Journal of Clinical Microbiology, Jan. 2010, p. 298–299 Vol. 48, No. 1
0095-1137/10/$12.00 doi:10.1128/JCM.01875-09
Copyright © 2010, American Society for Microbiology. All Rights Reserved.

Detection of SHV-Type Extended-Spectrum Beta-Lactamase in *Enterobacter* Isolates▽

Trent G. Towne,[1]† James S. Lewis II,[1,3] Monica Herrera,[3] Brian Wickes,[3] and James H. Jorgensen[2,3,4]*

Department of Pharmacy, University Health System, San Antonio, Texas,[1] and Departments of Microbiology,[2] Medicine,[3] and Pathology,[4] University of Texas Health Science Center, San Antonio, Texas 78229

Received 22 September 2009/Returned for modification 5 November 2009/Accepted 11 November 2009

One hundred four *Enterobacter* isolates were tested by standard CLSI disk diffusion methods for detecting extended-spectrum beta-lactamases (ESBLs) and with cefepime-clavulanate disk combinations. SHV-12 was produced by 8.7% of isolates. The cefepime-clavulanate combination provided 88% sensitivity and 91% specificity for the detection of SHV-12 ESBL.

Detection of extended-spectrum beta-lactamase (ESBL) production in *Enterobacter* spp. has not been undertaken by most clinical laboratories due to a lack of recommendations from the Clinical and Laboratory Standards Institute (CLSI) and potential interference in test interpretation from high-level production of an AmpC beta-lactamase (5). This inability to readily identify ESBLs in pathogens that potentially coproduce AmpC beta-lactamase and an ESBL presents a concern for providers considering cefepime therapy for these patients, since there is an inoculum effect with ESBLs that affects all cephalosporins (2, 6, 7, 13, 16). Detection of an ESBL could lead to selection of a carbapenem for therapy rather than a cephalosporin like cefepime due to a potentially poorer clinical response (4, 11, 14, 17), whereas *Enterobacter* isolates that produce only AmpC have been treated reliably with cefepime (12).

Enterobacter isolates recovered from blood cultures at the University Health System in San Antonio, TX, between 1 October 2004 and 31 December 2007 were examined in this study. These represented the first isolates from each patient and were nonrepetitive, with the exception of one patient, from whom two different *Enterobacter* spp. were identified. Initial identification and susceptibility testing were performed using a Vitek 2 instrument (GN13 cards; bioMerieux, Durham, NC) and/or standard CLSI disk diffusion methodologies (3). All isolates underwent ESBL phenotypic confirmatory disk testing utilizing the cefotaxime- and ceftazidime-clavulanate combinations as described by CLSI for *Escherichia coli*, *Klebsiella pneumoniae*, *Klebsiella oxytoca*, and *Proteus mirabilis* (3). In addition, cefepime disks (30 μg) were tested and zone diameters were recorded in a comparison with cefepime disks to which 10 μg of clavulanate was added in an attempt to improve the ability to detect ESBL production in organisms producing native AmpC beta-lactamase. *E. coli* ATCC 25922, *E. coli* ATCC 35218, and *K. pneumoniae* ATCC 700603 were included with each day's tests for quality control purposes. Zone diameters were recorded for the antimicrobial agent combinations with each isolate. Any isolate with a zone diameter that increased by at least 3 mm (the minimum zone difference that we felt could be reliably measured) with the addition of clavulanate for any of the three cephalosporins or for which no zone of inhibition was seen around any of the three cephalosporin disks was considered a possible ESBL producer and was subjected to further examination.

Using previously described methods, those isolates meeting the screening criteria and a random sampling of 15 isolates not meeting those criteria (control group) were further examined by PCR and gene sequencing to detect possible ESBLs of the three main families, i.e., CTX-M, SHV, and TEM (10). Isoelectric focusing was performed on selected isolates to confirm that all enzymes were detected.

One hundred four *Enterobacter* isolates were examined. *Enterobacter cloacae* represented 81/104 (77.9%) of those tested, with the remaining 23 (22.1%) being *Enterobacter aerogenes*. Sixteen percent ($n = 17$) of isolates were identified as either having a zone diameter change of ≥3 mm or greater or no zone of inhibition around any of the three cephalosporin disks (study group). Ten isolates in the study group had a zone diameter change of ≥5 mm for one or more of the three cephalosporins. Based upon PCR and sequencing, nine (8.7%) of the isolates harbored an ESBL; all harbored a single enzyme, SHV-12. Eight of the nine (89%) SHV-12-producing strains were *E. cloacae*. No CTX-M or TEM ESBLs were identified in the study group. In addition, no isolates in the control group were found to have an ESBL following molecular characterization.

