JOURNAL OF CLINICAL MICROBIOLOGY, Feb. 2005, p. 942–944 Vol. 43, No. 2
0095-1137/05/$08.00+0 doi:10.1128/JCM.43.2.942–944.2005

Evaluation of Etest MBL for Detection of *bla*$_{IMP-1}$ and *bla*$_{VIM-2}$ Allele-Positive Clinical Isolates of *Pseudomonas* spp. and *Acinetobacter* spp.

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Received 11 June 2004/Returned for modification 18 July 2004/Accepted 7 October 2004

The Etest MBL (AB BIODISK, Solna, Sweden) correctly differentiated all 57 isolates of *Acinetobacter* spp. and *Pseudomonas aeruginosa* with the *bla*$_{IMP-1}$ allele and 135 of 137 (98.5%) *Acinetobacter* spp. and *Pseudomonas* spp. isolates with the *bla*$_{VIM-2}$ allele. The Etest MBL was reliable for detecting the IMP-1- and VIM-2-producing *Pseudomonas* and *Acinetobacter* isolates.

The IMP- and VIM-type and other acquired class B metallo-β-lactamase (MBL)-producing gram-negative bacilli have been increasingly isolated from clinical specimens (10, 15). Among the imipenem-nonsusceptible isolates, 62.1% of the 58 *Pseudomonas aeruginosa* isolates had a VIM-type MBL in a Greek study (3), while 11.4% of 387 *Pseudomonas* spp. isolates and 14.2% of 267 *Acinetobacter* spp. isolates had either an IMP- or VIM-type MBL in a Korean study (7).

MBL can hydrolyze β-lactams from all classes except the monobactams (1). Higher mortality has been reported in patients infected with the IMP-1-producing strains (5). Therefore, the reliable detection of the MBL-producing strains is essential for the optimal treatment of infected patients and to control the nosocomial spread of resistance. However, the current NCCLS document (12) does not contain any method for detecting MBL.

MBL-producing strains can be detected with double-disk synergy tests (8, 13) and a microdilution method (11). While the former method is only qualitative, the latter is not simple for routine testing. Walsh et al. (16) reported that the Etest MBL strip (AB BIODISK, Solna, Sweden) had 100% sensitivity and specificity, but their findings were based on testing only 12 IMP-1-type strains and did not include the prevalent VIM-2-type strains. The performance of Etest MBL was influenced by the choice of the Mueller-Hinton agar brand (16) but may have also been influenced by the level of imipenem resistance of the MBL-producing strains (17, 18) and possibly by other testing conditions.

In this study, the performance of Etest MBL was evaluated with 199 *bla*$_{VIM-2}$ or *bla*$_{IMP-1}$ allele-positive isolates, and the factors that may influence the routine use of the test were also investigated.

(Part of this study was reported previously [A. Bolmström, A. Engelhardt, P. Ho, Y. Chong, and K. Lee., Abstr. 42nd Intersci. Conf. Antimicrob. Agents Chemother., abstr. D-532, 2002].)

Strains. The strains were isolated from the clinical specimens at Korean hospitals between 1995 and 2003, and imipenem susceptibility was tested with the disk diffusion method (12) using 10-μg disks and Mueller-Hinton agar (Becton Dickinson, Cockeysville, Md.). MBL screening was tested with the Hodge test and a double-disk synergy test (6, 8). The *bla*$_{IMP-1}$ and *bla*$_{VIM-2}$ alleles were detected by PCR (9). The isolates, which were imipenem resistant but MBL negative by the screening tests and PCR, were used as the negative controls. The test strains were stored at −76°C until used for the study.

Evaluation of Etest MBL. The evaluations were performed at two laboratories, one in Korea and the other in Sweden. The Swedish laboratory used the strains that were transported in semisolid cystine trypticase agar (Becton Dickinson) vials at ambient temperature. Several colonies from a 24-h culture plate were used to prepare the inoculum with a 0.5 McFarland standard density. Mueller-Hinton agar plates (Becton Dickinson), the recommended medium (16), were streaked by using cotton swabs (Korea) or a spiral inoculator (Sweden). The Etest MBL strips were then applied, and the plates were incubated at 35°C in air for 16 to 20 h. A ratio of the MICs of the imipenem (IP) to IP plus EDTA (IPI) of ≥8 or the presence of a phantom zone, i.e., an extra inhibition zone between the IP and IPI regions, or a deformation of the IP or IPI ellipses was interpreted as being positive for MBL production. The sensitivity and specificity of the Etest MBL were calculated based on the recommendations by Elder et al. (2).

Performance of Etest MBL. The Etest MBL detected all 57 *bla*$_{IMP-1}$ allele-positive isolates of *Acinetobacter* spp. and *P. aeruginosa* and 140 of 142 (98.6%) *bla*$_{VIM-2}$ allele-positive isolates of *Acinetobacter* spp., *Pseudomonas* spp., and *Enterobacteriaceae* (Table 1). The test correctly differentiated 71 of the 73 (97.3%) imipenem-resistant but non-MBL-producing isolates.

