Letters to the Editor

New Quality Control Strain for Use in Routine Testing for Production of Extended-Spectrum Beta-Lactamases by Enterobacteriaceae

The Clinical and Laboratory Standards Institute (CLSI) guidelines for the detection of extended-spectrum β-lactamases (ESBL) produced by Escherichia coli, Klebsiella pneumoniae, Klebsiella oxytoca, and Proteus mirabilis recommend the use of K. pneumoniae ATCC 700603 (an ESBL producer) as a quality control organism in tests to detect ESBL (1). A clinical isolate of Proteus mirabilis was studied to determine whether this isolate could be an alternative quality control organism in these tests. This isolate has been accepted by the American Type Culture Collection and is designated Proteus mirabilis ATCC BAA-856.

The Etest (AB Biodisk, Solna, Sweden) and disk diffusion tests were performed in accordance with the manufacturer’s instructions and CLSI recommendations, respectively (1). Ceftazidime, ceftazidime-clavulanic acid, ceftoxime, and ceftoxime-clavulanic acid were used in both susceptibility methods. Reproducibility of ESBL production by BAA-856 was studied by performing both susceptibility methods five times on each of four different days for a total of 20 replicates for each susceptibility method. The stability of ESBL expression was studied by subculturing BAA-856 20 consecutive times on solid 5% sheep blood medium. Colonies from passes 1, 5, 10, 15, and 20 were tested in duplicate by both susceptibility methods. Reproducibility of ESBL production by BAA-856 was studied by performing both susceptibility methods five times on each of four different days for a total of 20 replicates for each susceptibility method. The stability of ESBL expression was studied by subculturing BAA-856 20 consecutive times on solid 5% sheep blood medium. Colonies from passes 1, 5, 10, 15, and 20 were tested in duplicate by both susceptibility methods. The modal MICs (range) for the Etest system were the following: for cefotaxime, 0.75 g/ml (0.5 to 1.0 g/ml), and for ceftaxime-clavulanic acid, 0.032 g/ml (0.032 to 0.05 g/ml). All 20 replicate MICs for ceftazidime and ceftazidime-clavulanic acid were >32 and 0.125 g/ml, respectively. All MICs (±1 dilution) were stable throughout 20 passages. Use of the disk diffusion procedure to determine reproducibility of ESBL production yielded the following modal zone diameters (range): for cefotaxime, 28 mm (26 to 29 mm); for ceftaxime-clavulanic acid, 35 mm (32 to 36 mm); for ceftazidime, 17 mm (15 to 19 mm); and for ceftazidime-clavulanic acid, 30 mm (26 to 31 mm). The mean difference (range) between cefotaxime and ceftazidime-clavulanic acid was 5.7 mm (5 to 8 mm), and the mean difference between ceftazidime and ceftazidime-clavulanic acid was 11.4 mm (10 to 14 mm). Zone diameters showed consistency (±0.2 mm) throughout 20 passages.

Crude sonic extracts of BAA-856 were analyzed by analytical isoelectric focusing (IEF) in ampholine polyacrylamide gels (pH range, 3.5 to 9.5) on a Multiphor IEF apparatus (LKB, Rockville, MD). The following characteristics of ESBL produced by BAA-856 were determined: isoelectric points (pIs), ability to hydrolyze cefotaxime, and ability to be inhibited by clavulanic acid (2, 3). IEF lanes were overlaid with solutions of lithium clavulanate (1,000 μM), and ESBL bands were visualized by overlaying the IEF gel with a thin layer of nitrocefin agar which contained cefotaxime (0.75 μg/ml). Organisms with known β-lactamases (TEM-1, -2, and -3 and SHV-2, -3, -4, and -5) were used as IEF control standards. BAA-856 produced two β-lactamases with separate pIs of 5.6 and 8.0; both enzymes were inhibited by lithium clavulanate. The fact that only the pI 5.6 enzyme hydrolyzed cefotaxime suggested the presence of a TEM-ESBL.

DNA templates for PCR were prepared as previously described (2). Primers TEM15F (TGGGGAAATGTGCG) and TEMENDM (CGTTCCACTGAGCGTCAGAC) were used. A 3100-Avant genetic analyzer (ABI, Foster City, CA) was used for automated-cycle sequencing. BLASTn analysis identified the gene as blatEM-10 and the corresponding enzyme as TEM-10. The results of these studies suggest that BAA-856 produces a stable TEM-10 ESBL and that BAA-856 can be an acceptable isolate to use in tests to detect the production of ESBL.

REFERENCES


Joel E. Mortensen
Mavi Bernier
Cincinnati Children’s Hospital Medical Center
Cincinnati, Ohio 45229

Larry D. Gray*
Sharon Dolan
TriHealth Clinical Laboratories
Cincinnati, Ohio 45206

Nancy D. Hanson
Ellen S. Moland
Baha Abdalhamid
Creighton University School of Medicine
Omaha, Nebraska 68178

*Phone: (513) 569–5073
Fax: (513) 569–6243
E-mail: larry_gray@trihealth.com