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Detection of Carbapenemases in Clinical *Enterobacteriaceae* Isolates Using the VITEK AST-N202 Card

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Background: The rapid and accurate detection of carbapenemase-producing *Enterobacteriaceae* (CPE) in clinical microbiology laboratories is essential for the treatment and control of infections caused by these microorganisms. This study was performed to evaluate the ability of the VITEK AST-N202 card to detect CPE isolates.

Materials and Methods: A total of 43 (*Klebsiella pneumoniae*, n = 37; *Escherichia coli*, n = 3; and *Enterobacter cloacae*, n = 3) CPE isolates and 79 carbapenemase-non-producing *Enterobacteriaceae* (CNE) isolates were included in this study. The CPE isolates harbored KPC-2 (n = 11), KPC-3 (n = 20), GES-5 (n = 5), VIM-2 (n = 2), IMP-1 (n = 1), NDM-1 (n = 2), or OXA-232 (n = 2). Of the 79 CNE isolates, eight *K. pneumoniae* isolates were resistant to ertapenem, imipenem, and meropenem, while the remaining 71 isolates were susceptible to the carbapenems. Antimicrobial susceptibilities were tested using the VITEK AST-N202 card, and the results were interpreted as positive when the isolates showed resistant or intermediate results. Modified-Hodge tests (MHTs) were performed using ertapenem or meropenem disks for the screening of carbapenemase production. Polymerase chain reaction (PCR) and direct sequencing were used to identify β-lactamase genes.

Results: Sensitivity of MHT with ertapenem and meropenem disks for the detection of carbapenemase was 81.4% (35/43) and 81.4% (35/43), respectively, and a combination with both antibiotic disks increased the sensitivity to 88.4% (38/43). Specificity of the MHT was 100% (79/79) for the CNE isolates. Sensitivity of ertapenem, imipenem, and meropenem as assessed by the VITEK AST-N202 card was 100% (43/43), 93% (40/43), and 95.3% (41/43), respectively. Specificity (89.8%, 71/79) of the test with each carbapenem was improved to 100% (71/71) when eight carbapenem-resistant CNE isolates were excluded from the testing.

Conclusion: The VITEK AST-N202 card showed high sensitivity for the detection of carbapenemases in *Enterobacteriaceae* strains. PCR and sequencing experiments for the detection of carbapenemases are recommended when clinical *Enterobacteriaceae* isolates show non-susceptibility to carbapenems.

Key Words: Carbapenemase-producing *Enterobacteriaceae*; VITEK AST-N202 card; KPC; GES; NDM

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Introduction

Carbapenems have been used as the drug of choice for the treatment of infections caused by multi-drug resistant Gram-negative rods because these drugs easily permeate the porins of outer cellular membranes, exhibit high affinity with penicillin-binding proteins, and are stable against various β-lactamases produced by Gram-negative rods [1-4]. However, carbapenem-resistant *Enterobacteriaceae* (CRE) has appeared as a consequence of the frequent use of these drugs for the treatment of widespread extended-spectrum β-lactamase- and/or AmpC β-lactamase-producing *Enterobacteriaceae* [5]. Dissemination of CRE is considered a serious clinical threat because available antimicrobials for the treatment of infections caused by CRE are very limited.

Although *Enterobacteriaceae* can acquire carbapenem resistance via various mechanisms, the most important one is the production of plasmid-mediated carbapenemases [5]. Diverse types of carbapenemases have appeared in *Enterobacteriaceae*, including 1) KPC- and GES-type enzymes belonging to class A, 2) IMP-, VIM-, and NDM-type metallo-β-lactamases (MBLs) belonging to class B, and 3) OXA-48 and its variants belonging to class D. *Enterobacteriaceae* strains producing these various carbapenemases have already appeared in Korea [6].

