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Discrepancies and Interpretation Problems in Susceptibility Testing of VIM-1-Producing *Klebsiella pneumoniae* Isolates

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Susceptibilities to β-lactam antibiotics of five VIM-1-producing *Klebsiella pneumoniae* isolates were determined by broth microdilution, Etest, disk diffusion, and the automated systems Vitek 2, Phoenix, and MicroScan. Significant discrepancies were observed in the determination of susceptibility to imipenem and meropenem. Interpretation problems by the automated systems were also noted.

VIM metallo-β-lactamases (MBLs) exhibit a wide spectrum of hydrolytic activity against β-lactam antibiotics, including carbapenems. The respective *bla* genes are located in the variable region of class 1 integrons. Spread of VIM-encoding integrons occurs mainly among *Pseudomonas aeruginosa* strains in southern Europe and the Far East (11). However, sporadic isolation of enterobacteria carrying bla_{VIM} genes has also been reported in these geographic areas (4, 5, 7, 12, 14, 15).

VIM-producing *Klebsiella pneumoniae* (VPKP) isolates caused outbreaks in Greek hospitals in 2002 (4). Despite restriction measures, VPKP isolates continue to emerge sporadically in the intensive care units of various hospitals in the Athens metropolitan area (unpublished data). A collection of VPKP isolates is thus being formed in the National School of Public Health. It has been frequently observed that confirmed bla_{VIM}-carrying isolates referred to as resistant or intermediate to imipenem were susceptible to carbapenems by the microdilution method used in the National School of Public Health according to the NCCLS breakpoints (9).

In this study, we applied various susceptibility testing methods (Etest, disk diffusion, Vitek 2, Phoenix, and MicroScan) to five VPKP isolates exhibiting decreased susceptibility to imipenem (MIC = 2 to 4 μg/ml) as determined by broth microdilution (BMD) used as the reference method. To avoid inclusion of repetitive isolates, selection was based on typing by pulsed-field gel electrophoresis (PFGE) and analysis of bla_{VIM-1}-carrying plasmids as described previously (4). bla_{VIM-1} carriage and VIM-1 production were confirmed by DNA sequencing and analytical isoelectric focusing, respectively. The selected isolates represented three PFGE types. VIM-1 production was mediated by two apparently different multiresistant plasmids. One isolate also produced a TEM-1 β-lactamase (Table 1).

The BMD method was performed as described by the NCCLS, using Mueller-Hinton broth and inocula equal to 5×10^5 CFU/ml (9). The disk diffusion method was performed as recommended by the NCCLS (10). The Etest method was applied following the instructions of the manufacturer (AB Biodisk). MBL-detecting Etest strips containing imipenem plus EDTA were also used. Isolates were also examined by three automated systems: (i) Vitek 2 using the AST-N017 and AST-EXN2 susceptibility cards (bioMerieux), (ii) Phoenix with the NMIC/ID-12 panel (Becton Dickinson), and (iii) MicroScan (AUTOSCAN-4) with the Neg MIC type 30 panel (Dade MicroScan) according to the manufacturers' instructions. Susceptibility experiments by all methods were repeated three times. In the case of automated systems, the isolates were also tested by certified technicians from the manufacturing companies. Quality control was performed with the imipenem-susceptible *Escherichia coli* strain ATCC 25922 and a KPC-2-producing, imipenem-resistant *E. coli* strain (pST4707) (8).

The results of the β-lactam susceptibility testing are summarized in Table 2. Isolates were found resistant to penicillins, penicillin-inhibitor combinations, cefoxitin, cefotaxime, ceftriaxone, and ceftazidime and susceptible to aztreonam by all methods. The Vitek 2 MIC of cefepime for one isolate was significantly lower than the respective MICs determined by the other methods (8 versus ≥32 μg/ml). In the BMD reference method as well as disk diffusion, all isolates were classified as susceptible to imipenem and meropenem. The Etest MICs of carbapenems were the same as those determined by BMD, except for one strain, which was classified as intermediate to imipenem (Etest MIC = 8 μg/ml), while the BMD MIC was 2 μg/ml. In the MBL Etest, a synergy image between imipenem

TABLE 1. Characteristics of VIM-1-producing *K. pneumoniae* clinical isolates exhibiting decreased susceptibility to carbapenems

Isolate	PFGE type[a]	Plasmid type[a]	Acquired β-lactamases	Resistance to non-β-lactams[b]
VPKP1	A	1	VIM-1	Gm^r Net^r Tb^r An^r Sul^r Tp^r Cm^r
VPKP2	B	1	VIM-1	Gm^r Net^r Tb^r An^r Sul^r Tp^r Cm^r
VPKP3	B	2	VIM-1	Net^r Tb^r An^r Sul^r Tp^r
VPKP4	C	1	VIM-1, TEM-1	Gm^r Net^r Tb^r An^r Sul^r Tp^r Cm^r
VPKP5	C	2	VIM-1	Net^r Tb^r An^r Sul^r Tp^r

[a] PFGE types (A, B, and C) and VIM-encoding plasmid types (1 and 2) of the VPKP isolates have been defined previously (4).
[b] Gm, gentamicin; Net, netilmicin; Tb, tobramycin; An, amikacin; Sul, sulfonamides; Tp, trimethoprim; Cm, chloramphenicol.

