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Letters to the Editor

Detecting VIM-1 Production in *Proteus mirabilis* by an Imipenem-Dipicolinic Acid Double Disk Synergy Test[▽]

Gram-negative pathogens producing metallo-β-lactamases (MβLs) capable of hydrolyzing carbapenems have achieved worldwide spread (1). The most frequent MβLs reported so far belong to the VIM and IMP types. VIM-1 producers constitute the majority of the multiresistant *Klebsiella pneumoniae* isolates in Greek hospitals (6). MβL-positive isolates among other members of the family *Enterobacteriaceae* such as *Proteus mirabilis* have also sporadically occurred (8). Detection of MβLs is mostly based on carbapenem-EDTA synergy tests in various formats. There are, however, sensitivity problems due to low carbapenem MICs, as well as discrepancies between the various methods (1, 3).

P. mirabilis exhibiting resistance to newer β-lactams was recently noticed at Evgenidion General Hospital in Athens. The automated system used in the clinical laboratory (Vitek 2 using the AST-N103 and AST-EXN8 susceptibility cards; bioMérieux) invariably characterized these isolates as cephalosporinase positive. Sixteen *P. mirabilis* isolates from 2008 were studied. MICs of ceftazidime and cefotaxime were >64 mg/liter. Isolates were also resistant to penicillins, penicillin-clavulanate combinations, and cefoxitin. MIC ranges of cefepime and aztreonam were 8 to 16 and 2 to 4 mg/liter, respectively. Isolates were fully or intermediately susceptible to imipenem (MICs of 2 to 8 mg/liter) and meropenem (MICs of 0.25 to 2 mg/liter), as determined by broth microdilution. PCR assays specific for a variety of *bla* genes and sequencing of the amplicons showed that all isolates carried $bla_{\text{TEM-1}}$, $bla_{\text{CMY-16}}$, and $bla_{\text{VIM-1}}$. Isoelectric focusing of cell extracts confirmed production of the respective β-lactamases. Comparable imipenem-hydrolyzing activities were also observed by spectrophotometry in crude cell extracts from all isolates, although lower (10 to 15 U) than those usually found in VIM-1-producing *K. pneumoniae* (40 to 80 U; 1 U was the amount of enzyme hydrolyzing 1 nmol of imipenem/min/mg of protein) (5). All but two isolates appeared MβL negative by the imipenem-EDTA double disk synergy test (DDST) (2). The EDTA-imipenem combined disk test (2) failed to detect any of the isolates. The lack of sensitivity of the EDTA-based tests prompted us to evaluate synergy between dipicolinic acid (DPA) and imipenem. Disks containing 250 μg DPA and imipenem (10 μg) were placed at a distance of 8 mm (edge to edge) as recommended (Dipicolinic Acid Diatabs; Rosco Diagnostika, Taastrup, Denmark). All 16 isolates tested were MβL positive by this method, producing synergy images (Fig. 1). To validate the specificity of the DPA-imipenem synergy method, eight *P. mirabilis* strains from the collection of the Hellenic Pasteur Institute were used as negative controls. Of these, two strains produced TEM-1 (imipenem MICs were 0.25 and 1 mg/liter), four lacked any acquired β-lactamase (imipenem MIC range of 0.25 to 2 mg/liter), and the remaining two, also lacking detectable β-lactamase activity, were mutants selected *in vitro* on imipenem (MICs of 4 and 8 mg/liter). All eight control isolates were consistently negative by the DPA-imipenem synergy method. We also tried to apply a combined disk test using imipenem disks (10 μg) each supplemented with 200 μg of DPA (Sigma-Aldrich, St. Louis, MO). Although a slight increase in the imipenem inhibition zone was observed for all but three VIM-1 producers, reproducibility was low and further standardization was not attempted.

Imipenem-DPA synergy has been effectively used for MβL-producing *Pseudomonas* spp. and *Acinetobacter* spp (4, 7). To our knowledge, this is the first successful application of this method for *P. mirabilis*. Yet, it must be pointed out that the isolates examined were clonally related, as indicated by PFGE using NotI for generating macrorestriction fragments (data not shown). Thus, the DPA-based method requires further validation using epidemiologically distinct MβL-positive strains.

Both EDTA and DPA are chelators with high inhibitory activity against MβLs. Therefore, we cannot provide a plausible explanation for the markedly higher sensitivity of the DPA-imipenem DDST. Differences in the interaction of these agents with *P. mirabilis* cells, other than MβL inhibition, could be hypothesized. Irrespective of the underlying mechanism, it seems that the use of DPA can facilitate detection of VIM-positive *P. mirabilis* isolates that appear falsely negative by the EDTA-based tests. Also, these data

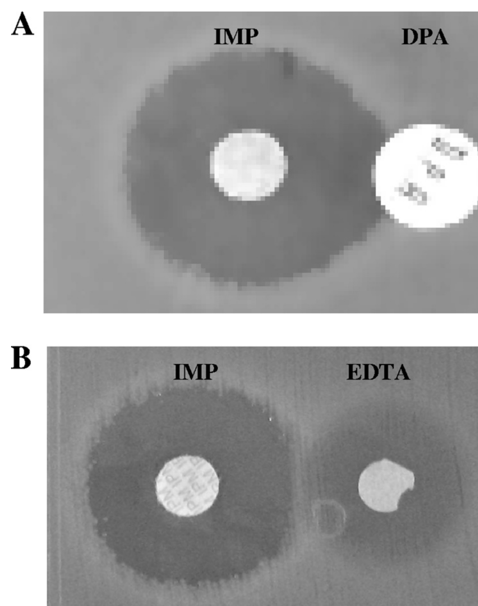

FIG. 1. (A) Positive synergy test using disks containing DPA (250 μg) and imipenem (IMP, 10 μg) for a *P. mirabilis* isolate producing VIM-1. (B) The same isolate appearing MβL negative by DDST using a disk containing EDTA (975 μg). Tests were performed with Mueller-Hinton agar and an inoculum of 5×10^5 cells/ml.

indicate an unnoticed spread of MβL-producing *P. mirabilis* that warrants investigation.

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