The rapid and accurate detection of carbapenemase-producing *Enterobacteriaceae* (CPE) in clinical laboratories is essential for the treatment of infections and infection control. However, the identification of CPE strains can be difficult because some CPE clinical isolates exhibit low-level resistance or susceptibility to carbapenems [7]. This study was performed to evaluate the ability of VITEK AST-N202 cards (bioMérieux, Marcy l'Etoile, France) to reliably detect CPE strains isolated from a clinical setting.

Materials and Methods

1. Bacterial strains and susceptibility testing

A total of 122 *Enterobacteriaceae* clinical isolates, 43 CPE and 79 carbapenemase-non-producing *Enterobacteriaceae* (CNE), were included in this study (Table 1). The CPE clinical isolates were identified as follows: 37 *Klebsiella pneumoniae*, three *Escherichia coli*, and three *Enterobacter cloacae*. The CPE clinical isolates produced KPC-2 (n = 11), KPC-3 (n = 20), GES-5 (n = 5), VIM-2 (n = 2), IMP-1 (n = 1), NDM-1 (n = 2), and OXA-232 (n = 2) carbapenemases. Of the 79 CNE clinical isolates, eight *K. pneumoniae* isolates were resistant to ertapenem, imipenem, and meropenem, while the remaining 71 isolates were susceptible to these carbapenems; *E. coli* (n = 35), *K. pneumoniae* (n = 17), *Klebsiella oxytoca* (n = 1), *E. cloacae* (n = 1), *Enterobacter aerogenes* (n = 6), *Enterobacter asburiae* (n = 2), *Serratia marcescens* (n = 4), *Citrobacter freundii* (n = 1), *Citrobacter koseri* (n = 1), *Morganella morganii* (n = 2), and *Proteus mirabilis* (n = 1). Bacterial species were identified using matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS) (Bruker Daltonics, Bremen, Germany) and by analysis of partial 16S rDNA sequences. Antimicrobial susceptibilities of the clinical isolates were determined using VITEK AST-N202 cards (bioMérieux, Table 2). Genes encoding β-lactamases, including TEM-, SHV-, CTX-M-, GES-, and KPC-types of class A; IMP-, VIM-, and NDM-types

Table 1. Characteristics of carbapenem-resistant *Enterobacteriaceae*

Strain	β-Lactamase		Modified-Hodge test		Antimicrobial susceptibility[a]		
	Carbapenemase	Others	Ertapenem	Meropenem	Ertapenem	Imipenem	Meropenem
Klebsiella pneumoniae	KPC-2	SHV-12, SHV-1, TEM-1	+	+	R	R	R
	KPC-2	SHV-12, SHV-1, TEM-1	+	+	R	R	R
	KPC-2	SHV-12, SHV-1, TEM-1	+	+	R	R	R
	KPC-2	SHV-12, SHV-1, TEM-1	+	+	R	R	R
	KPC-2	SHV-12, SHV-1, TEM-1	+	+	R	R	R
	KPC-2	SHV-12, SHV-11	+	-	R	R	R
	KPC-2	SHV-11, TEM-1	+	+	R	R	R
	KPC-2	TEM-1	+	+	R	R	R
	KPC-2		-	+	R	R	R
	KPC-2		+	+	R	R	R
	KPC-2		+	-	R	R	R