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TABLE 2. Susceptibilities to selected β-lactam antibiotics of VIM-1-producing *K. pneumoniae* clinical isolates as determined by various methods

Antibiotic	MIC range (μg/ml)					Zone diam mm in (DDT)
	BMD	Etest	Vitek 2	Phoenix	MicroScan	
Amoxicillin-clavulanate	≥64/32	≥128/64	≥32/16	≥32/16	≥32/16	<10
Ticarcillin-clavulanate	≥512/2	≥256/2	≥128/4		≥128/4	<10
Piperacillin	≥512	≥256	≥128	≥128	≥128	<10
Piperacillin-tazobactam	128/4–≥256/4	128/4–≥256/4	≥128/4	≥128/4	≥128/4	<10
Cefoxitin	≥512	≥256	≥64	≥32	≥32	<10
Cefotaxime	128–≥256	64–256	≥64	≥64	≥64	<10
Ceftriaxone	64–≥256	32–256	≥64		32–≥64	<10–14
Ceftazidime	256–≥512	128–≥256	≥64	≥32	≥32	<10
Cefepime	32–≥256	≥32	8–≥64	≥32	≥32	<10–15
Aztreonam	0.125–0.25	0.125–0.5	≤1	≤2	≤8	30–34
Imipenem	2–4	2–8	8–≥16	≥16	≤4	18–20
Meropenem	1–4	1–4	1–2	≥16	≤4	18–20

and EDTA, occurring as a "phantom zone" due to the low MICs of the antibiotic, was observed in all five isolates.

Major discrepancies changing the classification of the isolates from susceptible to resistant to carbapenems were observed with two of the automated systems, Vitek 2 and Phoenix. By Vitek 2, four isolates were consistently characterized as resistant to imipenem, the MICs for them being ≥16 μg/ml, and the remaining isolate was characterized as intermediate (MIC = 8 μg/ml). On the other hand, the Vitek 2 MICs of meropenem corresponded to those of the BMD method, ranging from 1 to 2 μg/ml. With the Phoenix system, all five isolates were found resistant to both imipenem and meropenem (MICs of ≥16 μg/ml). Carbapenem susceptibility data produced by the MicroScan were in agreement with those of the BMD method. Readings performed either by visual inspection of the panels or with the instrument of the latter system consistently indicated that the imipenem and meropenem MICs were ≤4 μg/ml.

Interpretations and suggestions given by the automated systems were also recorded. The advanced expert system (AES) of Vitek 2 (software version VTK-R01.02) interpreted the AST-N017 data (include imipenem but not meropenem and aztreonam) as fully consistent with the organism identification and did not suggest any corrections. However, considering the AST-EXN2 data (include meropenem and aztreonam but not imipenem), either alone or combined with AST-N017, the AES suggested either retesting or changing the MIC of meropenem (from 2 to 0.5 μg/ml) and the interpretation of the MIC of aztreonam (from susceptible to intermediate). In Phoenix, the relevant BDExpert-triggered rules (codes) were 1513 (suggesting confirmation of resistance to carbapenems and, if confirmed, consideration of the isolate as resistant to all β-lactams) and 106 (recommending testing for extended-spectrum β-lactamases). Extended-spectrum β-lactamase production was also indicated by MicroScan.

VPKP isolates frequently appear as susceptible to carbapenems. As shown here, the discrepancies between testing methods pose additional difficulties in the reliable identification of these isolates. Nevertheless, the BMD and Etest MICs as well as the disk inhibition zones of carbapenems were unusual for carbapenem-susceptible *K. pneumoniae*. Additionally, the isolates exhibited a characteristic resistance pattern compatible with the substrate specificity of VIM-1 β-lactamase: i.e., high activity against all β-lactams except aztreonam and resistance to β-lactamase inhibitors (3). These phenotypic traits may facilitate identification of VPKP. Also, application of the imipenem-EDTA Etest may be useful, although a strip containing lower concentrations of imipenem would be preferable. The efficacy of carbapenems in infections caused by VPKP has not been evaluated. It must be noted, however, that the BMD MICs of imipenem and meropenem increased to 16 to 32 μg/ml when an inoculum of 10^7 CFU/ml was used (data not shown). This marked "inoculum effect" is in line with the hypothesis that carbapemens may not be suitable for the treatment of the respective infections. Also, it may partly explain overdetection of resistance to imipenem in VPKP in the clinical laboratories.

The VPKP isolates were consistently classified by Vitek 2 using the AST-N017 card and Phoenix as carbapenem resistant. Overdetection of carbapenem resistance by automated systems has been attributed to errors such as high inocula, improper interpretation of the results, and antibiotic degradation (1, 2, 6, 13). Yet, the increased carbapenem MICs observed here may reflect a detectable bacterial growth at 8 μg of the antibiotic per ml. This issue was not examined further. As expected, none of the automated systems could correctly interpret the observed phenotypes. Consequently, all systems suggested unnecessary confirmatory tests. It must also be noted that the proposed change by the AES of Vitek 2 regarding meropenem is probably inappropriate. These findings may have implications for the management of infections caused by VPKP.

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