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Prevalence of Metallo-β-lactamases in *Pseudomonas aeruginosa* and *Acinetobacter baumannii*

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Background: Metallo-β-lactamases (MBLs) have been reported in gram negative bacilli and are becoming increasingly important clinically because the enzymes hydrolyse almost all β-lactams, including carbapenems. Thus, the present study was conducted to determine the prevalence of MBL types in imipenem-nonsusceptible *Pseudomonas aeruginosa* and *Acinetobacter baumannii* isolated from a tertiary teaching hospital.
Methods: Imipenem-nonsusceptible strains, 128 *P. aeruginosa* and 93 *A. baumannii*, were collected from clinical specimens. Identification and susceptibility tests were determined by Vitek GNI and GNS cards. MBL production was determined by modified Hodge test and imipenem-EDTA synergy test. Multiplex PCR amplification of MBL genes including bla_{IMP-1}, bla_{VIM-1} and bla_{VIM-2} were performed.
Results: Thirty-one *P. aeruginosa* (24.2%) isolates and 3 *A. baumannii* (3.2%) were found to be MBL producers. In *P. aeruginosa*, 20 (15.6%) and 11 (8.6%) isolates were positive for bla_{IMP-1} and bla_{VIM-2}, respectively whereas 1 (1.0%) and 2 (2.2%) isolates in *A. baumannii*, respectively.
Conclusion: IMP-1 is more prevalent MBL type than VIM-2 among imipenem-nonsusceptible *P. aeruginosa* unlike in other studies. Larger numbers of isolates and sequential studies are strongly recommended for the useful evaluation and monitoring of MBL production in the hospital setting to infection-control. **(Korean J Clin Microbiol 2010;13:169-172)**

Key Words: Multidrug resistance, *Pseudomonas aeruginosa, Acinetobacter baumannii*, Metallo-beta-lactamase (MBL), IMP-1, VIM-2

INTRODUCTION

Carbapenem is the most active antimicrobial agent against both gram negative and positive organisms and is used for the treatment of nosocomial infections especially due to multidrug- resistant *Pseudomonas aeruginosa* and *Acinetobacter baumannii*[1]. However, its efficacy is increasingly being limited because carbapenem-resistant isolates are emerging in many regions of the world[2,3]. The mechanisms for resistance to carbapenems were known to be the combined action of the acquisition of β-lactamases that hydrolyze carbapenems, Ambler class B (metalloenzymes, MBLs) and class D (oxacillinases) enzymes, mutations in genes coding for penicillin-binding proteins and decreased outer membrane permeability or overexpressed efflux pumps[2]. Among class B enzymes IMP and VIM types are frequent MBLs whereas OXA-23, OXA-24 and OXA-58 enzymes are three major subgroups of class D lactamases[4,5].

In Korea, recent studies reported imipenem resistance rates among nonfermenters were ranged from 18% up to 40%[6,7]. Imiepnem resistance rapidly increased from less than 5% in 2002 to 35% in 2009 in our hospital and became a great concern to treat multidrug-resistant *P. aeruginosa* and *A. baumannii*. According to the studies of carbapenem resistance, carbapenemase production accounts the most[2,3]. IMP-1, VIM-2 and OXA-23 are the most frequently encountered enzymes among multidrug-resistant *P. aeruginosa* and *A. baumannii* with proportions varied within Korea[8]. Therefore, we conducted this study to evaluate the prevalence and types of MBLs among class B group resulting in carbapenem resistance in our hospital.

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MATERIALS AND METHODS

A total of 128 *P. aeruginosa* and 93 *A. baumannii* consecutive, non-duplicated and imipenem-nonsusceptible were collected from May 2004 to April 2006. Identification was determined by Vitek GNI card (bioMerieux, Marcy l'Etoile, France). Antimicrobial susceptibility test was done both with disk-diffusion and microdilution methods depends on the specimen types. Microdilution method was performed by Vitek GNS card. Carbapenemase and MBL productions were screened by modified Hodge test and imipenem-EDTA synergy test, respectively. A suspension of *Escherichia coli* ATCC 25922, which was adjusted to the turbidity of the McFarland No. 0.5 tube was inoculated evenly on a Mueller-Hinton agar (MHA) plate. Then, an imipenem disk (30 μg, BBL)

was placed at the center of the plate and test strains were streaked from the edge of the disk to the end of the plate. The presence of a distorted inhibition zone after an incubation at 35°C for 18 hours was interpreted as a positive modified Hodge test. The test strains suspended to the McFarland No. 0.5 were used to inoculate on a MHA plate. A 30 μg imipenem disk and a blank filter paper disk were placed 10 mm apart from edge to edge on MHA plate. Ten microliters of 0.5 M EDTA solution was applied to the blank disk and then plates were incubated at 35°C for 18 hours. The presence of an enlarged zone of inhibition was interpreted as EDTA-synergy test positive.