Table 1. Continued

Strain	β-Lactamase		Modified-Hodge test		Antimicrobial susceptibility[a]		
	Carbapenemase	Others	Ertapenem	Meropenem	Ertapenem	Imipenem	Meropenem
Klebsiella pneumoniae	KPC-3	SHV-12, TEM-1, CMY-2	+	+	R	R	R
	KPC-3	SHV-12, SHV-1, TEM-1	+	+	R	R	R
	KPC-3	SHV-12, SHV-1, TEM-1	+	+	R	R	R
	KPC-3	SHV-12, SHV-1, TEM-1	+	+	R	R	R
	KPC-3	SHV-12, SHV-1, TEM-1	+	-	R	R	R
	KPC-3	SHV-12, SHV-1, TEM-1	+	+	R	R	R
	KPC-3	SHV-12, SHV-1, TEM-1	+	+	R	R	R
	KPC-3	SHV-12, SHV-1, TEM-1	+	+	R	R	R
	KPC-3	SHV-12, SHV-1, TEM-1	+	+	R	R	R
	KPC-3	SHV-12, SHV-1, TEM-1	+	+	R	R	R
	KPC-3	SHV-12, SHV-1, TEM-1	+	+	R	R	R
	KPC-3	SHV-12, SHV-1, TEM-1	+	+	R	R	R
	KPC-3	SHV-12, SHV-1, TEM-1	+	+	R	R	R
	KPC-3	SHV-12, SHV-1, TEM-1	+	+	R	R	R
	KPC-3	SHV-12, SHV-1, TEM-1	+	+	R	R	R
	KPC-3	TEM-1	+	+	R	R	R
	KPC-3		+	+	R	R	R
	KPC-3		+	+	R	R	R
	KPC-3		+	+	R	R	R
	KPC-3		+	+	R	R	R
	GES-5	SHV-12	+	+	R	R	R
	GES-5	SHV-12	+	+	R	R	R
	GES-5	SHV-12	-	+	R	I	R
	GES-5	SHV-12, CMY-2	-	-	R	I	R
	NDM-1		-	+	R	R	R
	NDM-1		+	+	R	R	R
Escherichia coli	GES-5		-	-	R	S	S
	OXA-232		+	+	R	S	R
	OXA-232		-	-	R	S	S
Enterobacter cloacae	VIM-2		-	-	R	R	R
	VIM-2		-	-	R	R	R
	IMP-1		+	+	R	R	R
Klebsiella pneumoniae		CTX-M-3, SHV-12, DHA-1, TEM-1	-	-	R	R	R
		CTX-M-14, SHV-2a, DHA-1	-	-	R	R	R
		CTX-M-14, SHV-12, DHA-1	-	-	R	R	R
		CTX-M-15, DHA-1	-	-	R	R	R
		SHV-12, DHA-1	-	-	R	R	R
		DHA-1	-	-	R	R	R
		DHA-1	-	-	R	R	R
		SHV-1, CMY-1	-	-	R	R	R

[a]Antimicrobial susceptibility was tested using the VITEK AST-N202 card.

Table 2. Carbapenem disk contents of the VITEK AST-N202 card

Carbapenem disk	Indication MIC (μg/mL)	MIC interpretive criteria (μg/mL)		FDA indication for use
		Susceptible	Resistance	
Ertapenem	0.5, 1, 6	0.5	8	*E. coli*, *K. pneumonia*, *Citrobacter freundii*, *Citrobacter koseri*, *Enterobacter aerogenes*, *E. cloacae*, Kox[a] (-ESBL), *Morganella morganii*, *Proteus mirabilis*, *Proteus vulgaris*, *S. marcescens*
Imipenem	1, 2, 6, 12	0.25	16	*Acinetobacter* spp., *Citrobacter* spp., *Enterobacter* spp., *E. coli*, *Klebsiella* spp., *M. morganii*, *P. vulgaris*, *Providencia rettgeri*, *P. aeruginosa*, *S. marcescens*, *Providencia stuartii*
Meropenem	0.5, 2, 6, 12	0.25	16	*E. coli*, *K. pneumoniae*, *P. aeruginosa*, *P. mirabilis*, *Acinetobacter* spp., *C. freundii*, *E. cloacae*, *K. oxytoca*, *M. morganii*, *P. vulgaris*, *S. marcescens*, *Aeromonas hydrophila*, *Citrobacter diversus*, *Hafnia alvei*, *Pasteurella multocida*, *Salmonella* spp., *Shigella* spp.

[a]*Klebsiella oxytoca* (excluding ESBL-producing strains)
MIC, minimal inhibitory concentration; FDA, Food and Drug Administration; ESBL, extended-spectrum β-lactamase.