The Etest MBL strip was reported to be an acceptable diagnostic reagent for detecting the MBL-producing isolates (16). The test was successfully used to screen the VIM-1-producing *Klebsiella pneumoniae* strains for which imipenem

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TABLE 1. Accuracy of Etest MBL for differentiating MBL-producing isolates

Allele of MBL gene	Organism (no. of isolates)	No. (%) of isolates with Etest result that was:		Sensitivity (%)	Specificity (%)
		Positive	Negative		
bla_{IMP-1}	*Acinetobacter* spp. (56)	56 (100)	0 (0)	100	NA
	P. aeruginosa (1)	1 (100)	0 (0)	100	NA
bla_{VIM-2}	*Acinetobacter* spp. (21)	20 (95.2)	1 (4.8)	95.2	NA
	P. aeruginosa (102)	101 (99.0)	1 (1.0)	99.0	NA
	P. putida (14)	14 (100)	0 (0)	100	NA
	Enterobacteriaceae[a] (5)	5 (100)	0 (0)	100	NA
	Total (199)	197 (99.0)	2 (1.0)	99.0	NA
None[b]	*Acinetobacter* spp. (44)	1 (2.3)	43 (97.7)	NA[c]	97.7
	P. aeruginosa (23)	1 (4.3)	22 (95.7)	NA	95.7
	Enterobacteriaceae (6)	0 (0)	6 (100)	NA	100
	Total (73)	2 (2.7)	71 (97.3)	NA	97.3

[a] One each of *K. pneumoniae* and *E. cloacae* isolates and three *Serratia marcescens* isolates.
[b] Imipenem-resistant isolates. An *Acinetobacter* isolate had the OXA-23 gene. The numbers of *Enterobacteriaceae* isolates were five *K. pneumoniae* isolates and one *S. marcescens* isolate.
[c] NA, not applicable.

MICs were 4 to 32 μg/ml (4). In our study, one isolate of *Enterobacter cloacae* for which the imipenem MIC was relatively low (8 μg/ml) was Etest MBL positive, but the test became negative with a 10-fold reduction in the inoculum (data not shown). Although an imipenem MIC of 0.5 μg/ml could be estimated on the IPI side, the strip needs to be modified in order to detect the MBL-producing strains for which the imipenem MICs were <4 μg/ml (Fig. 1A).

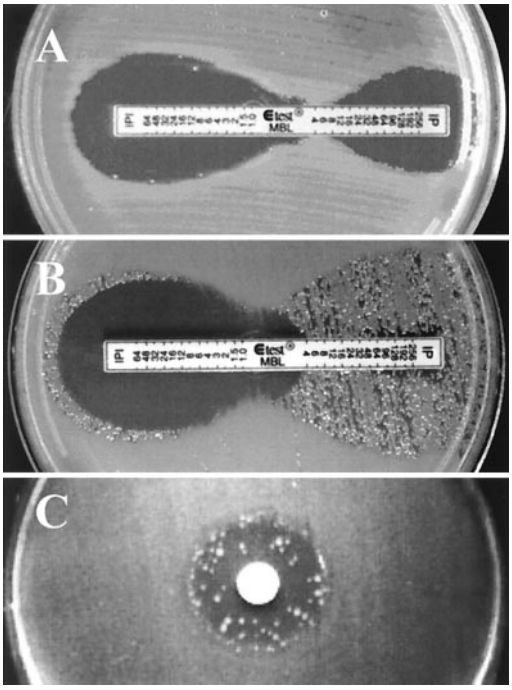

FIG. 1. (A) An MBL-producing isolate for which the imipenem MIC was 8 μg/ml could be detected because the IPI side has the lowest imipenem MIC limit of 1 μg/ml. However, an improvement of the strip is required to detect the MBL-producing strains for which imipenem MICs are ≤4 μg/ml. (B) An MBL-producing strain with a clear inhibition ellipse for IPI and a discernible ellipse for IP alone with inner colonies suggesting the presence of imipenem-susceptible and -resistant cells. (C) A strain retaining only a small proportion of the MBL gene-carrying cells. This type of appearance could be mistakenly attributed to the strain being contaminated.

Another important factor was found to influence the performance of the Etest MBL. Some strains lost the bla_{IMP-1} or bla_{VIM-2} genes partially or completely at ambient temperature: 29 MBL gene-positive isolates of *P. aeruginosa* and *Acinetobacter* spp. for which imipenem MICs were 16 to 256 μg/ml at the Korean laboratory gave negative Etest MBL results and lower imipenem MICs (<4 to 8 μg/ml) at the Swedish laboratory (data not shown). Some of the strains showed imipenem ellipses with either inner colonies (Fig. 1B) or a few resistant colonies in the inhibition zone of the disk diffusion test (Fig. 1C). Retesting of these strains using fresh subcultures from frozen stock gave the original higher imipenem MICs and positive Etest MBL results (data not shown). Takahashi et al. (14) observed a loss of bla_{IMP} genes in 19 of 28 *A. baumannii* isolates after storage in Casitone medium at room temperature for a year or more and considered that the gene could be retained only in the presence of imipenem. These results indicate the importance of the simultaneous testing of phenotypic MBL production and gene detection.

In conclusion, the Etest MBL is highly sensitive and specific for detecting bla_{IMP-1} and bla_{VIM-2} allele-positive isolates of *Pseudomonas* spp. and *Acinetobacter* spp. The strains kept at ambient temperature may give negative results when the Etest MBL is used due to the loss of MBL genes.

This work was supported in part by the Brain Korea 21 Project for Medical Sciences, Yonsei University, in 2004.

We thank Myung Sook Kim for collecting the isolates and Yonghee Suh and Chasoon Lee for screening the MBL-producing isolates.

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