ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Feb. 2007, p. 800–801 Vol. 51, No. 2
0066-4804/07/$08.00+0 doi:10.1128/AAC.01143-06

Letters to the Editor

Klebsiella pneumoniae Isolate Producing at Least Eight Different β-Lactamases, Including AmpC and KPC β-Lactamases▽

Reports of the production of multiple β-lactamases in a single gram-negative pathogen are increasing (1, 2, 6). Isolates of *Klebsiella* species producing KPC-2, SHV extended-spectrum β-lactamases (ESBLs), and inhibitor-resistant TEM-30 β-lactamases have been reported as endemic in New York City (1). The present report identifies a *Klebsiella pneumoniae* isolate from New York City which produced up to 10 different β-lactamases, including a FOX-like plasmid-mediated AmpC, in addition to the previously reported KPC, SHV ESBL, and IRT β-lactamases (1).

The *K. pneumoniae* isolate was obtained from the sputum of a patient with a mesothelioma. No carbapenem antibiotics were given to the patient prior to the isolation of the *K. pneumoniae* isolate. CLSI (formerly NCCLS) disk diffusion and broth microdilution assays (4) revealed that the *K. pneumoniae* isolate lacked susceptibility to levofloxacin (>16 μg/ml), amikacin (32 μg/ml), piperacillin-tazobactam (>128 μg/ml), ceftazidime (128 μg/ml), cefotaxime (64 μg/ml), aztreonam (>128 μg/ml), and cefoxitin (>64 μg/ml) but was susceptible by microbroth tests to tigecycline (0.5 μg/ml), minocycline (4 μg/ml), polymyxin B (1 μg/ml), cefepime, and imipenem. However, when broth microdilution tests were performed with an inoculum of 10^7 CFU/ml instead of 10^5 CFU/ml, the MICs of both imipenem and cefepime increased (4 μg/ml to 32 μg/ml and 8 μg/ml to >128 μg/ml, respectively). The MICs of tigecycline, minocycline, and polymyxin B were not affected when a higher inoculum was used. The presence of an ESBL was indicated by the CLSI ESBL confirmatory disk tests but not by the CLSI ESBL broth microdilution confirmatory tests (4).

TABLE 1. Identification of the β-lactamases produced by the *K. pneumoniae* isolate

pI[a]	Inhibition by:[b]		Hydrolysis of cefotaxime overlay on IEF[c]	PCR-positive result[e]	Likely enzyme based on pI, inhibitor profile, and PCR
	Clavulanate	Cloxacillin			
8.2	Yes	No	Yes	SHV	SHV-12-like
8.0	Yes	No	No	ND	ND
7.6	Yes	No	No	SHV	SHV-1-like
7.2	No	Yes	Yes	FOX	FOX-like
6.9	Slightly	No	ND[d]	OXA	OXA-9[f]
6.7	Slightly	No	Yes[d]	KPC	KPC-like
5.7+	Yes	No	No	ND	ND
5.7	Slightly	No	No	PSE	PSE-1-like
5.4	Yes	No	No	TEM	TEM-1-like
5.25	Slightly	No	No	TEM	TEM-30-like

[a] pI values obtained by IEF. The + indicates that the pI values were in the same range but that the unidentified band was slightly more alkaline than the 5.7 band representing PSE-1.
[b] Inhibitor profiles on the IEF gel, in the absence or presence of 1 mM clavulanate or cloxacillin.
[c] Cefotaxime (CTX) hydrolysis was determined on the IEF gel using 0.75 μg/ml of CTX in an agar overlay combined with *Escherichia coli* ATCC 25922, with growth of the organism as an indicator of hydrolysis. ND, not determined.
[d] Determining the contribution of the CTX hydrolysis from each enzyme observed on the gel was difficult. Previous reports have indicated that OXA-9 does not hydrolyze CTX; therefore, the CTX hydrolysis observed was most likely due to the KPC enzyme.
[e] PCR-positive results with family-specific primers.
[f] Determined by partial sequence analysis.

Isoelectric focusing (IEF) indicated that this isolate produced up to 10 different β-lactamases. Characterization of these enzymes was performed, as previously described, using IEF, inhibitor profiles, cefotaxime gel hydrolysis assays, and PCR (5–7, 8, 9). Using these techniques, 8 of 10 β-lactamases were identified. Three of these enzymes hydrolyzed cefotaxime. The pI and inhibitor profiles of these enzymes suggested the production of an SHV-12-like ESBL, a KPC-like carbapenem-hydrolyzing enzyme, a FOX-like AmpC, a PSE-1-like β-lactamase, and an OXA β-lactamase. In addition, TEM-1-like, TEM-30-like, and SHV-1-like enzymes were also identified (Table 1). PCR amplification using family-specific primers substantiated the presence of these genes within the *K. pneumoniae* isolate and identified the OXA β-lactamase as an OXA-9-like β-lactamase. Sequence data generated using the same primers that amplified the bla_{OXA} product also suggested that the gene was $bla_{OXA\text{-}9}$.

