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 carbapenemase (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

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 (penicillinas); B (metallo-β-lactamas, 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were reported in 2010 and 2011 (Interscience Conference on Antimicrobial Agents and Chemotherapy 2011 poster c2-652, unpublished observation) [9, 10].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 blaGES1-9 and blaGES11-20 (GES-F: 5’-GGTCGTGTTTCCCTTTAGCC-3’; product size: 399 bp), SME primers for blaSME1-3 (SME-F1: 5’-ACTTTGATGGGAGGATTGGC-3’; product size: 551 bp), and KPC primers for blaKPC1-13 (KPC-F2: 5’-GTATCCGCGCTGATCTGCC-3’; KPC-R1: 5’-GTTCCGTTTCTCTTACC-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 5 min was added.

The GenBank nucleotide sequence accession numbers for the sequences studied here were as follows: GES-1 (AF156486); GES-2 (AF326395); 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-10 (FJ854362); GES-11 (DF554543); GES-12 (GU169702); GES-13 (GUJ207844); GES-15 (GU208678); GES-16 (HM173356); GES-17 (HQ874631); GES-18 (LQ028729); 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 (AY854237); KPC-2 (AY033484); 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 1 SME-1, 1 IMI-1, 1 NMC-A, 1 KPC-2, and 1 GES-5-type enzyme-producing strain was 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.

### 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 |
| 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 |
| SIM | 1 | Acinetobacter baumannii | SIM-1 like | Our laboratory |
| Non-carbapenemase | | | | |
| ESBL | 1 | Escherichia coli | 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 ß-lactamase originated from E. cloacae.

Abbreviation: ESBL, extended-spectrum ß-lactamase.
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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