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# 요 검체에서 분리된 대장균의 항균제 내성 기전

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# Antibiotic Resistance Mechanisms of *Escherichia coli* Isolates from Urinary Specimens

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**Background :** This study was designed to characterize urinary isolates of *Escherichia coli* that produce extended-spectrum β-lactamases (ESBLs) and to determine the prevalence of other antimicrobial resistance genes.

**Methods :** A total of 264 non-duplicate clinical isolates of *E. coli* were recovered from urine specimens in a tertiary-care hospital in Busan in 2005. Antimicrobial susceptibility was determined by disk diffusion and agar dilution methods, ESBL production was confirmed using the double-disk synergy (DDS) test, and antimicrobial resistance genes were detected by direct sequencing of PCR amplification products. *E. coli* isolates were classified into four phylogenetic biotypes according to the presence of *chuA*, *yjaA*, and TSPE4.

**Results :** DDS testing detected ESBLs in 27 (10.2%) of the 264 isolates. The most common type of ESBL was CTX-M-15 (N=14), followed by CTX-M-3 (N=8) and CTX-M-14 (N=6). All of the ESBL-producing isolates were resistant to ciprofloxacin. PCR experiments detected genes encoding DHA-1 and CMY-10 AmpC β-lactamases in one and two isolates, respectively. Also isolated were 5 isolates harboring 16S rRNA methylases, 2 isolates harboring Qnr, and 19 isolates harboring AAC(6')-Ib-cr. Most ESBL-producing isolates clustered within phylogenetic groups B2 (N=14) and D (N=7).

**Conclusion :** CTX-M enzymes were the dominant type of ESBLs in urinary isolates of *E. coli*, and ESBL-producing isolates frequently contained other antimicrobial resistance genes. More than half of the urinary *E. coli* isolates harboring CTX-M enzymes were within the phylogenetic group B2. *(Korean J Lab Med 2009;29:17-24)*

**Key Word :** *Escherichia coli, Extended-spectrum β-lactamases (ESBL), Phylogenetic group*

## INTRODUCTION

*Escherichia coli* is the most important pathogen involved in urinary tract infections (UTIs) [1]. β-Lactam antibiotics have been widely used to treat *E. coli* infections; however, treatment of UTIs has become increasingly problematic due to the spread of extended-spectrum β-lactamases (ESBLs), including the TEM, SHV, and CTX-M families. The genes

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encoding CTX-M enzymes have spread rapidly in the past few decades in many parts of the world and are now the predominant type of ESBLs in *E. coli* [2, 3]. CTX-M enzymes, which now exceed 70 different types, can be divided into six clusters based on amino acid sequence similarities, namely, CTX-M-1, CTX-M-2, CTX-M-8, CTX-M-9, CTX-M-25, and CTX-M-45 [2]. The CTX-M-1 and CTX-M-9 clusters are distributed throughout many parts of the world, while CTX-M-2 and CTX-M-8 clusters are focally distributed in Europe [4]. In Korea, CTX-M-15 and CTX-M-3 were the most prevalent ESBLs according to a 2003 study of clinical *E. coli* isolates [5].

Plasmid-mediated AmpC $\beta$-lactamases can also hydrolyze expanded-spectrum cephalosporins, but unlike ESBLs, they are not inactivated by clavulanic acid and are active against cephamycins. Since the initial finding of CMY-1 in Korea, various types of AmpC $\beta$-lactamases, including MIR, MOX, FOX, LAT, BIL, and DHA, have been reported [6]. In Korea, cefoxitin-resistant *E. coli* has become increasingly common, and a study performed in 2003 showed that a high portion (46.6%, 62/116) of cefoxitin resistance in *E. coli* were due to the production of plasmid-mediated AmpC $\beta$-lactamase [7].

