

**H-1****Identification of Novel Beta-lactamases with RFLD-PCR Method from Korean Clinical Isolates**

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The diagnostic ability of restriction fragment length dimorphism (RFLD)-PCR method in clinical samples was evaluated. Sixteen primer pairs, newly designed from 145 beta-lactamase genes encoding extended-spectrum beta-lactamases (ESBLs), were used to differentiate ten genes encoding TEM-1a, SHV-12, OXA-2, FOX-3, MOX-1, CMY-3, MIR-1, IMP-1, IMI-1, and Toho-1 beta-lactamases. The RFLD-PCR was carried out successfully, and these genes were rapidly and effectively differentiated by the sizes of their PCR product and by digesting with unique restriction enzyme sites. And this discriminatory detection of the genes was also confirmed by sequencing the PCR products. Among 65 clinical isolates from January 1999 to December 1999 in Kosin Medical Center, South Korea, we detected 28 strains producing TEM, 29 strains producing SHV, 16 strains producing CMY, and 6 strains producing AmpC beta-lactamase using the method. Until now, we identified six novel beta-lactamases (TEM-17b, CMY-10, 11, 12, and two different AmpCs). Nucleotide and amino acid substitutions between *bla*<sub>CMY-10</sub>, *bla*<sub>CMY-11</sub>, and *bla*<sub>CMY-12</sub> were shown in Table 1. In conclusion, RFLD-PCR method provides a promising prospect for the diagnosis of various ESBLs in clinical isolates and the identification of a novel beta-lactamase.

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Table 1. Nucleotide and amino acid substitutions between *bla*<sub>CMY-10</sub>, *bla*<sub>CMY-11</sub>, *bla*<sub>CMY-11</sub>, and *bla*<sub>CMY-12</sub>.

| Gene (enzyme)                         | Accession number or source | Nucleotide (amino acid) <sup>a</sup> |            |            |
|---------------------------------------|----------------------------|--------------------------------------|------------|------------|
|                                       |                            | 1276 (315)                           | 1429 (366) | 1431 (367) |
| <i>bla</i> <sub>CMY-10</sub> (CMY-1)  | X92508                     | T (Ile)                              | A (Asn)    | (Glu)      |
| <i>bla</i> <sub>CMY-10</sub> (CMY-10) | This study                 | T (Ile)                              | T (Ile)    | G (Glu)    |
| <i>bla</i> <sub>CMY-11</sub> (CMY-11) | This study                 | G (Ser)                              | T (Ile)    | G (Glu)    |
| <i>bla</i> <sub>CMY-12</sub> (CMY-12) | This study                 | G (Ser)                              | T (Ile)    | A (Lys)    |

<sup>a</sup>Nucleotide and amino acid numbering are according to the nucleotide sequence of the *bla*<sub>CMY-10</sub> gene (X92508) and the amino acid sequence of the CMY-1 beta-lactamase, respectively, of *Klebsiella pneumoniae* CHO (Bauernfeind et al., 1998).

**H-2****Development of Broad-Spectrum ELISA Using 19kDa Lipoprotein Antigen of *Legionella pneumophila* for Diagnosis of Legionella Infection**

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Although Legionella is an important cause of severe pneumonia, difficulty still exists in its diagnosis. Because at least 80% of patient with Legionellosis excrete the Legionella antigen in their urine, the tests for urinary antigen detection have been developed and commercialized. However, the tests have disadvantage only reliably to detect urinary *L. pneumophila* serogroup 1 antigen. In this study we developed an enzyme-linked immunosorbent assay (ELISA) for urinary antigen detection, based on the 19 kDa soluble lipoprotein antigen that is highly conserved among Legionella species. The 19 kDa lipoprotein of *L. pneumophila* serogroup 1 was expressed in *E. coli*, and the resulting recombinant protein was purified and used for raising antibody in a rabbit. A "sandwich" ELISA coated with purified anti-lipoprotein IgG in a microtiter plate (lipoprotein-based ELISA) was developed and evaluated for detection of Legionella urinary antigen. Seventeen urine samples were obtained from guinea pigs inoculated intraperitoneally with each Legionella species including pneumophila serogroups 1, 3, 6, *L. oakridgensis*, *L. sainthelens*, *L. anisa* and *L. jordanis*, and 258 urine specimens from healthy subjects and patients with either non-Legionella pneumonia or urinary tract infections. As a standard test, the ELISA using soluble antigens extracted from 12 different Legionella species was used. The test performance of the lipoprotein-based ELISA showed that the sensitivity and specificity were 76.5% and 100%, respectively. Detection limit of the test was 20 ng/mL. And the lipoprotein based ELISA detected urinary lipoprotein antigen of different Legionella species. In conclusion, the ELISA using 19 kDa lipoprotein antigen is a useful broad-spectrum diagnostic method for Legionella infection.