Sensitivity and specificity were evaluated for each of the disk diffusion tests at each of the zone diameter changes (Table 1). Sensitivity analyses revealed that both ceftazidime and cefepime exhibited excellent sensitivity for detection of SHV-12 if a zone diameter change of >3 mm was employed as opposed to the usual ≥5-mm change advocated by the CLSI for other organisms. Specificity was highest overall in the cefotaxime arm (100%), but poor sensitivity (66%) limits the applicability of this substrate for the detection of ESBL in *Enterobacte*r spp. The cefepime-clavulanate combination at a zone diameter change of ≥3 mm exhibited the highest combination of sensi-

* Corresponding author. Mailing address: University of Texas Health Science Center, Department of Pathology, 7703 Floyd Curl Drive, San Antonio, Texas 78229. Phone: (210) 567-4088. Fax: (210) 567-2367. E-mail: jorgensen@uthscsa.edu.

† Present address: Department of Pharmacy Practice/Pharmacy Administration, Philadelphia College of Pharmacy, Philadelphia, PA.

▽ Published ahead of print on 18 November 2009.

TABLE 1. Sensitivity and specificity of ESBL detection based upon disk diffusion zone diameter changes with addition of clavulanate[a]

Test parameter	Value with criterion					
	CTX zone diam change with CLA (mm) of:		CAZ zone diam change with CLA (mm) of:		FEP zone diam change with CLA (mm) of:	
	≥3	≥5	≥3	≥5	≥3	≥5
% Sensitivity	66	66	88	55	88	77
% Specificity	96	100	82	91	91	100

[a] CLA, clavulanate; CTX, cefotaxime; CAZ, ceftazidime; FEP, cefepime. Thirty-three tests were carried out.

tivity (88%) and specificity (91%) of all combinations. Unexpectedly, tests utilizing ceftazidime and ceftazidime-clavulanate also provided very good results, especially if a zone diameter change of ≥3 mm was used (sensitivity of 88%; specificity of 82%).

While reports of ESBL production in *Enterobacter* spp. have been noted worldwide, the true scope of the problem in the United States remains poorly defined. Recently published data from France, South Korea, and Algeria have demonstrated a wide range (4.4% to 17.7%) of ESBL production among clinical *E. cloacae* isolates (1, 8, 9). In the United States, Szabó and colleagues have previously utilized the CLSI double-disk diffusion methods (including the cefepime-clavulanate combination) applied to *Enterobacter* spp. and determined a rate of 33% (15/45 isolates) harboring an SHV-type ESBL (15). In addition, a sensitivity of only 75% was observed with the incorporation of the cefepime-clavulanate combination. The present study, conducted at a single U.S. center, identified ESBL-producing *Enterobacter* spp. as an infrequent occurrence and demonstrated effective detection utilizing a zone diameter change (≥3 mm) with either ceftazidime or cefepime substrates.

Consistent with other reports from around the world, SHV-12 was the major ESBL identified in our *Enterobacter* bloodstream isolates (1, 4, 8, 9). This is in spite of data from Lewis and colleagues (10) at the same institution indicating that a few *Enterobacter* isolates produced CTX-M ESBLs. Isolates from the present study were restricted to those from blood cultures of hospitalized patients instead of urinary samples from ambulatory patients, as frequently encountered in the prior study (10).

The high predictive value of both the ceftazidime-clavulanate and cefepime-clavulanate combinations for *Enterobacter* isolates producing SHV-12 suggests this may be a practical tool for identification of ESBL production in *Enterobacter* isolates.

This study was supported in part by a grant from Merck, Inc.

We thank Letitia Fulcher and M. Leticia McElmeel for excellent technical support and the UHS Clinical Microbiology Laboratory technologists for assistance with identification and retrieval of isolates. We also thank Anne Marie Queenan of Johnson and Johnson Pharmaceutical Research Institute for performing isoelectric focusing on selected isolates.