Acquisition of fluoroquinolone (FQ) resistance in *Enterobacteriaceae* is usually due to accumulation of mutations in chromosome-mediated target enzymes (DNA gyrase and topoisomerase IV) and/or to decreasing intracellular drug accumulation by upregulation of native efflux pumps either alone or in combination with decreased expression of outer membrane porins [8]. Thus, it was widely believed that FQ resistance was transferred only vertically. However, the recent discovery of plasmid-mediated FQ resistance determinants, specifically the Qnr protein and AAC(6')-Ib-cr, has made it clear that FQ resistance can be transferred horizontally as well [9, 10]. Indeed, a study reported the detection of *qnr* genes in 2 of 260 clinical *E. coli* isolates obtained in Korea between 2001-2003 [11].

Aminoglycosides are widely used to treat severe infections involving gram-negative bacteria, often in combination with broad-spectrum $\beta$-lactam antibiotics [12]. Aminoglycosides bind to the aminoacyl site of 16S ribosomal RNA (rRNA) within 30S ribosomal subunits and interfere with protein synthesis [13]; however, a high level resistance to aminoglycosides mediated by methylation of 16S rRNA has recently emerged among gram-negative bacilli [14].

The spread of plasmids containing multidrug-resistance determinants, including ESBL genes, is an emerging threat [10, 15]. Furthermore, ESBL-producing isolates usually show resistance to other antibiotics, including aminoglycosides and quinolones. The aims of the present study were to describe the characteristics of ESBL-producing urinary *E. coli* isolates from a Korean hospital, and to determine the prevalence of genes encoding AmpC $\beta$-lactamases, Qnr, AAC(6')-Ib-cr, and 16S rRNA methylases in ESBL-producing isolates.

## MATERIALS AND METHODS

### 1. Bacterial strains

A total of 264 non-duplicate clinical isolates of *E. coli* were recovered from urine samples in a tertiary-care hospital in Busan, Korea in 2005. The isolates were identified with conventional biochemical tests and Vitek GNI cards (bioMérieux Vitek Inc., Hazelwood, MO, USA). *E. coli* ATCC 25922 was used as an MIC reference strain and *E. coli* J53 Azide$^R$ as a recipient strain for conjugative transfer.

### 2. Antimicrobial susceptibility testing

Antibiotic-containing disks (BBL, Cockeysville, MD, USA) were used for routine antibiograms by the CLSI disk diffusion assay [16]. The double-disk synergy (DDS) test was carried out on Mueller-Hinton agar (Difco Laboratories, Detroit, MI, USA) with disks containing 30 $\mu$g of ceftazidime, cefotaxime, or aztreonam, placed at a distance of 15 mm (center to center) from a disk containing amoxicillin-clavulanic acid (20 $\mu$g/10 $\mu$g) located in the center of the plate. MICs were determined by the agar dilution method on Mueller-Hinton agar with an inoculum of $10^4$ CFU/spot. MICs of cefotaxime (Handok, Seoul, Korea) and ceftazidime (Hanmi, Seoul, Korea) were determined alone or in combination with a fixed concentration (4 $\mu$g/mL) of clavulanic acid

(Sigma Chemical Co., St. Louis, MO, USA) [16].

### 3. Mating-out assay

Conjugation experiments were carried out using the azide-resistant recipient strain *E. coli* J53 by the broth mating method [17]. Transconjugants were selected on MacConkey agar containing azide (100 μg/mL) and cefotaxime (2 μg/mL).