Multiplex PCR ampilification of MBL genes including bla_{IMP-1}, bla_{VIM-1}, and bla_{VIM-2} were performed[9,10](Table 1). The amplification conditions were: initial denaturation at 94°C for 5 minutes, 30 cycles of 94°C for 25 seconds, 52°C for 40 seconds, 72°C for 50 seconds, and a final elongation at 72°C for 7 minutes. Sequencing analysis was then perform to determine specific enzymes of MBLs as described previously[11] sending to the reference laboratory.

RESULTS

One hundred twenty-eight *P. aeruginosa* isolates were from sputum (58), urine (45), body fluid (9), wound (7), blood (3), catheter tip (3), and others (3). Ninety-three *A. baumannii* isolates were from sputum (79), urine (5), wound (4), body fluid (2), catheter tip (2) and throat (1).

Thirty-one isolates (24.2%) of 128 imipenem-nonsusceptible *P. aeruginosa* were found to be MBL producers. Eleven isolates (8.6%) were bla_{VIM-2} positive and 20 (15.6%) were bla_{IMP-1} positive (Table 2). Among 93 imipenem-nonsusceptible *A. baumannii*, 3 isolates (3.2%) were positive for modified Hodge and imipenem-EDTA synergy test. Gene of bla_{VIM-2} was positive in 2 *A. baumannii* isolates and only one was bla_{IMP-1} positive (Table 3). By DNA sequencing analysis of MBL producing strains in *P. aeruginosa,* 5 out of 20 bla_{IMP-1} like positive isolates were IMP-6 and the rest were IMP-1. Eleven of bla_{VIM-2}-like positive were VIM-2.

Table 1. Sequences of primers used in this study

Enzyme	Primer	Sequence (5'→3')	Size (bp)	Reference
Class B	IMP-1F	CTACCGCAGCAGAGTCTTTGC	578	
	IMP-1R	GAACAACCAGTTTTGCCTTACC		
	VIM-1F	TCTACATGACCGCGTCTGTC	748	10, 11
	VIM-1R	TGTGCTTTGACAACGTTCGC		
	VIM-2F	ATGTTCAAACTTTTGAGTAAG	801	
	VIM-2R	CTACTCAACGACTGAGCG		

Table 2. Characteristics of metallo-β-lactamase producing *P. aeruginosa*

Isolate	Type of specimen	MHT	IEST	bla_{IMP-1} like	bla_{VIM-2} like
DSH005	Urine	+	+	+	
DSH006	Urine	+	+	+	
DSH009	Bile	+	+	+	
DSH010	Sputum	+	+	+	
DSH012	Urine	+	+		+
DSH014	Sputum	+	+		+
DSH015	Sputum	+	+	+	
DSH020	Urine	+	+	+	
DSH025	Urine	+	+		+
DSH026	Blood	+	+	+	
DSH027	Sputum	+	+	+	
DSH033	Sputum	+	+	+	
DSH055	Urine	+	+		+
DSH060	Urine	+	+		+
DSH063	Urine	+	+	+	
DSH064	Wound	+	+		+
DSH068	Urine	+	+		+
DSH078	Urine	+	+		+
DSH083	Urine	+	+		+
DSH092	Urine	+	+	+	
DSH093	Urine	+	+	+	
DSH096	Urine	+	+	+	
DSH105	Urine	+	+	+	
DSH108	Urine	+	+	+	
DSH110	Urine	+	+	+	
DSH113	Urine	+	+		+
DSH115	Wound	+	+	+	
DSH122	Wound	+	+	+	
DSH126	Urine	+	+		+
DSH127	Sputum	+	+	+	
DSH128	Urine	+	+	+	

Abbreviations: MHT, modified Hodge test; IEST, imipenem-EDTA synergy test.

Table 3. Metallo-β-lactamase genes detected in imipenem-nonsusceptible *P. aeruginosa* and *A. baumannii* by PCR

Organism (No.)	No. of isolates with MHT positive	No. of isolates with IEST	No. of isolates with positive genes for			Total
			bla_{IMP-1}-like	bla_{IMP-6}*	bla_{vim-2}-like	
P. aeruginosa (128)	51	31	15	5	11	31 (24.2%)
A. baumannii (93)	79	3	1	0	2	3 (3.2%)
Total (221)	130	34	16	5	13	34 (15.4%)

*Identified and confirmed as bla_{IMP-6}[16].
Abbreviations: MHT, modified Hodge test; IEST, imipenem-EDTA synergy test.

All the bla_{IMP-1}-like and bla_{VIM-2}-like in *A. baumannii* were IMP-1 and VIM-2, respectively.