REFERENCES

1. **Biendo, M., C. Manoliu, G. Laurans, S. Castelain, B. Canarelli, D. Thomas, F. Hamdad, F. Rousseau, and F. Eb.** 2008. Molecular typing and characterization of extended-spectrum TEM, SHV, and CTX-M β-lactamases in clinical isolates of *Enterobacter cloacae*. Res. Microbiol. **159:**590–594.
2. **Bradford, P. A.** 2001. Extended-spectrum ß-lactamases in the 21st century; characterization, epidemiology, and detection of this important resistance threat. Clin. Microbiol. Rev. **14:**933–951.
3. **Clinical and Laboratory Standards Institute.** 2009. Performance standards for antimicrobial susceptibility testing. Approved standard M100-S19. Clinical and Laboratory Standards Institute, Wayne, PA.
4. **Crowley, B., and G. Ratcliffe.** 2003. Extended-spectrum beta-lactamases in *Enterobacter cloacae*: underestimated but clinically significant! J. Antimicrob. Chemother. **51:**1316–1317.
5. **Drieux, L., F. Brossier, W. Sougakoff, and V. Jarlier.** 2008. Phenotypic detection of extended-spectrum β-lactamase production in *Enterobacteriaceae*: review and bench guide. Clin. Microbiol. Infect. **14**(Supp 1)**:**90–103.
6. **Jacoby, G. A., and I. Carreras.** 1990. Activities of ß-lactam antibiotics against *Escherichia coli* strains producing extended-spectrum ß-lactamases. Antimicrob. Agents Chemother. **34:**858–862.
7. **Jacoby, G. A., and L. S. Munoz-Price.** 2005. The new ß-lactamases. New Engl. J. Med. **352:**380–391.
8. **Labadene, H., Y. Messai, H. Ammari, N. Ramdani-Bouguessa, S. Lounes, R. Bakour, and G. Arlet.** 2008. Dissemination of ESBL and Qnr determinants in *Enterobacter cloacae* in Algeria. J. Antimicrob. Chemother. **62:**133–136.
9. **Ko, K. S., M. Y. Lee, J. H. Song, H. Lee, D. S. Jung, S. I. Jung, S. W. Kim, J. S. Chang, Y. S. Kim, H. K. Ki, D. R. Chung, K. T. Kwon, K. R. Peck, and N. Y. Lee.** 2008. Prevalence and characterization of extended-spectrum beta-lactamase-producing *Enterobacteriaceae* isolated in Korean hospitals. Diagn. Microbiol. Infect. Dis. **61:**453–459.
10. **Lewis, J. S., II, M. Herrera, B. Wickes, J. A. Patterson, and J. H. Jorgensen.** 2007. First report of the emergence of CTX-M-type extended-spectrum β-lactamases (ESBLs) as the predominant ESBL isolated in a U.S. health care system. Antimicrob. Agents Chemother. **51:**4015–4021.
11. **Paterson, D. L., W.-C. Ko, A. Von Gottberg, J. M. Casellas, L. Mulazimoglu, K. P. Klugman, R. A. Bonomo, L. B. Rice, J. G. McCormack, and V. L. Yu.** 2001. Outcome of cephalosporin treatment for serious infections due to apparently susceptible organisms producing extended-spectrum ß-lactamases: implications for the clinical microbiology laboratory. J. Clin. Microbiol. **39:** 2206–2212.
12. **Pfaller, M. A., H. S. Sader, T. R. Fritsche, and R. N. Jones.** 2006. Antimicrobial activity of cefepime tested against ceftazidime-resistant Gram-negative clinical strains from North American Hospitals: report from the SENTRY Antimicrobial Surveillance Program (1998–2004). Diagn. Microbiol. Infect. Dis. **56:**63–68.
13. **Queenan, A. M., B. Foleno, C. Gownley, E. Wira, and K. Bush.** 2004. Effects of inoculum and β-lactamase activity in AmpC- and extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* and *Klebsiella pneumoniae* clinical isolates tested by using NCCLS ESBL methodology. J. Clin. Microbiol. **42:**269–275.
14. **Song, W., E. S. Moland, N. D. Hanson, J. S. Lewis, J. H. Jorgensen, and K. S. Thomson.** 2005. Failure of cefepime therapy in treatment of *Klebsiella pneumoniae* bacteremia. J. Clin. Microbiol. **43:**4891–4894.
15. **Szabó, D., R. A. Bonomo, F. Silveira, A. W. Pasculle, C. Baxter, P. K. Linden, A. M. Hujer, K. M. Hujer, K. Deeley, and D. L. Paterson.** 2005. SHV-type extended-spectrum beta-lactamase production is associated with reduced cefepime susceptibility in *Enterobacter cloacae*. J. Clin. Microbiol. **43:**5058–5064.
16. **Thomson, K. S., and E. S. Moland.** 2001. Cefepime, piperacillin-tazobactam, and the inoculum effect in tests with extended-spectrum ß-lactamase-producing *Enterobacteriaceae*. Antimicrob. Agents Chemother. **45:**3548–3554.
17. **Zanetti, G., F. Bally, G. Greub, J. Garbino, T. Kinge, D. Lew, J.-A. Romand, J. Bille, D. Aymon, L. Stratchounski, L. Krawczyk, E. Rubinstein, M.-D. Schaller, R. Chiolero, M.-P. Glauser, and A. Cometta.** 2003. Cefepime versus imipenem-cilastatin for treatment of nosocomial pneumonia in intensive care unit patients: a multicenter, evaluator-blind, prospective, randomized study. Antimicrob. Agents Chemother. **47:**3442–3447.