### 4. PCR and sequencing experiments

Detection of genes coding ESBLs, AmpC β-lactamases,

Table 1. Primers used in this study

| PCR target | Primer name | Primer sequence | Reference |
|---|---|---|---|
| *bla*$_{CTX-M}$ (CTX-M-1 cluster) | CTX-M-1F | 5′-GGACGTACAGCAAAAACTTGC-3′ | |
| | CTX-M-1R | 5′-CGGTTCGCTTTCACTTTTCTT-3′ | |
| *bla*$_{CTX-M}$ (CTX-M-2 cluster) | CTX-M-2F | 5′-CGGTGCTTAAACAGAGCGAG-3′ | |
| | CTX-M-2R | 5′-CCATGAATAAGCAGCTGATTGCCC-3′ | |
| *bla*$_{CTX-M}$ (CTX-M-8 cluster) | CTX-M-8F | 5′-ACGCTCAACACCGCGATC-3′ | |
| | CTX-M-8R | 5′-CGTGGGTTCTCGGGGATAA-3′ | |
| *bla*$_{CTX-M}$ (CTX-M-9 cluster) | CTX-M-9F | 5′-GATTGACCGTATTGGGAGTTT-3′ | |
| | CTX-M-9R | 5′-CGGCTGGGTAAAATAGGTCA-3′ | |
| *bla*$_{TEM}$ | TEM-F | 5′-ATGAGTATTCAACATTTCCGT-3′ | [18] |
| | TEM-R | 5′-TTACCAATGCTTAATCAGTGA-3′ | |
| *bla*$_{SHV}$ | SHV-F | 5′-CCGGGTTATTCTTATTTGTCGCT-3′ | |
| | SHV-R | 5′-TAGCGTTGCCAGTGCTCG-3′ | |
| *bla*$_{VEB}$ | VEB-F | 5′-ACCAGATAGGAGTACAGACATATGA-3′ | |
| | VEB-R | 5′-TTCATCACCGCGATAAAGCAC-3′ | |
| *bla*$_{GES/IBC}$ | GES/IBC-F | 5′-GTTAGACGGGCGTACAAAGATAAT-3′ | |
| | GES/IBC-R | 5′-TGTCCGTGCTCAGGATGAGT-3′ | |
| *bla*$_{TLA}$ | TLA-F | 5′-CGCGAAAATTCTGAAATGAC-3′ | |
| | TLA-R | 5′-AGGAAATTGTACCGAGACCCT-3′ | |
| *bla*$_{DHA-1}$-like | DHA-F | 5′-GGGGAGATAACGTCTGACCA-3′ | |
| | DHA-R | 5′-TAGCCAGATCCAGCAATGTG-3′ | |
| *bla*$_{CMY-1}$-like | CMY-1F | 5′-TCACATCGGCTTCACAGAGC-3′ | |
| | CMY-1R | 5′-CCATGGTGATGCTGTCAAAGA-3′ | |
| *bla*$_{CMY-2}$-like | CMY-2F | 5′-CAACACGGTGCAAATCAAAC-3′ | [19] |
| | CMY-2R | 5′-CATGGGATTTTCCTTGCTGT-3′ | |
| *bla*$_{ACT-1}$-like | ACT-1F | 5′-CGTCATGGTCTCGTCCGTTAG-3′ | |
| | ACT-1R | 5′-CCTTGACCTCATCCGGTACCT-3′ | |
| *qnrA1* to *qnrA6* | QnrAm-F | 5′-AGAGGATTTCTCACGCCAGG-3′ | |
| | QnrAm-R | 5′-TGCCAGGCACAGATCTTGAC-3′ | |
| *qnrB1* to *qnrB6* | QnrBm-F | 5′-GGMATHGAAATTCGCCACTG-3′ | |
| | QnrBm-R | 5′-TTTGCYGYYCGCCAGTCGAA-3′ | [20] |
| *qnrS1* to *qnrS2* | QnrSm-F | 5′-GCAAGTTCATTGAACAGGGT-3′ | |
| | QnrSm-R | 5′-TCTAAACCGTCGAGTTCGGCG-3′ | |
| *aac(6′)-Ib* | AAC(6′)-IbF | 5′-TGACCAACAGCAACGATTCC-3′ | [21] |
| | AAC(6′)-IbR | 5′-TTAGGCATCACTGCGTGTTC-3′ | |
| *armA* | armA-F | 5′-TATGGGGGTCTTACTATTCTGCCTAT-3′ | |
| | armA-R | 5′-TCTTCCATTCCCTTCTCCTTT-3′ | |
| *rmtA* | rmtA-F | 5′-CTAGCGTCCATCCTTTCCTC-3′ | |
| | rmtA-R | 5′-TTTGCTTCCATGCCCTTGCC-3′ | |
| *rmtB* | rmtB-F | 5′-TCAACGATGCCCTCACCTC-3′ | |
| | rmtB-R | 5′-GCAGGGCAAAGGTAAAATCC-3′ | [22] |
| *rmtC* | rmtC-F | 5′-GCCAAAGTACTCACAAGTGG-3′ | |
| | rmtC-R | 5′-CTCAGATCTGACCCAACAAG-3′ | |
| *rmtD* | rmtD-F | 5′-CTGTTTGAAGCCAGCGGAACGC-3′ | |
| | rmtD-R | 5′-GCGCCTCCATCCATTCGGAATAG-3′ | |
| *npmA* | npmA-F | 5′-CTCAAAGGAACAAAGACGG-3′ | |
| | npmA-R | 5′-GAAACATGGCCAGAAACTC-3′ | |

