tested in triplicate for 3 days by both the Vitek ESBL test and the 2-disk test at all three trial sites. Nine strains produced ESBLs, and two did not. Results were reproducible day to day and site to site (data not shown).

Quality control. A strain of *E. coli* producing TEM-3 (1076) has been chosen as the quality control organism for the Vitek ESBL test as results generated during phases 1, 2, and 3 with this strain were reproducible and the strain was found to be stable and well suited for quality control testing.

DISCUSSION

There is currently a great need for a reliable test to detect ESBLs in clinical isolates of *Enterobacteriaceae*. The test also needs to be practical for routine use in the clinical laboratory. Although the 2-disk test performed as well overall as the Vitek ESBL test, it did not discriminate between strains of *K. oxytoca* possessing ESBLs and high levels of the non-ESBL Bush group 2be enzymes and those that did not. Furthermore, interpretation of results with the 2-disk test were highly subjective in many instances since what defines enhancement of the zone of inhibition is not quantitatively defined. Furthermore, for the 2-disk test to be as reliable as possible, it requires placement of the clavulanic acid-containing disk at a distance from the test drug disk that will allow visualization of enhancement. If the test strain produces a large zone with the test drug, this distance must be fairly large. Conversely, if the test strain gives a small zone with the test drug, this distance must be much smaller. Thus, to get maximal correct information, the 2-disk test may have to be run several times to get the best distance between the disks for detecting enhancement. Additional problems include the great variations in the shapes of the enhanced zones that may be seen (Fig. 1). Rarely are these areas of enhancement uniform. Thus, the 2-disk test requires that the user be very familiar with the test and the various appearances of positive results indicating enhancement. With some strains, the test may need to be repeated on successive days in order to identify the best distance for disk placement as well.

TABLE 3. Characteristics of 60 strains of *K. oxytoca* grouped by β -lactamase produced

Parameter	Wild type	Wild type plus Bush group 2b	Wild type plus ESBL	High-level non-ESBL Bush group 2be
No. of strains	37	4	6	13
No. of strains with decreased S or R ^a	0	0	6	12
No. of strains positive by Vitek ESBL test	0	0	6	12 ^b
No. of strains positive by 2-disk test	7	3	6	2 ^c

^a Decreased susceptibility (S) or resistance (R) to expanded-spectrum cephalosporins or aztreonam was demonstrated by standard microbroth dilution tests.

^b Twelve of 12 strains with decreased susceptibility or resistance gave positive results.

^c Only 5 of 13 strains could be tested by this method because of the small zones of inhibition surrounding the test disks.

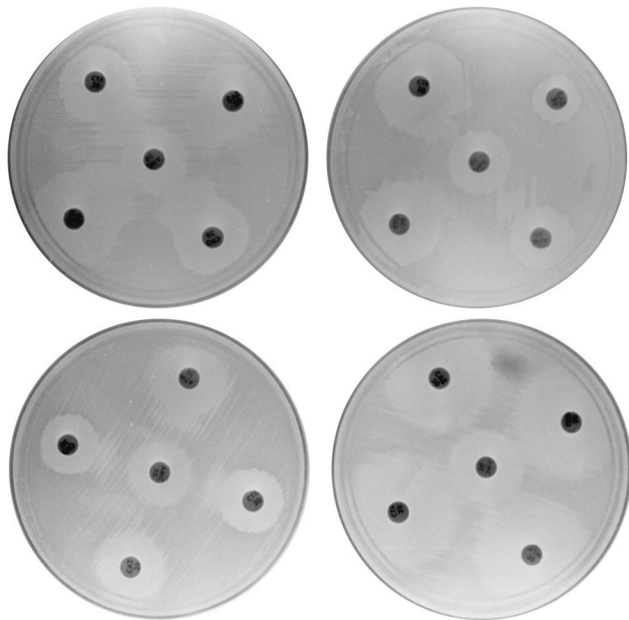


FIG. 1. Photographs of results obtained by the 2-disk test. The center disk contains clavulanic acid. A positive result indicating the presence of an ESBL is an enhancement of the zone of inhibition produced by any one of the four test disks surrounding the clavulanic acid disk in the area between the two disks. (Top left) Results obtained with a strain containing an ESBL. The enhancement of zones is very apparent. (Top right) Results obtained with a strain containing an ESBL. The area of enhancement is very irregular and occurs with three of the four test disks. (Bottom left) Results obtained with a strain containing an ESBL. The area of enhancement is very small because of small zones produced by test disks. (Bottom right) Results obtained with a strain that does not contain an ESBL. Enhancement appears to be occurring because of the intersecting of zones of inhibition.

