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Multiplex PCR for Rapid Detection of Genes Encoding Class A Carbapenemases

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In recent years, there have been increasing reports of KPC-producing *Klebsiella pneumoniae* in Korea. The modified Hodge test can be used as a phenotypic screening test for class A carbapenamase (CAC)-producing clinical isolates; however, it does not distinguish between carbapenemase types. The confirmation of type of CAC is important to ensure optimal therapy and to prevent transmission. This study applied a novel multiplex PCR assay to detect and differentiate CAC genes in a single reaction. Four primer pairs were designed to amplify fragments encoding 4 CAC families (SME, IMI/NMC-A, KPC, and GES). The multiplex PCR detected all genes tested for 4 CAC families that could be differentiated by fragment size according to gene type. This multiplex PCR offers a simple and useful approach for detecting and distinguishing CAC genes in carbapenem-resistant strains that are metallo-β-lactamase nonproducers.

Key Words: Carbapenemase, Multiplex PCR, KPC, GES

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Carbapenems are important antibiotics for the treatment of infections caused by multidrug-resistant gram-negative bacilli [1, 2]; however, carbapenem-resistance is increasing, causing infections that are difficult to treat [2-4]. Bacterial production of carbapenemases is one of the most important mechanisms of carbapenem resistance [1]. There are 3 molecular classes of carbapenemases: A (penicillinases); B (metallo-β-lactamases, MBLs); and D (oxacillinases). The class A carbapenemases (CACs) include the SME, IMI/NMC-A, SFC, BIC, KPC, and some type of GES family proteins. The genes for the SME, IMI/NMC-A (except IMI-2), SFC, and BIC enzymes are chromosomal, and the genes for KPC and GES are carried on plasmids [1]. KPC producers have caused severe treatment problems in hospitals around New York and have also been reported in Europe, South America, and China [5-8]. In Korea, KPC-producing *Klebsiella pneumoniae* have rarely been detected. However, several cases were reported in 2010 and 2011 (Interscience Conference on Antimicrobial Agents and Chemotherapy 2011 poster c2-652, unpublished observation) [9, 10].

The modified Hodge test (MHT) can be used as a phenotypic confirmatory test for suspected carbapenemase production in *Enterobacteriaceae* [11]. However, it is reported that the MHT shows approximately 25% false positive results among carbapenemase nonproducers, mainly AmpC hyperproducers and strains harboring CTX-M [12]. Moreover, it does not distinguish between carbapenemase types or CAC types.

The major concern from the therapeutic and epidemiologic perspective is with transmissible and not chromosomal carbapenemases [1, 2], and this information cannot be acquired by the phenotypic methods. Confirmation of the CAC type is important to ensure optimal therapy and to prevent transmission [3]. In this study, we developed a multiplex PCR assay to detect and differentiate multiple CAC genes in a single reaction.

Eleven CAC producers (1 SME-producing *Serratia marce-*

scens, 2 IMI/NMC-A-producing *Enterobacter cloacae*, 2 KPC-producing *Enterobacteriaceae*, and 6 GES-producing *Klebsiella pneumoniae*); 7 MBL producers (3 VIM-producing *Pseudomonas aeruginosa*, 2 IMP-producing *P. aeruginosa*, 1 IMP-producing *Acinetobacter baumannii*, and 1 SIM-producing *A. baumannii*); and 5 non-carbapenemase-producing *Enterobacteriaceae* were studied (Table 1).

The bacterial cells were lysed by heating at 95°C for 10 min, and cellular debris was removed by centrifugation at 13,000 rpm for 5 min. The supernatant was used as the source of amplification templates. PCR was performed with a final volume of 20 μL in 0.2 mL thin-walled tubes (Accupower™ HotStart PCR PreMix; Bioneer, Daejeon, Korea).

We designed 4 primer pairs for 4 CAC families (SME, IMI/NMC-A, KPC, and GES). The genes encoding IMI and NMC-A type CACs are similar to each other and could not be differentiated by conventional PCR. Therefore, a single primer pair for the detection of these 2 CAC families was designed. The SFC-1 and the BIC-1 enzymes have been found in environmental isolates, and the corresponding genes are chromosomally encoded [13, 14]. For this reason, we did not design pairs of primers for these genes. The primers used in this study were GES primers for bla_{GES1-9} and $bla_{GES11-20}$ (GES-F: 5′-GCTTCATTCACGCACTATT-3′; GES-MR: 5′-CGATGCTAGAAACCGCTC-3′; product size: 323 bp), IMI/NMC-A primers for bla_{IMI1-3} and bla_{NMC-A} (IMI(NMC)-F1: 5′-TGCGGTC-GATTGGAGATAAA-3′; IMI(NMC)-R1: 5′-CGATTCTTGAAGCTTCT-GCG-3′; product size: 399 bp), SME primers for bla_{SME1-3} (SME-F1: 5′-ACTTTGATGGGAGGATTGGC-3′; SME-R1: 5′-ACGAATTCGAG-CATCACCAG-3′; product size: 551 bp), and KPC primers for $bla_{KPC2-13}$ (KPCF2: 5′-GTATCGCCGTCTAGTTCTGC-3′; KPCFR: 5′-GGTCGTGTTTCCCTTTAGCC-3′; product size 638 bp). The PCR program consisted of an initial denaturation step at 94°C for 5 min, followed by 25 cycles of DNA denaturation at 94°C for 30 sec, primer annealing at 50°C for 30 sec, and primer extension at 72°C for 1 min. After the last cycle, a final extension step at 72°C for 7 min was added.