Accurate β-lactam susceptibility testing can be expected to become increasingly difficult over the next few years due to an increase in isolates producing multiple β-lactamases. The numbers and types of β-lactamases produced by the *K. pneumoniae* isolate from New York described in this report are a cause for concern, especially with respect to detecting ESBL, AmpC, and KPC-type enzymes. The CLSI microbroth ESBL confirmatory tests were unable to detect the presence of the SHV ESBL in this isolate, and these tests also suggested imipenem susceptibility, even though the isolate produced a KPC-type enzyme. As the number of pathogens producing multiple β-lactamases continues to rise, the difficulties in identifying the mechanisms responsible for β-lactam MICs will increase (1, 2, 6, 10). Clinical laboratories need the option of molecular testing in addition to phenotypic testing for the identification of resistance mechanisms that may be masked by the production of multiple enzymes. Additional testing options for these highly resistant pathogens may help avert future outbreaks like the ones reported for KPC-producing *K. pneumoniae* isolates in New York (1–3).

REFERENCES

1. **Bradford, P. A., S. Bratu, C. Urban, M. Visalli, N. Mariano, D. Landman, J. J. Rahal, S. Brooks, S. Cebular, and J. Quale.** 2004. Emergence of carbapenem-resistant *Klebsiella* species possessing the class A carbapenem-hydrolyzing KPC-2 and inhibitor-resistant TEM-30 beta-lactamases in New York City. Clin. Infect. Dis. **39:**55–60.
2. **Bratu, S., D. Landman, R. Haag, R. Recco, A. Eramo, M. Alam, and J. Quale.** 2005. Rapid spread of carbapenem-resistant *Klebsiella pneumoniae* in New York City: a new threat to our antibiotic armamentarium. Arch. Intern. Med. **165:**1430–1435.
3. **Bratu, S., M. Mooty, S. Nichani, D. Landman, C. Gullans, B. Pettinato, U. Karumudi, P. Tolaney, and J. Quale.** 2005. Emergence of KPC-possessing *Klebsiella pneumoniae* in Brooklyn, New York: epidemiology and recommendations for detection. Antimicrob. Agents Chemother. **49:**3018–3020.
4. **Clinical and Laboratory Standards Institute.** 2006. Performance standards for antimicrobial susceptibility testing. CLSI/NCCLS M100-S16. Clinical and Laboratory Standards Institute, Wayne, PA.
5. **Hanson, N. D., E. Smith Moland, and J. D. Pitout.** 2001. Enzymatic characterization of TEM-63, a TEM-type extended spectrum beta-lactamase

expressed in three different genera of Enterobacteriaceae from South Africa. Diagn. Microbiol. Infect. Dis. **40:**199–201.

6. **Hanson, N. D., K. S. Thomson, E. S. Moland, C. C. Sanders, G. Berthold, and R. G. Penn.** 1999. Molecular characterization of a multiply resistant *Klebsiella pneumoniae* encoding ESBLs and a plasmid-mediated AmpC. J. Antimicrob. Chemother. **44:**377–380.
7. **Moland, E. S., N. D. Hanson, J. A. Black, A. Hossain, W. Song, and K. S. Thomson.** 2006. Prevalence of newer beta-lactamases in gram-negative clinical isolates collected in the United States in 2001 to 2002. Antimicrob. Agents Chemother. **44:**3318–3324.
8. **Perez-Perez, F. J., and N. D. Hanson.** 2002. Detection of plasmid-mediated AmpC beta-lactamase genes in clinical isolates by using multiplex PCR. J. Clin. Microbiol. **40:**2153–2162.
9. **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.
10. **Tenover, F. C., R. K. Kalsi, P. P. Williams, R. B. Carey, S. Stocker, D. Lonsway, J. K. Rasheed, J. W. Biddle, J. E. McGowan, Jr., and B. Hanna.** 2006. Carbapenem resistance in *Klebsiella pneumoniae* not detected by automated susceptibility testing. Emerg. Infect. Dis. **12:**1209–1213.

Ellen Smith Moland
Seong Geun Hong
Kenneth S. Thomson
Department of Medical Microbiology and Immunology
Center for Research in Anti-Infectives and Biotechnology
Creighton University School of Medicine
Omaha, Nebraska

Davise H. Larone
Department of Pathology and Laboratory Medicine
Weill Cornell Medical Center
New York-Presbyterian Hospital
New York, New York

Nancy D. Hanson*
Department of Medical Microbiology and Immunology
Center for Research in Anti-Infectives and Biotechnology
2500 California Plaza
Creighton University School of Medicine
Omaha, Nebraska 68178

*Phone: (402) 280-5837
Fax: (402) 280-1875
E-mail: ndhanson@creighton.edu

▽ Published ahead of print on 4 December 2006.