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Supplementation of Growth Media with Zn^{2+} Facilitates Detection of VIM-2-Producing *Pseudomonas aeruginosa*▽

Isolation rates of *Pseudomonas aeruginosa* producing zinc-dependent class B metallo-β-lactamases (MBLs) mainly of the VIM and IMP types are increasing worldwide (7). These enzymes exhibit wide hydrolysis spectra, including carbapenems, and are strongly inhibited by chelating agents such as EDTA. Based on the latter property, various MBL-detecting assays have been developed (1). EDTA-imipenem synergy tests are widely utilized in hospitals in Greece, where the incidence of VIM-positive *P. aeruginosa* is considered among the highest in Europe (www.rivm.nl/earss). However, observations in various hospitals in Athens and the reference laboratory of the National School of Public Health (NSPH) indicate suboptimal sensitivity of the EDTA-based methods. In this study, we attempted to evaluate the effect of zinc supplementation of the test medium on the performance of these methods.

Forty-three *P. aeruginosa* isolates submitted for testing in the NSPH from 13 hospitals during 2006 due to difficulties in interpreting the results of the EDTA-imipenem synergy methods were included in the study. MICs of β-lactams were determined by the Etest (AB Biodisk, Solna, Sweden). PCR assays for detection of MBL genes were performed as described previously (5, 6). The identities of MBL genes were confirmed by sequencing of the respective amplicons. Phenotypic detection of MBLs was performed in Mueller-Hinton agar (MHA) as well as in the same medium in which 70 mg/liter $ZnSO_4 \cdot 7H_2O$ had been incorporated (Zn^{2+} at a final concentration of 250 μM) as suggested by Lee et al. (4). Imipenem-EDTA synergy was assessed with the MBL-Etest (with a ≥8-fold decrease in the MIC of imipenem in the presence of EDTA considered a positive result) as well as two in-house techniques: the double-disk synergy test (DDST), using imipenem (10 μg) and EDTA disks (930 μg) in a 20-mm center-to-center distance, and the combination disk test (CDT), using an imipenem (10 μg) disk alone and containing 930 μg EDTA (with a ≥7-mm increase in inhibition zone considered a positive result) (3). The effects of the Zn^{2+} supplementation on the Etest MICs of imipenem and ceftazidime were also determined.

Twenty-seven (63%) of the 43 isolates carried bla_{VIM-2} (group A). The remaining 16 isolates (37%) were negative for MBL genes (group B). Imipenem MICs for group A isolates ranged from 1 to >32 μg/ml. The respective range for group B isolates was 4 to 32 μg/ml. Imipenem MICs were in good agreement with those reported by the hospital laboratories. Sensitivity problems (false negatives) were noticed with all three EDTA-based methods employed. The higher sensitivity score was observed with DDST followed by MBL-Etest and CDT. Also four of the group B isolates appeared false positive (Table 1), producing slight although reproducible synergy images. Imipenem MICs of the false-positive isolates ranged from 8 to 32 μg/ml.

Incorporation of Zn^{2+} in the growth medium resulted in a significant increase in the sensitivity of all three MBL detection methods without compromising specificity. More specifically, in Zn^{2+}-supplemented MHA, the MBL-Etest and CDT correctly identified 27 (sensitivity 100%) and 26 (sensitivity 96%) group A isolates, respectively, while performance of the conventional testing techniques was poor. Likewise, zinc supplementation increased the number of group A isolates characterized as MBL positive by the DDST from 12 to 18, thus improving sensitivity from 44 to 67% (Table 1). A plausible explanation for the positive effect of Zn^{2+} on MBL detection in *P. aeruginosa* is that Zn^{2+} may facilitate formation of functional MBL molecules in the periplasmic space. Also, the relatively high Zn^{2+} concentrations during growth reduce expression of *P. aeruginosa* porins and consequently carbapenem diffusion rates (2), further enhancing the effects of carbapenemase activity. This explanation is compatible with the increase in the apparent resistance levels to imipenem and ceftazidime that was more pronounced among VIM-2 producers (Table 1).

TABLE 1. Properties of 43 *P. aeruginosa* isolates

Isolate type (*n*)[a]	MIC range (μg/ml)		No. of isolates with positive result				
						MBL	
	Imipenem	Ceftazidime	MBL-Etest	DDST	CDT	All 3 assays	At least 1 assay
Group A (27)							
MHA	1–>32	4–256	4	12	3	2	15
MHA + Zn^{2+}	>32	32–>256	27	18	26	18	27
Group B (16)							
MHA	4–32	4–>256	1	1	2	0	4
MHA + Zn^{2+}	4–>32	8–>256	1	1	2	0	3

[a] Group A is VIM-2 positive, and group B is MBL negative.

Twenty-seven of the 43 submitted *P. aeruginosa* isolates (15 group A and 12 group B) were readily and correctly characterized in the NSPH by at least one conventional EDTA-based phenotypic method, likely suggesting technical problems in the hospital laboratories. Nevertheless, in a number of isolates, MBL production was not apparent. Despite the limitations of this preliminary study (a relatively small number of VIM-producing isolates), our findings suggest that Zn^{2+} supplementation may be a useful adjunct for MBL detection in *P. aeruginosa* and warrants further investigation.

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REFERENCES

1. **Arakawa, Y., N. Shibata, K. Shibayama, H. Kurokawa, T. Yagi, H. Fujiwara, and M. Goto.** 2000. Convenient test for screening metallo-β-lactamase-producing gram-negative bacteria by using thiol compounds. J. Clin. Microbiol. **38:**40–43.
2. **Conejo, M. C., I. Garcia, L. Martinez-Martinez, L. Picabea, and A. Pascual.** 2003. Zinc eluted from siliconized latex urinary catheters decreases OprD expression, causing carbapenem resistance in *Pseudomonas aeruginosa*. Antimicrob. Agents Chemother. **47:**2313–2315.
3. **Gupta, V., P. Datta, and J. Chander.** 2006. Prevalance of metallo-β-lactamase (MBL) producing *Pseudomonas* spp. and *Acinetobacter* spp. in a tertiary care hospital in India. J. Infect. **52:**311–314.
4. **Lee, K., Y. S. Lim, D. Yong, J. H. Yum, and Y. Chong.** 2003. Evaluation of the Hodge test and the imipenem-EDTA double-disk synergy test for differentiating metallo-β-lactamase-producing isolates of *Pseudomonas* spp. and *Acinetobacter* spp. J. Clin. Microbiol. **41:**4623–4629.
5. **Poirel, L., T. Naas, D. Nicolas, L. Collet, S. Bellais, J.-D Cavallo, and P. Nordman.** 2000. Characterization of VIM-2, a carbapenem-hydrolyzing metallo-β-lactamase and its plasmid- and integron-borne gene from a *Pseudo-*

monas aeruginosa clinical isolate in France. Antimicrob. Agents Chemother. **44:**891–897.

6. **Senda, K., Y. Arakawa, S. Ichiyama, K. Nakashima, H. Ito, S. Ohsuka, K. Shimokata, N. Kato, and M. Ohta.** 1996. PCR detection of metallo-β-lactamase gene (bla_{IMP}) in gram-negative rods resistant to broad-spectrum β-lactams. J. Clin. Microbiol. **34:**2909–2913.
7. **Walsh, T. R., M. A. Toleman, L. Poirel, and P. Nordmann.** 2005. Metallo-β-lactamases: the quiet before the storm? Clin. Microbiol. Rev. **18:**306–325.

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