

## Differences in Antimicrobial Resistance Phenotypes by the Group of CTX-M Extended-Spectrum $\beta$ -Lactamase

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**Background:** *Escherichia coli* and *Klebsiella pneumoniae* clinical isolates producing CTX-M extended-spectrum  $\beta$ -lactamases (ESBLs) were assessed for antimicrobial resistance phenotypes varied by group of enzymes.

**Methods:** A total of 1,338 blood isolates, including 959 *E. coli* and 379 *K. pneumoniae*, were studied. All the strains were collected between January and July 2017 from eight general hospitals in South Korea. The species were identified by matrix-assisted laser desorption ionization-time of flight mass spectrometry. Antimicrobial susceptibilities were determined by disk diffusion methods and ESBL phenotypes by double-disk synergy tests using disks containing cefotaxime, ceftazidime, cefepime, aztreonam, and clavulanic acid (CA). The genes for  $\beta$ -lactamases were identified by PCR and sequencing.

**Results:** Of total microbes, 31.6% (303/959) *E. coli* and 24.0% (91/379) *K. pneumoniae* were resistant to cefotaxime and 28.1% (269/959) *E. coli* and 20.1%

(76/379) *K. pneumoniae* were CTX-M-type ESBL producers. Among the detected CTX-M ESBLs, 58.0% (156/269) in *E. coli* and 86.8% (66/76) in *K. pneumoniae* belonged to group 1, 46.8% (126/269) in *E. coli* and 14.5% (11/76) in *K. pneumoniae* were group 9. Ten *E. coli* and one *K. pneumoniae* isolates co-produced both groups of CTX-M ESBL. The group 1 CTX-M producers had a higher level of resistance to cefotaxime, ceftazidime, cefepime, and aztreonam and exhibited stronger synergistic activities when combined with CA compared to group 9.

**Conclusion:** ESBL phenotypes differ by CTX-M ESBL group and phenotype testing with drugs including 4<sup>th</sup> generation cephalosporins and monobactams is critical for screening CTX-M-producers with better sensitivity. (Ann Clin Microbiol 2019;22:1-8)

**Key Words:** CTX-M, *Escherichia coli*, Extended-spectrum  $\beta$ -lactamase, *Klebsiella pneumoniae*

### INTRODUCTION

Extended-spectrum cephalosporins are one of the preferred choice for empirical therapy for infections by Gram-negative bacteria. However, dissemination of the extended-spectrum  $\beta$ -lactamases (ESBLs) conferring resistance to wide-range of  $\beta$ -

lactams including the 3<sup>rd</sup> and 4<sup>th</sup> generation cephalosporins and monobactams, complicates antimicrobial therapy in the clinical settings [1].

Among over 10 families of ESBLs documented to date [2], the plasmid-mediated cefotaximase CTX-M is the most rapidly growing family with a significant clinical impact [3]. The

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CTX-M ESBL was first identified in Japan [4] and designated in Germany [5]. The growing family consists with heterogeneous groups of enzymes. The amino acid sequence alignment of CTX-M variants categorized the enzymes into five groups, *i.e.*, 1, 2, 8, 9, and 25, sharing >94% amino acid identity within a group and ≤90% amino acid identity between groups [6]. The groups are also differed by the hydrolytic property. The first cefotaximase CTX-M-3, renamed from FEC-1, did not confer resistance to ceftazidime [4] and the following CTX-M variants also confer resistance to cefotaxime. The C7  $\beta$ -amino thiazol-oxyimino-amide side chain protected the ceftazidime against majority of the CTX-M ESBLs [7]. However, some CTX-M ESBLs, mostly belonging to the group 1, had broader substrate-spectrum including ceftazidime resulting in varied resistance phenotypes. For instance, the dominant CTX-M ESBL CTX-M-15 a representative group 1 enzyme confers resistance to ceftazidime, while the other dominant CTX-M-14 a representative group 9 CTX-M ESBL does not.

Clavulanic acid (CA) is an effective inhibitor for ESBL including CTX-M, and the combination with amoxicillin and ticarcillin, which are both good substrates for the ESBLs, are used in the clinical settings for the infection treatment [8]. By using this activity, CA is widely used for synergy test to detect ESBL producers in the laboratory [9]. CA induces the AmpC production, which can mask the synergistic effects of cephalosporins with CA by inhibiting ESBLs when a bacterial host carries both an ESBL and an AmpC [10].