Qnr, AAC(6')-Ib-cr, and 16S rRNA methylases was performed by PCR amplification with the primers listed in Table 1. Following amplification, the PCR products were subjected to direct sequencing. Both strands of each PCR product were sequenced in duplicate with an automatic sequencer (Model 3730×l; Applied Biosystems, Weiterstadt, Germany).

### 5. Phylogenetic grouping

*E. coli* isolates were classified into phylogenetic biotypes (A, B1, B2, and D) according to the presence of *chuA*, *yjaA*, and TSPE4 using multiplex PCR as described previously [23]. Group B2 possesses both *chuA* and *yjaA* genes, and group D contains *chuA* gene but not the *yjaA* gene. Group A harbors neither *chuA* gene nor TSPE4, and group B1 contains TSPE4 but not the *chuA* gene.

## RESULTS

### 1. Prevalence of ESBLs in urinary *E. coli* isolates

The DDS test detected ESBLs in 27 (10.2%) of the 264 isolates tested. Transfer of ESBL genes to the *E. coli* J53

Table 2. Characteristics of urinary *E. coli* isolates containing genes encoding ESBLs

| Strain | MICs (mg/L) | | | | | | Susceptibility* | | | Trans-Conjugability | Antimicrobial resistance genes encoding | | | | | Phylogenetic group |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | CAZ | CAZ/CA | CTX | CTX/CA | FOX | CIP | G | NN | AN | | ESBL | AmpC | Qnr | AAC(6')-Ib-cr | Methylase | |
| KU05/10441 | 128 | 128 | >256 | >256 | >256 | >256 | R | R | R | + | CTX-M-3 | CMY-10 | | | *armA* | B2 |
| KU05/17759 | 64 | 2 | 256 | 1 | 128 | >256 | R | R | R | + | CTX-M-3 | DHA-1 | QnrB4 | + | *armA* | A |
| KU05/18969 | 16 | 1 | 128 | 0.3 | 16 | >256 | R | R | I | + | CTX-M-3 | | | + | *armA* | A |
| KU05/23567 | 64 | 8 | >256 | 4 | 32 | >256 | R | R | R | + | CTX-M-3 | | | | | B2 |
| KU05/24485 | 128 | 256 | >256 | >256 | >256 | >256 | R | R | I | + | CTX-M-3 | CMY-10 | | + | | B2 |
| KU05/27254 | 16 | 1 | >256 | 0.5 | 16 | >256 | R | R | S | + | CTX-M-3 | | | | | D |
| KU05/14811 | 32 | 0.3 | 256 | 0.3 | 32 | >256 | R | R | S | - | CTX-M-15 | | | + | | B2 |
| KU05/16013 | 64 | 0.5 | >256 | 0.3 | 8 | >256 | R | R | S | - | CTX-M-15 | | QnrS1 | + | | B2 |
| KU05/17301 | 256 | 0.5 | >256 | 1 | 8 | >256 | R | R | S | - | CTX-M-15 | | | | | B2 |
| KU05/19020 | >256 | 2 | >256 | 1 | 32 | >256 | R | R | S | - | CTX-M-15 | | | + | | B2 |
| KU05/21856 | 32 | 0.5 | 128 | 0.1 | 4 | 16 | R | R | S | + | CTX-M-15 | | | + | | B1 |
| KU05/22571 | 256 | 2 | >256 | 1 | 8 | >256 | R | R | S | + | CTX-M-15 | | | + | | A |
| KU05/23521 | 64 | 0.5 | 128 | 0.1 | 8 | >256 | R | R | S | - | CTX-M-15 | | | + | | A |
| KU05/23984 | 256 | 2 | >256 | 1 | 32 | >256 | R | R | S | + | CTX-M-15 | | | + | | B2 |
| KU05/24852 | 128 | 1 | >256 | 1 | 32 | >256 | R | R | S | - | CTX-M-15 | | | + | | B2 |
| KU05/27014 | 64 | 1 | 256 | 0.3 | 4 | >256 | R | R | S | - | CTX-M-15 | | | + | | B2 |
| KU05/29305 | 64 | 1 | >256 | 0.3 | 64 | >256 | R | R | S | - | CTX-M-15 | | | + | | B2 |
| KU05/29630 | 64 | 4 | >256 | 2 | 256 | >256 | R | R | R | + | CTX-M-15 | | | + | *rmtB* | D |
| KU05/28700 | >256 | 2 | >256 | 0.5 | 32 | 64 | R | R | S | + | CTX-M-15 | | | + | | B2 |
| KU05/19028 | 4 | 0.5 | >256 | 0.3 | 32 | >256 | R | S | S | + | CTX-M-14 | | | | | D |
| KU05/27080 | 16 | 2 | >256 | 1 | 32 | >256 | S | S | S | - | CTX-M-14 | | | | | D |
| KU05/29253 | 16 | 2 | 256 | 1 | 16 | >256 | S | R | R | - | CTX-M-14 | | | + | | D |
| KU05/14517 | 256 | 1 | >256 | 1 | 32 | >256 | R | R | S | - | TEM-52 | | | + | | B2 |
| KU05/30403 | 64 | 1 | >256 | 0.5 | 16 | >256 | R | R | S | - | CTX-M-14+ CTX-M-15 | | | | | D |
| KU05/31131 | 16 | 1 | >256 | 0.5 | 16 | >256 | R | R | S | + | CTX-M-3+ CTX-M-14 | | | | | D |
| KU05/15422 | 256 | 1 | >256 | 0.5 | 16 | >256 | R | R | I | - | CTX-M-14+ SHV-12 | | | + | | B2 |
| KU05/21306 | 256 | 1 | 128 | 0.1 | 4 | >256 | R | R | R | + | CTX-M-3+ CTX-M-9+SHV-12 | | | + | *armA* | B1 |