Table 3. Nucleotide sequences of primers used in this study

Primer name	Target gene	Nucleotide sequence (5' to 3')	Product size (bp)	Reference
KPC-F	KPC-type	GTCACTGTATCGCCGTCTAGTTC	909	This study
KPC-R		TGGTGGGCCAATAGATGATT		
GES-F	GES-type	CGCTTCATTCACGCACTATT	855	[8]
GES-R		GTCCGTGCTCAGGATGAGTT		
IMP-1F	IMP-1 variants	AAGGCGTTTATGTTCATACTTCG	605	This study
IMP-1R		TTTAACCGCCTGCTCTAATGTAA		
VIM-2F	VIM-2 variants	ATCATGGCTATTGCGAGTCC	749	[9]
VIM-2R		ACGACTGAGCGATTTGTGTG		
NDM-F	NDM-type	GCCCAATATTATGCACCCGG	738	This study
NDM-R		CTCATCACGATCATGCTGGC		
OXA-48F	OXA-48 variants	GATTATCGGAATGCCTGCGG	845	[10]
OXA-48R		CTACAAGCGCATCGAGCATCA		
TEM-F	TEM-type	CTTGAAGACGAAAGGGCCTC	997	[11]
TEM-R		TGACTCCCCGTCGTGTAGAT		
SHV-F	SHV-type	CGCCGGGTTATTCTTATTTG	936	This study
SHV-R		CGGCTTAGCGTTGCCAGT		
CTXM-1F	CTXM-1 variants	CCGTCACGCTGTTGTTAGG	782	[8]
CTXM-1R		ACGGCTTTCTGCCTTAGGTT		
CTXM-9F	CTXM-9 variants	CAAAGAGAGTGCAACGGATG	862	[8]
CTXM-9R		CCTTCGGCGATGATTCTC		
CMY-1F	CMY-1 variants	CAACGACAATCCATCCTGTG	1,007	This study
CMY-1R		GAGCCGGTCTTGTTGAAGAG		
CMY-2F	CMY-2 variants	AGTAAGACGTTTAACGGCGTGT	749	
CMY-2R		TTATGCACCCATGAGGCTTT		
DHA-F	DHA type	ACAATCGCCACCTGTTTTTC	976	This study
DHA-R		TGGTGGACAGCACCATTAAA		

of class B; CMY-1-, CMY-2-, and DHA-types of class C; and OXA-48-types of class D, were identified by PCR and sequencing (Table 3) [8-11].

2. Detection of carbapenemases

Carbapenemases were screened using VITEK AST-N202 cards and modified Hodge tests (MHTs). If an isolate exhibited intermediate or resistance designations to more than one of the carbapenems, ertapenem, imipenem, or meropenem, based on the VITEK AST-N202 card, then the isolate was considered CPE. MHTs were performed with ertapenem and meropenem disks, separately, as described previously [12]. Briefly, a suspension of *E. coli* ATCC 25922 at a 0.5 McFarland turbidity unit concentration was spread on the entire surface of a MacConkey agar (Becton and Dickinson Company, Sparks, MD, USA) plate. Disks containing ertapenem or meropenem (10 μL, Becton and Dickinson Company) were placed on the center of the agar using a cotton swab, and then the clinical isolates were thickly inoculated from the edge of the disk to the periphery of the agar using a platinum loop. After overnight incubation, if a thickening of the inoculation line of a clinical isolate was observed on the edge of an inhibition zone, then the isolate was considered a CPE strain.

