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Performance of Methods for Detection of Extended-Spectrum β-Lactamases Applied to Clinical Enterobacterial Strains Producing IBC-Type β-Lactamases

IBC-1 is a novel extended-spectrum β-lactamase (ESBL) that was found recently in an *Enterobacter cloacae* clinical strain from Greece (1). The enzyme is highly similar to GES-1, an ESBL produced by a sporadic *Klebsiella pneumoniae* strain from Guinea (5). Hydrolysis experiments and resistance phenotypes suggested that both β-lactamases are highly active against ceftazidime but affect cefotaxime, ceftriaxone, and particularly aztreonam to a lesser extent. Also, compared with the classical ESBL of the TEM and SHV types, they are less susceptible to inhibition by clavulanic acid (1, 5).

We present here the results of various ESBL-detecting tests applied to five *Escherichia coli* and four *K. pneumoniae* strains producing IBC-type enzymes. The strains were isolated in four hospitals in Athens between 2001 and 2002 in the context of an ongoing surveillance study concerning the spread of ESBL-producing enterobacteria. IBC-1 production in these strains had been confirmed by bla_{IBC}-specific PCR and isoelectric focusing.

Susceptibility to the β-lactams of the nine IBC producers was determined by an agar dilution technique (3). MIC ranges were as follows: ceftazidime, 32 to >128 μg/ml; cefotaxime, 2 to 8 μg/ml; aztreonam, 0.5 to 8 μg/ml; cefepime, 0.12 to 0.5 μg/ml; amoxicillin-clavulanate (2:1), 8 to 32 μg/ml; ticarcillin-clavulanate, 8:2 to 64:2 μg/ml; and piperacillin-tazobactam, 4:4 to 8:4 μg/ml.

Production of ESBL was examined by using the following methods and/or materials: Etest strips containing clavulanate plus ceftazidime or cefotaxime (AB Biodisk, Solna, Sweden); the double-disk synergy test (DDST) (2), combining disks of ceftazidime or cefotaxime with amoxicillin-clavulanate (2:1); and ESBL confirmatory test disks (Becton Dickinson, Sparks, Md.) (4). Strains were also tested by using the following automated systems: the Phoenix NMIC/ID-5 panel (Becton Dickinson); VITEK 2 GNS AST-N card (bioMérieux S.A., Marcy l'Etoile, France); Wider I C093-31/W panel (Francisco Soria Melguizo S.A., Madrid, Spain); and MicroScan B1017-308 panel (Dade Behring, Deerfield, Ill.).

Etest assays combining ceftazidime and clavulanate detected all nine ESBL producers (sensitivity, 100%). The same was the case with the respective ESBL confirmatory test disks (a ≥7-mm increase in the zone diameter of ceftazidime in the presence of clavulanate). On the other hand, the sensitivity of the cefotaxime-clavulanate combination was lower with both methods. In three strains exhibiting a cefotaxime MIC of 2 μg/ml, clavulanate reduced resistance levels but this reduction was less than that required to consider the strain ESBL positive by Etest (a less than 3 $\log_2$ dilution reduction of the cephalosporin MIC). False-negative results were observed with all nine strains by the cefotaxime-clavulanate disk test (a less than 3-mm increase in the cefotaxime inhibition zone). Also, the performance of DDST was poor. Only two *E. coli* strains appeared ESBL positive with this method. It is likely that the problems encountered with the ESBL-detecting methods that are based on clavulanate inhibition were due mainly to the relatively low susceptibility of the IBC β-lactamases to this inhibitor.

Three of the automated systems, Phoenix, VITEK 2, and Wider I, readily recognized all nine IBC-producing strains as ESBL positive and included the appropriate corrections for cephalosporins and aztreonam in the final susceptibility report. MicroScan reported all nine strains as suspected ESBL producers and suggested further confirmatory testing.

Notwithstanding the above-described detection problems, the low prevalence of IBC producers suggests that misclassifications are rare. Additionally, the characteristic IBC phenotype, i.e., high-level resistance to ceftazidime and susceptibility to aztreonam, which is rare with the common TEM and SHV "ceftazidimases," may facilitate the detection of IBC producers. Special attention, however, must be given in hospital laboratories using DDST or other methods based on cefotaxime-clavulanate synergy.

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E. Lebessi
G. Stamos
M. Foustoukou
Laboratory of Microbiology
P. & A. Kyriakou Children's Hospital

S. Vourli
N. J. Legakis
L. S. Tzouvelekis*
Department of Microbiology
Medical School
University of Athens
Athens 11521, Greece

*Phone: 30 1 746 2011
Fax: 30 1 746 2124
E-mail: ltzouvel@cc.uoa.gr