*Antimicrobial susceptibilities of *E. coli* isolates determined by disk diffusion assay.
Abbreviations: MIC, minimum inhibitory concentration; CAZ, ceftazidime; CA, clavulanic acid; CTX, cefotaxime; FOX, cefoxitin; CIP, ciprofloxacin; G, gentamicin; NN, tobramycin; AN, amikacin; AmpC, AmpC β-lactamase; R, resistant; I, intermediately resistant; S, susceptible; ESBL, extended-spectrum β-lactamase.

Azide$^R$ recipient by conjugation was successful in only 14 of the 27 isolates with an ESBL phenotype, despite multiple attempts. PCR experiments detected genes encoding members of the CTX-M-1 and CTX-M-9 clusters in 22 (81.5%) and 7 (25.9%) isolates, respectively. CTX-M-15 (N=14) was the most common type of ESBL, although genes encoding CTX-M-3 (N=8), CTX-M-14 (N=6), and CTX-M-9 (N=1) were also detected. Genes encoding TEM-type β-lactamases were detected in 20 of the 27 isolates, although all but one were identified as TEM-1; the isolate KU05/14517 carried the *bla*$_{TEM-52}$ gene. The *bla*$_{SHV-12}$ gene was detected in two isolates. Four isolates carried multiple ESBL genes. Non-TEM and non-SHV ESBLs including VEB, GES, and TLA enzymes, as well as members of the CTX-M-2 and CTX-M-8 clusters, were not detected in this study.

### 2. Other antimicrobial resistance genes in ESBL-producing isolates

Genes encoding DHA-1 and CMY-10 AmpC β-lactamases were detected in one and two CTX-M-3-harboring isolates, respectively. The *qnrB4* and *qnrS1* genes were detected each in one isolate harboring CTX-M-3 and CTX-M-15, respectively. Genes encoding AAC(6')-Ib-cr were detected in 19 (70.4%) isolates, irrespective of the ESBL genotype. Lastly, the *rmtB* and *armA* genes, both of which encode a 16S rRNA methylase, were detected in one and four isolates, respectively.