Results

1. Ability of MHTs to identify CPE strains

Of the 43 CPE isolates, 35 (81.4%) exhibited positive results in the MHTs with ertapenem or meropenem disks. In the ertapenem MHTs, eight CPE isolates showed false-negative results, including KPC-2-producing *K. pneumoniae* (n = 1), GES-5-producing *K. pneumoniae* (n = 2), NDM-1-producing *K. pneumoniae* (n = 1), GES-5-producing *E. coli* (n = 1), OXA-232-producing *E. coli* (n = 1), and VIM-2-producing *E. cloacae* (n = 2). In the meropenem MHTs, eight CPE isolates showed false-negative results, including KPC-2-producing *K. pneumoniae* (n = 2), KPC-3-producing *K. pneumoniae* (n = 1), GES-5-producing *K. pneumoniae* (n = 1), GES-5-producing *E. coli* (n = 1), OXA-232-producing *E. coli* (n = 1), and VIM-2-producing *E. cloacae* (n = 2). Five CPE isolates showed false-negative results in both ertapenem and meropenem MHTs, including GES-5-producing *K. pneumoniae* (n = 1), GES-5-producing *E. coli* (n = 1), OXA-232-producing *E. coli* (n = 1), and VIM-2-producing *E. cloacae* (n = 2). All 79 CNE isolates showed negative results regardless of carbapenem susceptibility. Sensitivities of the ertapenem and meropenem MHTs for CPE were both 81.4% (35/43), and that was increased to 88.4% (38/43) when the MHTs were performed with both antibiotics. Specificity of the MHTs was 100% (79/79) (Table 4).

2. Ability of the VITEK AST-N202 card to identify CPE strains

In antimicrobial susceptibility testing using VITEK AST-N202 cards, all 43 CPE isolates exhibited resistance to ertapenem, while only 38 and 41 of the CPE isolates showed resistance to imipenem and meropenem, respectively. Two and three CPE isolates exhibited intermediate and susceptibility patterns to imipenem, respectively, and two isolates were susceptible to meropenem. Three isolates exhibiting susceptibility patterns to imipenem included one GES-5-producing *E. coli* isolate and two OXA-232-producing *E. coli* isolates. Two isolates exhibited susceptibility to meropenem, including one GES-5-producing *E. coli* isolate and one OXA-232-producing *E. coli* isolate. Of the 79 CNE isolates, eight exhibited resistance to ertapenem, imipenem, and meropenem, while the remaining 71 isolates were susceptible to these carbapenems. Sensitivities to ertapenem, imipenem, and meropenem of the CPE strains when assessed using the VITEK AST-N202 cards were 100% (43/43), 88.4% (38/43), and 95.3% (41/43), respectively. Sensitivity of the VITEK 2 AST-N202 cards for the CPE

Table 4. Sensitivity and specificity of modified-Hodge test and VITEK AST-N202 cards for the detection of CPE strains

Methods	Antimicrobial agent (s)	Sensitivity	Specificity
Modified-Hodge test	Ertapenem	81.4% (35/43)	100% (79/79)
	Meropenem	81.4% (35/43)	
	Ertapenem + meropenem	88.4% (38/43)	
VITEK AST-N202 card	Ertapenem	100.0% (43/43)	89.9% (71/79)[a]
	Meropenem	95.3% (41/43)	
	Imipenem	93.0% (40/43)	

CPE, carbapenemase-producing enterobacteriaceae.
[a]The results of eight carbapenem-resistant clinical CRE isolates are excluded.

strains when using all three carbapenems was 100%. Specificities of the three carbapenems for CPE strains using the VITEK AST-N202 cards were all 89.8% (71/79); however, that reached 100% (71/71) when the eight carbapenem-resistant clinical CNE isolates were excluded (Table 4).

