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. Jorgensen2,3,4*

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

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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 produce 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 cepotaxime- 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 cepotaxime arm (100%), but poor sensitivity (66%) limits the applicability of this substrate for the detection of ESBL in Enterobacter 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.
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The high predictive value of both the ceftazidime-clavulanate and ceftazidime-clavulanate combinations for Enterobacter isolates producing SHV-12 suggests this may be a practical tool for identification of ESBL production in Enterobacter isolates.

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REFERENCES


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