The Vitek ESBL test, on the other hand, proved to be reliable and easy to perform without any subjective interpretation of results. It was able to detect ESBLs in clinical strains of *E. coli*, *K. pneumoniae*, and *K. oxytoca*. Additionally, it could detect hyperproduction of the non-ESBL Bush group 2be β -lactamase in *K. oxytoca*, a situation that could lead to therapeutic consequences similar to those produced by the presence of an ESBL because of the reduced susceptibilities of such isolates to newer cephalosporins and/or aztreonam (6–8). The reliability of the Vitek ESBL test in detection of ESBLs in other species is unknown, since too few strains from other species with ESBLs were encountered in this study.

One might question the utility of a test proven to date to be reliable with only three species of gram-negative organisms. However, *E. coli* and *Klebsiella* spp. are a few of the most commonly recovered gram-negative pathogens and they are the organisms in which ESBLs are most common (9, 18, 20). These are also the two groups responsible for most of the clinical problems encountered to date. ESBLs in other species of gram-negative bacteria, like *Enterobacter* spp. and *Serratia* spp., are extremely rare and have usually been detected only in retrospective surveys for these enzymes initiated after a *K. pneumoniae* or *E. coli* outbreak has been reported (9, 18, 20). Thus, the Vitek ESBL test is a reliable indicator of the presence of ESBLs in the three most common species producing such enzymes and in those species for which ESBLs pose therapeutic challenges for physicians.

Other tests for the detection of ESBL-producing *Enterobacteriaceae* are under evaluation (3, 24, 26). A three-dimensional test developed by Thomson and Sanders (26), although highly

reliable in detection of ESBL-producing *Enterobacteriaceae*, has not been refined to the point of being able to be adopted for routine use in the clinical microbiology laboratory. The Etest ESBL strip has recently been shown to be able to detect ESBLs in 21 of 22 strains known to produce such enzymes, but more extensive testing will be required to assess its overall reliability (3). Reduced susceptibility to cefpodoxime in routine disk diffusion susceptibility tests has recently been proposed as a marker for the presence of ESBLs in *E. coli* and *K. pneumoniae* (24). This proposal too will require more extensive testing to assess overall reliability.

The high level of interest in developing tests for the detection of ESBL-producing *Enterobacteriaceae* underscores the great need for such tests. Until such tests become available, hospitals will continue to run the risk that dissemination of resistant strains will escape detection. More importantly, patients will continue to be at risk of becoming infected with strains that will not respond to seemingly appropriate therapy. Since patients most likely to become infected with ESBL-producing *Enterobacteriaceae* are those with prolonged stays in the intensive care unit and those who have had invasive procedures, any delay in detection of this often hidden mechanism of resistance could have dire consequences (13, 25). Too many instances of therapeutic failures have already occurred for this situation to be allowed to continue (5, 11, 12, 14, 15, 25).

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REFERENCES

- Bradford, P. A., N. V. Jacobus, N. Bhachech, and K. Bush. 1996. TEM-28 from an *Escherichia coli* clinical isolate is a member of the His-164 family of TEM-1 extended-spectrum β -lactamases. *Antimicrob. Agents Chemother.* **40**:260–262.
- Bradford, P. A., C. Urban, A. Jaiswal, N. Mariano, B. A. Rasmussen, S. J. Projan, J. J. Rahal, and K. Bush. 1995. SHV-7, a novel cefotaxime-hydrolyzing β -lactamase, identified in *Escherichia coli* isolates from hospitalized nursing home patients. *Antimicrob. Agents Chemother.* **39**:899–905.
- Brown, D. F. J., A. Wattam, and E. Walpole. 1995. Detection of extended spectrum β -lactamases (ESBLs) with the Etest ESBL strip, abstr. D12, p. 68. In *Abstracts of the 35th Interscience Conference on Antimicrobial Agents and Chemotherapy*. American Society for Microbiology, Washington, D.C.
- Bush, K., G. A. Jacoby, and A. A. Medeiros. 1995. A functional classification scheme for β -lactamases and its correlation with molecular structure. *Antimicrob. Agents Chemother.* **39**:1211–1233.
- Casellas, J. M., and M. Goldberg. 1989. Incidence of strains producing extended spectrum β -lactamases in Argentina. *Infection* **17**:434–436.
- Chardon, H., C. Pachetti, L. Collet, O. Bellon, and E. Lagier. 1993. Isoelectric point determination of beta-lactamases from 67 strains of *Klebsiella oxytoca* and activity of eight beta lactam antibiotics. *Pathol. Biol.* **41**:343–348.
- Fournier, B., P. H. Lagrange, and A. Philippon. 1996. β -Lactamase gene promoters of 71 clinical strains of *Klebsiella oxytoca*. *Antimicrob. Agents Chemother.* **40**:460–463.
- Fournier, B., P. H. Roy, P. H. Lagrange, and A. Philippon. 1996. Chromosomal β -lactamase genes of *Klebsiella oxytoca* are divided into two main groups, *bla*_{OXY-1} and *bla*_{OXY-2}. *Antimicrob. Agents Chemother.* **40**:454–459.
- Jacoby, G. A., and A. A. Medeiros. 1991. More extended-spectrum β -lactamases. *Antimicrob. Agents Chemother.* **35**:1697–1704.
- Jarlier, V., M.-H. Nicolas, G. Fournier, and A. Philippon. 1988. Extended broad-spectrum β -lactamases conferring transferable resistance to newer β -lactam agents in *Enterobacteriaceae*: hospital prevalence and susceptibility patterns. *Rev. Infect. Dis.* **10**:867–878.
- Karas, J. A., D. G. Pillay, D. Muckart, and A. W. Sturm. 1996. Treatment failure due to extended spectrum β -lactamase. *J. Antimicrob. Chemother.* **37**:203–204.
- Katsanis, G. P., J. Spargo, M. J. Ferraro, L. Sutton, and G. A. Jacoby. 1994. Detection of *Klebsiella pneumoniae* and *Escherichia coli* strains producing

