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Rare Case of Failure by an Automated System To Detect Extended-Spectrum β-Lactamase in a Cephalosporin-Resistant *Klebsiella pneumoniae* Isolate

Several tests used for the detection of extended-spectrum-β-lactamase (ESBL)-producing enterobacterial isolates have been evaluated in past studies. Detection of ESBLs by the Vitek GNS-506 panel (Bio-Mérieux, Marcy l'Etoile, France) is based on the comparison of the reduction in growth by cefotaxime-clavulanate and ceftazidime-clavulanate with the reduction caused by the cephalosporins alone. The test has been proven reliable for the detection of ESBLs in *Klebsiella pneumoniae* and *Escherichia coli* and more effective than the double-disk synergy test (DDST) (3). The Vitek system is widely used in Greek hospitals.

In the context of the study of the epidemiology of bacterial resistance in Athens hospitals, we are evaluating randomly selected multidrug-resistant strains in our laboratories. During the course of these studies, we have noticed failure of the Vitek system to detect ESBL in a *K. pneumoniae* strain. This isolate was resistant to cefoxitin (MIC $\geq$ 32 μg/ml) and ceftazidime (MIC $\geq$ 32 μg/ml) and susceptible to cefotaxime (MIC $\leq$ 4 μg/ml). Resistance to cefoxitin indicated the production of plasmidic class C β-lactamase(s). These enzymes are frequently encountered among *K. pneumoniae* and *E. coli* isolates in Greek hospitals and are closely related to the *Citrobacter freundii* chromosomal cephalosporinase (1, 2). Testing of the strains by the the disk diffusion method confirmed the resistance phenotype. No synergy between ceftazidime or cefotaxime and amoxicillin-clavulanate (AMC) disks was observed. However, the DDST was positive when AMC was combined with cefepime. The latter antibiotic can be hydrolyzed efficiently by various ESBLs but, unlike ceftazidime and cefotaxime, resists hydrolysis by class C β-lactamases. Subsequent experiments, including isoelectric focusing of crude enzyme preparations from the wild strain and transconjugant clones, showed that the *K. pneumoniae* isolate simultaneously produced a class C β-lactamase with a highly basic isoelectric point (pI $>$ 9) together with an extended-spectrum enzyme focused at 8.2 (presumably an SHV-5-type β-lactamase).

It is likely that the false-negative result obtained with the Vitek ESBL test, and also with the DDST routinely in use in Greek hospitals, was due to interference of the class C β-lactamases, which are not inhibited by clavulanate. It should be noted, however, that other *K. pneumoniae* isolates from the same setting, when examined by Vitek, exhibited similar resistance phenotypes but were reported as ESBL positive. Therefore, it may be postulated that the detection system can be deceived when the quantity of AmpC produced, relative to that of ESBL, is such that the presence of the latter enzyme is obscured. The evaluation of this window of error, which is bound to be narrow, is currently under way.

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