### 3. Phenotypic characteristics of ESBL-producing isolates

The cefotaxime MICs for three isolates harboring CTX-M-14 were ≥256 μg/mL, which was more than 16 times higher than ceftazidime MIC (4-16 μg/mL) (Table 2). The isolates harboring CTX-M-15 exhibited higher levels of resistance (MIC, 32→256 μg/mL) to ceftazidime than those harboring CTX-M-14. Clavulanic acid restored the activities of cefotaxime and ceftazidime in all but two of the ESBL-producing isolates, both of which contained CMY-10. All of the 27 isolates harboring ESBLs were resistant (MIC, ≥16 μg/mL) to ciprofloxacin, irrespective of the presence of *qnr* or *aac(6')-Ib-cr* genes. The five isolates producing 16S rRNA methylases were resistant or intermediate to amikacin.

Table 3. Phylogenetic background of the 27 ESBL-producing urinary *E. coli* isolates

| ESBL gene | Phylogenetic group | | | | Total |
|---|---|---|---|---|---|
| | A | B1 | B2 | D | |
| *bla*$_{CTX-M-3}$ | 2 | | 3 | 1 | 6 |
| *bla*$_{CTX-M-15}$ | 2 | 1 | 9 | 1 | 13 |
| *bla*$_{CTX-M-14}$ | | | | 3 | 3 |
| *bla*$_{TEM-52}$ | | | 1 | | 1 |
| *bla*$_{CTX-M-14}$+*bla*$_{CTX-M-15}$ | | | | 1 | 1 |
| *bla*$_{CTX-M-3}$+*bla*$_{CTX-M-14}$ | | | | 1 | 1 |
| *bla*$_{CTX-M-14}$+*bla*$_{SHV-12}$ | | | 1 | | 1 |
| *bla*$_{CTX-M-3}$+*bla*$_{CTX-M-9}$+*bla*$_{SHV-12}$ | | 1 | | | 1 |
| Total | 4 | 2 | 14 | 7 | 27 |

Abbreviation: ESBL, extended-spectrum β-lactamase.

### 4. Phylogenetic groups

Most of the ESBL-producing isolates were within phylogenetic groups B2 (N=14, 51.9%) and D (N=7, 25.9%) (Table 3). Five (83.3%) of the six isolates producing CTX-M-14 were within group D, while nine (64.3%) of the fourteen isolates producing CTX-M-15 were within group B2.

## DISCUSSION

UTI is a common cause of morbidity in either healthy persons or in patients with various underlying diseases. The *E. coli* associated UTIs can progress to bacteremia, which are often treated with different broad-spectrum antibiotics because of concerns about infections with various resistant mechanisms [24]. Many antibiotics used in UTIs are usually capable of reaching and maintaining high urinary concentrations that are much greater than the MIC of the causative *E. coli* [25]. It might be argued that MIC and other pharmacokinetic parameters are less useful for the treatment of UTIs [25].

In 2003, we showed that only 3.3% (8/246) of clinical *E. coli* isolates produced CTX-M ESBLs [18]; however, in the present study, the prevalence of these enzymes had increased to 9.8% (26/264), indicating that CTX-M enzymes have become the dominant type of ESBL in Korea as shown by

another recent study [26]. Compared with our 2003 survey, the incidence of CTX-M-3 and CTX-M-15 within the CTX-M-1 cluster increased from 1.2% (3/246) to 3.0% (8/264) and from 1.6% (4/246) to 5.7% (15/264), respectively. The incidence of CTX-M-14 within the CTX-M-9 cluster increased from 0.4% (1/246) to 2.3% (6/264). Previous reports found that the most common ESBL in *E. coli* isolates from Korea was TEM-52 [7]; however, that particular ESBL was detected in only one isolate in our study. Furthermore, SHV-12, which was the most common ESBL in *K. pneumoniae* in 2003 [27], was detected in only two isolates in this study.