Discussion

Although the prevalence of CPE strains is still low (<1%) in Korea, various types of carbapenemases have been identified (Table 4). Since the first isolation of *S. marcescens*-producing class B VIM-2 MBL in Korea in 2002, VIM-2-producing *E. cloacae* have repeatedly been reported (Table 5) [13-25]. An outbreak of NDM-1-producing *K. pneumoniae* sequence type 340 (ST340) was reported in 2012, and the NDM-1-producing *E. coli* ST101 strain appeared in Korea in 2013 [26]. In a nationwide survey of antimicrobial resistance performed in 2003, a class A GES-5 carbapenemase was first identified in two clinical *K. pneumoniae* isolates from a hospital in Gyeonggi province, and an outbreak caused by the strain occurred in that same hospital in the next year [19]. Furthermore, a GES-5-producing *E. coli* ST131 was detected in 2011 [18]. An infection caused by the KPC-2-producing *K. pneumoniae* ST11 strain was first reported in 2010; thereafter, outbreaks caused by the KPC-2-producing *K. pneumonia* ST258 strain have been repeatedly reported in several hospitals in Korea [13, 14]. In 2014, an outbreak caused by *K. pneumoniae* ST14-producing OXA-232, a variant of class D OXA-48, occurred in a hospital in Seoul, Korea, and *E. coli* isolates producing this carbapenemase were also detected from rectal swab specimens [25]. Therefore, the development of rapid and accurate methods for the detection of CPE is needed for adequate treatment of infections caused by these microorganisms and for prevention of further dissemination.

Recently, the Clinical and Laboratory Standard Institute (CLSI) recommended MHTs with ertapenem disks as the standard detection method for CPE strains [12]. This study was performed to compare the ability of ertapenem and meropenem MHTs with VITEK AST-N202 cards. Both ertapenem and meropenem MHTs detected 35/43 (81.4%) CPE isolates, and sensitivity was increased to 88.4% (38/43) when the MHTs were performed with both carbapenem disks. The results indicate that the detection of CPE strains is dependent on whether MHTs fail in some cases, especially in cases of CPE strains harboring carbapenemases (GES-5, OXA-232, and MBLs) other than KPC. Ertapenem susceptibility testing using the VITEK AST-N202 card detected all 43 CPE isolates as resistant, while three and two CPE isolates respectively exhibited susceptibility patterns to imipenem and meropenem using this commercial card. CPE isolates harboring GES-5 or OXA-232 exhibited susceptibility to these carbapenems. The results suggest that CPE detection must be conducted based on ertapenem susceptibility when using VITEK AST-N202 cards.

Table 5. Carbapenemase-producing *Enterobacteriaceae* in Korea

Carbapenemase	Species	Strain	MIC (μg/mL)		Reference
			Imipenem	Meropenem	
KPC-2	*K. pneumoniae*	KPN-DK2	16	16	[13]
KPC-2	*K. pneumoniae*	KPN1010	>256	>256	[14]
KPC-2	*K. pneumoniae*	6 isolates	32-128	64-256	[15]
KPC-2	*K. pneumoniae*	3 isolates	2-4	2-16	[16]
KPC-2	*K. pneumoniae*	MP14	ND	≥16	[17]
GES-5	*K. pneumoniae*	2 isolates	ND	ND	[18]
GES-5	*K. pneumoniae*	6 isolates	0.5-1	ND	[19]
GES-5	*E. coli*	BD07372	0.5	0.25	[20]
VIM-2	*S. marcescens*	YMC00/4/1591	64	64	[21]
VIM-2	*E. cloacae*	KU680	4	4	[22]
VIM-2	*E. cloacae*	YMC08/12/3793	4	0.75	[23]
VIM-2	*E. cloacae*	YMC03/4/397	4	4	[23]
NDM-1	*K. pneumoniae*	4 isolates	1- >128	2- >128	[24]
OXA-232	*E. coli* *K. pneumoniae*	2 isolates 16 isolates	1 4-16	0.5-1 8-16	[25]

In conclusion, the VITEK AST-N202 card showed excellent performance for the detection of CPE strains. It is recommended that ertapenem-resistant *Enterobacteriaceae* clinical isolates should be directly subjected to molecular diagnostic methods for the identification of carbapenemase genes, because MHTs did now show sufficient sensitivity for the detection of CPEs.

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