**H-3****Species-specific Identification of *Mycobacterium tuberculosis* by Polymerase Chain Reaction Using a Gene for Methionyl-tRNA Synthetase**

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Gene encoding methionyl-tRNA synthetase (MetRS) was cloned from a pathogenic organism, *Mycobacterium tuberculosis* and its sequence was determined. Four sets of primers specific for the MetRS gene were designed based on the alignment of seventeen MetRSs to detect *M. tuberculosis* by polymerase chain reaction (PCR). Although all the tested primer sets generated the expected PCR products, only one set of primers specific to the sequence of the N-terminal domain of the enzyme showed specificity to *M. tuberculosis* among 10 different species of Mycobacteria. The PCR assay using this primer pair was sensitive enough to detect 125 fg of chromosomal DNA isolated from *M. tuberculosis*. This work provides an additional option to improve the accuracy and sensitivity for the diagnosis of tuberculosis that still remains as a major cause for human death.

**H-4****Rapid Detection of Drug Resistant *Mycobacterium tuberculosis* Using an Oligonucleotide Chip - in the case of Rifampin-resistance through *rpoB* Gene Mutation**

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Tuberculosis (TB) is one of the most common human infectious diseases, causing three million deaths a year worldwide. Although many antibiotic drugs for TB are available, the emergence of drug-resistant *Mycobacterium tuberculosis* created additional concern in the event of TB control. Therefore the rapid and accurate detection of drug-resistant tuberculosis has become increasingly important. Rifampin resistance is known to be associated with genetic alterations in 157 bp core region of *rpoB* gene. In this study, an oligonucleotide chip was used to detect rifampin- and/or rifabutin-resistant *M. tuberculosis*. The chip contained two types of probes which enabled to detect rifampin-resistance and/or rifabutin-resistance: one for 10 different wild type sequences and the other for 20 different mutant sequences. Two marker probes for the evaluation of bacterial species were also included. Polymerase Chain Reaction (PCR) products from clinical samples were determined the resistance of rifampin and/or rifabutin by fluorescence intensity. The mutations of resistant isolates were confirmed by DNA sequencing. Total 55 clinical cultured samples were analyzed. Thirty eight samples were successfully evaluated as mutants, and 26 of 38 mutants were confirmed by DNA sequencing. These results suggest that the oligonucleotide chip can be a useful method for the rapid and accurate diagnosis of drug-resistant *M. tuberculosis*.

**H-5****Neutrophil Antimicrobial Proteins in the Serum and the Cerebrospinal fluid of Children with Meningitis****G.Aleshina\*, N.Boterashvili, M.Sorokina**

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Neutrophils contain an array of constitutive antimicrobial proteins and peptides including myeloperoxidase (MPO), lactoferrin (LF) and defensins, when released into the extracellular fluids, they can kill microbes or inhibit the growth. We measured concentrations of MPO and LF in the serum and the cerebrospinal fluid (CSF) of children with meningitis, both bacterial and aseptic, using enzyme-linked immunosorbent assay. MPO and LF concentrations in the serum of all patient groups were significantly higher than those in healthy blood donors; furthermore MPO concentration in the serum of children with bacterial meningitis was significantly higher than that in children with aseptic meningitis. The concentrations of both MPO and LF in CSF were elevated significantly in children with bacterial meningitis compared with patients with bacterial meningitis. MPO and LF levels in the CSF of children with bacterial meningitis ranged from 26 ng/mL to 3,900 ng/mL with a mean of 703 ng/mL (SE+/-240) and from 0 ng/mL to 22,000 ng/mL with a mean of 5,384 ng/mL (SE+/-1453), respectively, whereas MPO and LF levels in the CSF of children with aseptic meningitis ranged from 0 ng/mL to 80 ng/mL with a mean of 16 ng/mL (SE+/-5) and from 0 ng/mL to 1,000 ng/mL with a mean of 138 ng/mL (SE+/-60), respectively. Therefore, based on our findings, we propose that the increased level of these proteins may reflect the important role of antimicrobial molecules of neutrophils in the infectious process.

**H-6****Efficient Multiplex PCR for the Identification of Vancomycin-Resistant *Enterococcus* Species and the Detection of Vancomycin Resistant Genotypes****S.-H. KIM\*, D.-G. LEE, J.-H. CHOI, W.-S. SHIN and M.-W. KANG**The Clinical Research Institute\*, St. Mary's Hospital  
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**BACKGROUND** Dukta-Malen *et al.* used multiplex PCR to diagnose vancomycin resistant genotype on the standard strains of *Enterococcus* species. Since we found that there are some misinformed primer sequences and the interference between primers, we wanted to modify original method.