DISCUSSION

Carbapenems such as imipenem and meropenem generally represent last resources for the treatment of nosocomial infections produced by multidrug-resistant Gram-negative bacteria due to their broad antimicrobial activity spectrum and stability against most common β-lactamases[12]. However, emergence of resistance to these drugs becomes a threatening for the treatment of Gram-negative bacterial infection worldwide[4,10]. As the mechanisms of the carbapenem resistance have been studied, they are due to specific reductions in outer membrane permeability, efflux pump, alteration of penicillin-binding proteins and also presence of carbapenem-hydrolysing enzymes[5]. Production of carbapenemases, especially MBLs, is becoming a leading cause of the resistance and considered to be more important than other mechanisms due to the horizontal spread of plasmids[2,3].

Ambler class B carbapenemases, MBLs, such as IMP, VIM and SIM are frequently detected enzyme types in imiepnem-resistant *P. aeruginosa* and *A. baumannii* in Korea[12-14]. For class B carbapenem-hydrolysing enzymes, IMP-1 is found out to be more prevalent MBL type than VIM-2 type among imipenem-nonsusceptible *P. aeruginosa* in our study. The results were consistent with other reports of Daegu and Busan, near city in Korea[14,15]. We were able to find an outbreak of IMP-6 positive *P. aeruginosa* retrospectively during this study and reported for the first time in Korea[16]. However, VIM-2 type was the most frequently detected in other regions of Korea[8,17,18]. Only three isolates of *A. baumannii* had MBLs, 2 for VIM-2 and 1 for IMP-1 in this study. Considering that the isolates of imipenem-resistant *A. baumannii* have increased abruptly in our hospital since 2003, carbapenemases other than MBLs and or other resistant mechanisms could be present and further affect the prevalence of carbapenem nonsusceptibility. It was very regretful that we could not perform any epidemiological studies to determine the clonality of the imipenem-nonsusceptible isolates. Therefore, our infection control committee recently started to surveillance the prevalence and outbreaks since then and to collect the isolates to perform the moleculoepidemiological studies, if possible.

Early recognition of carbapenemase producers among pathogenic isolates and a preliminary characterization of the type of enzyme produced are considered to an essential step for infection control in the hospital. Guiding the use of antibiotics that may favor the spread of carbapenemase producing bacteria also plays an important role in infection-control measures[4]. We give attention to increased prevalence of any multidrug-resistant organisms and also to outbreaks, especially in intensive care units. Larger numbers of isolates including closer districts within Daegu and sequential studies for annual prevalence are strongly recommended for the useful evaluation and monitoring of carbapenemase producing Gram-negative bacteria.

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=국문초록=

*Pseudomonas aeruginosa*와 *Acinetobacter baumannii*에서의 Metallo-β-lactamase의 유병률

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배경: 그람음성막대균에서의 metallo-β-lactamase에 대한 연구가 지속되고 있으며 카바페넴을 포함한 대부분의 β-lactam 제제를 가수분해하므로 임상적 중요성이 점차 증가되고 있다. 본 연구는 일개 대학병원에서 분리된 이미페넴에 비감수성 *Pseudomonas aeruginosa*와 *Acinetobacter baumannii*를 대상으로 metallo-β-lactamase의 유병률을 조사하였다.

방법: 임상검체에서 분리된 이미페넴 비감수성인 128주의 *P. aeruginosa*와 93주의 *A. baumannii*를 대상으로 Vitek GNI와 GNS 카드를 이용하여 균의 동정과 감수성검사를 실시하였다. Hodge 변법과 imipenem-EDTA synergy 검사를 이용하여 metallo-β-lactamase의 생성유무를 확인하였다. bla_{IMP-1}, bla_{VIM-1} 그리고 bla_{VIM-2} 유전형검사는 다중분획중합효소연쇄반응법을 이용하였다

결과: 총 31주의 *P. aeruginosa* (24.2%)와 3 *A. baumannii* (3.2%)에서 metallo-β-lactamase를 생성하였다. *P. aeruginosa* 31주 중 20 (15.6%)주에서 bla_{IMP-1}를 11 (8.6%)주에서는 bla_{VIM-2}를 생성하였으며 *A. baumannii*에서는 1 (1.0%)주에서 bla_{IMP-1}를, 2 (2.2%)주에서 bla_{VIM-2}를 생성하였다.

결론: 본 연구결과, 다른 연구와는 달리 이미페넴 비감수성 *P. aeruginosa*에서 IMP-1이 VIM-2유전자보다 더 흔한 것으로 나타났다. 병원 내 감염관리와 metallo-β-lactamase의 유전형의 지역적 역학분석을 위하여 보다 많은 균주를 대상으로 지속적인 연구가 필요할 것으로 생각한다. **[대한임상미생물학회지 2010;13:169-172]**

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