The epidemiology of CTX-M enzyme-producing organisms is different from those of TEM or SHV ESBL-producing organisms [28]. Community-acquired infections caused by organisms producing CTX-M enzymes have been described frequently, typically as urinary tract infections by *E. coli*. Organisms producing CTX-M enzymes frequently carry other antimicrobial resistance genes. Indeed, genes encoding plasmid-mediated AmpC β-lactamases and methylase were detected in three (11.5%) and five (19.2%) of the 26 *E. coli* isolates harboring CTX-M enzymes, respectively. It was surprising that all (N=27) of the ESBL-producing *E. coli* isolates were also resistant to FQs, irrespective of the presence of *qnr* and/or *aac(6')-Ib-cr* genes. These results indicate that the primary mechanism of FQ resistance might be through the accumulation of mutations in genes encoding DNA gyrase and topoisomerase IV.

Virulent extra-intestinal strains belong mainly to group B2 and, to a lesser extent, group D, whereas most commensal strains belong to group A and B1 [29]. A past report showed that most FQ-resistant *E. coli* isolates that cause UTIs are from non-B2 groups (notably, group A, D, and B1) [30]. However, more than half (14/27) of our FQ-resistant *E. coli* isolates harboring CTX-M enzymes were within group B2. *E. coli* isolates within group B2 harbor many virulent factors [1]. Furthermore, strains producing CTX-M enzymes are more likely to cause repeated UTIs than strains that do not produce these enzymes, because strains producing CTX-M enzymes commonly contain *iha*, which encodes an adhesin-siderophore receptor associated with an increased risk for recurrent UTIs [31].

In conclusion, CTX-M enzymes are the dominant type of ESBL found in urinary *E. coli* isolates. All of the isolates harboring CTX-M enzymes were resistant to ciprofloxacin, and it was not uncommon for these strains to also contain other antimicrobial resistance genes encoding AmpC β-lactamases, Qnr, AAC(6')-Ib-cr, and 16S rRNA methylases. More than half of the urinary *E. coli* isolates harboring CTX-M enzymes were within the phylogenetic group B2, and might therefore cause problematic and recurrent UTIs.

## 요 약

**배경 :** 본 연구에서는 요 검체에서 분리된 extended-spectrum β-lactamase (ESBL) 생성 *Escherichia coli*의 내성 특성을 조사하고, 이들 ESBL 생성 균주의 다른 항균제 내성 유전자의 보유율을 알아보았다.

**방법 :** 2005년 국내 3차 병원의 요 검체에서 분리된 *E. coli* 264주를 대상으로 하였다. 항균제 감수성은 디스크 확산법 및 한천희석법으로 시험하였으며, double-disk synergy (DDS)법을 사용하여 ESBL 생성을 확인하였다. PCR 및 염기서열 분석으로 항균제 내성의 유전형을 분석하였다. 균주의 *chuA*, *yjaA* 및 TSPE4 보유 여부에 따라 계통발생학적 분류를 하였다.

**결과 :** 총 264주 중 27주가 DDS 양성이었으며, CTX-M-15 (N=14), CTX-M-3 (N=8), CTX-M-14 (N=6)의 순으로 ESBL이 검출되었으며, ESBL 생성 균주 모두는 ciprofloxacin에 내성이었다. ESBL 생성 균주 중 각 1주와 2주에서 DHA-1과 CMY-10 AmpC β-lactamase, 5주에서 16S rRNA methylase, 2주에서 Qnr, 19주에서 AAC(6')-Ib-cr를 생성하였다. ESBL 생성 균주 대부분은 계통발생학적 B2군(N=14, 51.9%) 혹은 D군(N=7, 25.9%)에 속하였다.

**결론 :** 요 검체에서 분리된 *E. coli*가 생성하는 ESBL의 다수는 CTX-M형이었으며, ESBL 생성 균주 중 타 계열의 항균제에 내성을 부여하는 유전자를 지닌 경우가 흔하였다. CTX-M형 ESBL 생성 균주의 반 이상이 계통발생학적 B2군에 속하였다.

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