Table 1. Bacterial strains used for class A carbapenemase multiplex PCR

Enzyme family	N of strains	Strain	Enzyme type*	Source†
Class A carbapenemase				
SME	1	*Serratia marcescens*	SME-1	CRAB
IMI	1	*Enterobacter cloacae*	IMI-1	CRAB
NMC-A	1	*Enterobacter cloacae*	NMC-A	CRAB
KPC	1	*Klebsiella pneumoniae*	KPC-2	CRAB
	1	*Escherichia coli*	KPC-3	CRAB
GES	6	*Klebsiella pneumoniae*	GES-5 like	Our laboratory
Metallo-ß-lactamase				
VIM	3	*Pseudomonas aeruginosa*	VIM-2 like	Our laboratory
IMP	2	*Pseudomonas aeruginosa*	IMP-6 like	Our laboratory
	1	*Acinetobacter baumannii*	IMP-25 like	Our laboratory
SIM	1	*Acinetobacter baumannii*	SIM-1 like	Our laboratory
Non-carbapenemase				
ESBL	1	*Escherichia coli*	TEM type	Our laboratory
	1	*Escherichia coli*	SHV type	Our laboratory
	1	*Klebsiella pneumoniae*	SHV type	Our laboratory
	1	*Citrobacter freundii*	TEM type	Our laboratory
ESBL+AmpC (EBC‡)	1	*Klebsiella pneumoniae*	SHV type	Our laboratory

*ESBLs were detected by CLSI phenotypic confirmatory tests and type specific PCR; †CRAB, Center for Research in Anti-Infectives and Biotechnology, Department of Medical Microbiology and Immunology, School of Medicine, Creighton University, Omaha, Nebraska; ‡EBC is a group of AmpC β-lactamase originated from *E. cloacae*.
Abbreviation: ESBL, extended-spectrum β-lactamase.

The GenBank nucleotide sequence accession numbers for the sequences studied here were as follows: GES-1 (AF156486); GES-2 (AF326355); GES-3 (AB113580); GES-4 (AB116260); GES-5 (AY494717); GES-6 (AY494718); GES-7 (IBC-1, AF208529); GES-8 (IBC-2, AF329699); GES-9 (AY920928); GES-11 (FJ854362); GES-12 (FN554543); GES-13 (GU169702); GES-14 (GU207844); GES-15 (GU208678); GES-16 (HM173356); GES-17 (HQ874631); GES-18 (JQ028729); GES-19 (JN596280); GES-20 (JN596280); IMI-1 (U50278); IMI-2 (DQ173429); IMI-3 (GU015024); NMC-A (Z21956); SME-1 (Z28968); SME-2 (AF275256); SME-3 (AY584237); KPC-2 (AY034847); KPC-3 (AF395881); KPC-4 (AY700571); KPC-5 (EU400222); KPC-6 (EU555534); KPC-7 (EU729727); KPC-8 (FJ234412); KPC-9 (FJ624872); KPC-10 (GQ140348); KPC-11 (HM066995); KPC-12 (HQ342889); and KPC-13 (HQ342890).

The CAC families could be differentiated into 4 groups, SME, IMI/NMC-A, KPC, and GES, by the PCR product size (Fig. 1). None of the non-CAC producers included in this study produced PCR product bands. Not all genotypes of CAC were tested: only SME-1, IMI-1, NMC-A, KPC-2, KPC-3, and GES-5-type enzyme-producing strains were included in this study. The primers for the genotypic detection of SME, KPC and GES enzymes were exactly complementary to the corresponding GenBank sequences, but the primers for IMI/NMC-A were not complementary at 1 base each in the forward and reverse sequences of IMI-3 and NMC-A. Therefore, it is somewhat uncertain whether this multiplex PCR assay would be able to detect all of the genotypes of CACs described above.

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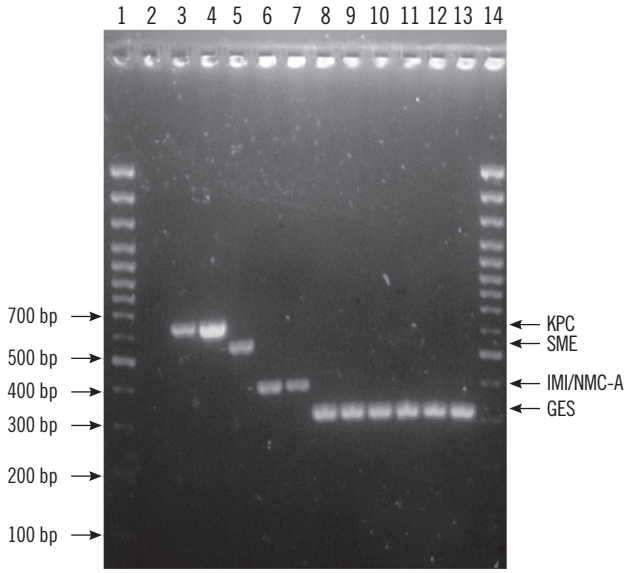

Fig. 1. Results of multiplex PCR for class A carbapenemase (CAC)-producing strains. Multiplex PCR products were separated on a 2% agarose gel. Lanes 1 and 14 show the 100-bp DNA ladder; lane 2, the PCR product of the negative control (distilled water); lanes 3 and 4, KPC-type enzyme-producing strains; lane 5, SME-type; lanes 6 and 7, NMC-A and IMI-type, respectively; lanes 8-13, GES-type. The amplified product from each PCR is indicated on the right, and the size of the marker in base pairs is shown on the left.

In summary, this multiplex PCR method appears to be a simple and useful approach for detecting and distinguishing CAC genes in MBL-negative carbapenem-resistant strains. Therefore, this method should be helpful for characterization of CACs and prevention of the spread of pathogens producing these enzymes.

Authors' Disclosures of Potential Conflicts of Interest

No potential conflicts of interest relevant to this article were reported.

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