**MATERIALS and METHODS** Standard & clinical isolates of *Enterococcus* species were identified by conventional method. For the identification of *E. faecium* and *E. faecalis*, each species-specific primers, *ddl<sub>E.faecium</sub>* and *ddl<sub>E.faecalis</sub>* have been used. The major differences in PCR compared with previous method are *ddl<sub>E.faecium</sub>* has been modified that 526bp product was obtained as a result of redesigning the primer, which produce 24 bp sorter product than that of Dukta-Malen's and bovine serum albumin was used as an adjuvant for PCR reaction.

**RESULTS** Evidently, this method is able to identify known standard strains and detect each vancomycin-resistant genotypes when applied to clinical specimens. Identification of species and differentiation between genotypes was remarkable. Some drawbacks in this study are that auxiliary experiments, motility and pigment production, have to be added to evaluate *Enterococcus* species other than *E.faecium* and *E.faecalis*.

**CONCLUSION** We established rapid multiplex PCR with confidence to diagnose VRE and genotypes after modification of the previous method. In the long run, using this method, more diagnosis and epidemiologic study are needed to elucidate the eligibility of this method.

**H-7****Multiplex PCR for the Detection of Genes Encoding Methicillin Resistance and Aminoglycoside Modifying Enzymes among *Staphylococcus aureus* and Coagulase Negative Staphylococci****S-M Choi, S-H KIM\*, H-J KIM\*, D-G LEE, J-H CHOI, W-S SHIN and M-W KANG**The Clinical Research Institute, St. Mary's Hospital\*  
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**BACKGROUND** Current identification and antibiotic susceptibility test for staphylococci mainly depend on conventional microbiological and biochemical assays so far. However, these assays take at least 36 hours to complete. Considering the importance of antibiotic resistance for treatment, we have developed multiplex PCR to detect resistance genes simultaneously, especially aminoglycoside modifying enzyme (AME) and methicillin resistant gene.

**MATERIALS and METHODS** *Staphylococcus* species used in this study has been identified by Microscan WalkAway-96. Agar dilution method has been performed. Purification of DNA was done and then carried out multiplex PCR.

**RESULTS** The PCR result is listed below (the parenthesis indicate MIC ( $\mu$ g/mL) of gentamicin(GM) and oxacillin (OX) ).



- 1-2 MSSA (GM<0.125, OX 0.25) (GM 128, OX 0.25)  
3-5 MRSA (GM 0.25, OX >125) (GM 64, OX 16) (GM 256, OX 256)  
6-8 MSSA (GM<0.125, OX <0.125) (GM 128, OX 0.5) (GM 32, OX 0.25)  
9-10 MSCNS (GM<0.125, OX <0.125) (GM 256, OX 8)  
11-12 MRCNS (GM 8, OX 128) (GM 256, OX >256)  
13-15 MSCNS (GM<0.125, OX <0.125) (GM 128, OX 2) (GM 128, OX 2)

**CONCLUSION** We have developed multiplex PCR to detect AMEs, such as, *aac(6')-aph(2'')*, *ant(4')*, *aph(3')* and methicillin resistant gene, *mecA*. To elucidate the eligibility of this method, Epidemiological study is undergoing now.

**H-8****Set up the Routine RT-PCR Technique for Diagnosis of Hepatitis C Virus (HCV)****NO THO THANH THUY\*, PHAM HUNG VAN \***\*Doctor, lecturer of University of Medicine and Pharmacy  
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Hepatitis C disease is a status which is especially paid attention of clinicians and epidemiologists. Nowadays, HCV is considered a main cause of hepatofibrosis and hepatoma.

**PURPOSE** to set up the routine RT-PCR technique for diagnosis of HCV-RNA. We also assessed the result of this technique at University of Medicine-Pharmacy (UMP) and HoaHao Diagnostic Centre (HHDC)

**METHOD** A sectionally prospective study.

**RESULT** The RT-PCR was set up and performed on 1198 patients at UMP and HHDC which were diagnosed C hepatitis based on clinical symptoms and anti HCV(+). RT-PCR(+) were 66% and 64% in UMP and HHDC, respectively.

**DISCUSSION and CONCLUSION** The result of the RT-PCR technique for detection HCV-RNA in 2 centers demonstrates the effectiveness of the technique allowing to direct detect HCV-RNA from sera. The technique should be developed in many laboratories and medical centers to define HCV infection.