- extended-spectrum β -lactamases. *J. Clin. Microbiol.* **32**:691-696.
13. **Lucet, J.-C., S. Chevreton, D. Decré, D. Vanjak, A. Macrez, J.-P. Bédos, M. Wolff, and B. Regnier.** 1996. Outbreak of multiply resistant Enterobacteriaceae in an intensive care unit: epidemiology and risk factors for acquisition. *Clin. Infect. Dis.* **22**:430-436.
 14. **Medeiros, A. A.** 1993. Nosocomial outbreaks of multiresistant bacteria: extended-spectrum beta-lactamases have arrived in North America. *Ann. Intern. Med.* **119**:428-430.
 15. **Meyer, K. S., C. Urban, J. A. Eagan, B. J. Berger, and J. J. Rahal.** 1993. Nosocomial outbreak of *Klebsiella* infection resistant to late-generation cephalosporins. *Ann. Intern. Med.* **119**:353-358.
 16. **National Committee for Clinical Laboratory Standards.** 1993. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard M7-A3. National Committee for Clinical Laboratory Standards, Villanova, Pa.
 17. **National Committee for Clinical Laboratory Standards.** 1993. Performance standards for antimicrobial disk susceptibility tests. Approved standard M2-A5. National Committee for Clinical Laboratory Standards, Villanova, Pa.
 18. **Philippon, A., R. Labia, and G. A. Jacoby.** 1989. Extended-spectrum β -lactamases. *Antimicrob. Agents Chemother.* **33**:1131-1136.
 19. **Sanders, C. C., J. P. Iaconis, G. P. Bodey, and G. Samonis.** 1988. Resistance to ticarcillin-potassium clavulanate among clinical isolates of the family *Enterobacteriaceae*: role of PSE-1 β -lactamase and high levels of TEM-1 and SHV-1 and problems with false susceptibility in disk diffusion tests. *Antimicrob. Agents Chemother.* **32**:1365-1369.
 20. **Sanders, C. C., and W. E. Sanders, Jr.** 1992. β -Lactam resistance in gram-negative bacteria: global trends and clinical impact. *Clin. Infect. Dis.* **15**:824-839.
 21. **Sanders, C. C., W. E. Sanders, Jr., and E. S. Moland.** 1986. Characterization of β -lactamases in situ on polyacrylamide gels. *Antimicrob. Agents Chemother.* **30**:951-952.
 22. **Sanders, C. C., K. S. Thomson, and P. A. Bradford.** 1993. Problems with detection of β -lactam resistance among nonfastidious gram-negative bacilli. *Infect. Dis. Clin. N. Am.* **7**:411-425.
 23. **Siroton, D. L., F. W. Goldstein, C. J. Soussy, A. L. Courtieu, M. O. Husson, J. Lemozy, M. Meyran, C. Morel, R. Perez, C. Quentin-Noury, M. E. Reverdy, J. M. Scheffel, M. Rosebaum, and Y. Rezvani.** 1992. Resistance to cefotaxime and seven other β -lactams in members of the family *Enterobacteriaceae*: a 3-year survey in France. *Antimicrob. Agents Chemother.* **36**:1677-1681.
 24. **Thomson, K. S.** 1995. Improved detection of extended-spectrum β -lactamases (ESBLs) from disk diffusion susceptibility tests, abstr. C-87, p. 16. *In* Abstracts of the 95th General Meeting of the American Society for Microbiology 1995. American Society for Microbiology, Washington, D.C.
 25. **Thomson, K. S., A. M. Prevan, and C. C. Sanders.** 1996. Novel plasmid-mediated β -lactamases in Enterobacteriaceae: emerging problems for new β -lactam antibiotics. *Curr. Clin. Top. Infect. Dis.* **16**:151-163.
 26. **Thomson, K. S., and C. C. Sanders.** 1992. Detection of extended-spectrum β -lactamases in members of the family *Enterobacteriaceae*: comparison of the double-disk and three-dimensional tests. *Antimicrob. Agents Chemother.* **36**:1877-1882.