The differed activity and spectrum between the groups of CTX-M ESBLs are empirical facts, however the phenotypic differences are indeed controversial. Thereby, this study was designed to evaluate the differences in resistance phenotypes by the group of CTX-M ESBLs.

## MATERIALS AND METHODS

### 1. Bacterial strains

Non-duplicate 1,338 blood isolates including 959 *E. coli* and 379 *K. pneumoniae* were collected between January and July 2017 from eight general hospitals participating in Korea GLobal Antimicrobial resistance Surveillance System. The species were identified by matrix assisted laser desorption ionization-time of flight mass spectrometry using MALDI Biotyper® (Bruker Daltonik GmbH, Bremen, Germany) and/or 16S rDNA sequencing.

### 2. Antimicrobial susceptibility and double-disk synergy testing

Antimicrobial susceptibilities to cefotaxime, ceftazidime, cefepime, and aztreonam were tested by the disk diffusion method on Mueller-Hinton agar (Difco Laboratories, Detroit, MI, USA), following the Clinical and Laboratory Standards Institute guidelines [11]. Further ESBL-testing was conducted for the isolates non-susceptible to both cefotaxime and/or ceftazidime, following the EUCAST guidelines [12] with slight modification. Both *E. coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used for quality control of each batch of experiment. Double-disk synergy test (DDST) was performed using disks (Oxoid Ltd., Basingstoke, UK) containing cefotaxime (30  $\mu$ g), ceftazidime (30  $\mu$ g), cefepime (30  $\mu$ g), aztreonam (30  $\mu$ g), and CA (10  $\mu$ g). The CA disk was freshly made using 6 mm-sized filter disks (Adventec Toyo Kaisha, Ltd., Tokyo, Japan). The inhibition zone diameters of each drug and enlarged zone of inhibition toward a CA disk were recorded and the synergy by CA (SC) was determined by dividing the enlarged zone diameter toward a CA disk with the original zone diameter.

**Table 1.** The 3<sup>rd</sup> and 4<sup>th</sup> generation cephalosporins and monobactam susceptibilities of *Escherichia coli* and *Klebsiella pneumoniae* blood isolates\*

| Isolates                        | Cefotaxime     |              |                | Ceftazidime    |              |                  | Cefepime       |               |                | Aztreonam      |              |                  |
|---------------------------------|----------------|--------------|----------------|----------------|--------------|------------------|----------------|---------------|----------------|----------------|--------------|------------------|
|                                 | R              | I            | S              | R              | I            | S                | R              | I             | S              | R              | I            | S                |
| <i>E. coli</i><br>(n=959)       | 303<br>(31.6%) | 14<br>(1.5%) | 642<br>(66.9%) | 106<br>(11.0%) | 70<br>(7.3%) | 783<br>(81.6%)   | 189<br>(19.7%) | 92<br>(9.5%)  | 678<br>(70.7%) | 180<br>(18.8%) | 38<br>(4.0%) | 741<br>(77.3%)   |
| <i>K. pneumoniae</i><br>(n=379) | 91<br>(24.0%)  | 0<br>(0%)    | 288<br>(76%)   | 76<br>(20.0%)  | 5<br>(1.3%)  | 298<br>(78.6%)   | 74<br>(19.5%)  | 13<br>(3.4%)  | 292<br>(77%)   | 81<br>(21.4%)  | 2<br>(0.5%)  | 296<br>(78.1%)   |
| Total<br>(n=1,338)              | 394<br>(29.5%) | 14<br>(1.0%) | 930<br>(69.5%) | 182<br>(13.6%) | 75<br>(5.6%) | 1,081<br>(80.8%) | 263<br>(19.7%) | 105<br>(7.8%) | 970<br>(72.5%) | 261<br>(19.5%) | 40<br>(3.0%) | 1,037<br>(77.5%) |

\*The breakpoints were applied according to the Clinical and Laboratory Standards Institute guideline [11].

Abbreviations: R, resistant; I, intermediate; S, susceptible.

### 3. DNA manipulation and PCR

Total DNA was extracted by using MG Cell genomic DNA extraction kit (MGmed Inc., Seoul, South Korea) and, by using the extracted total DNA, PCR and sequencing were carried out to detect the genes for carbapenemases, *i.e.*, *bla*<sub>KPC</sub>, *bla*<sub>NDM</sub>, *bla*<sub>IMP</sub>, *bla*<sub>VIM</sub>, *bla*<sub>GES</sub>, and *bla*<sub>OXA-48</sub> [13]; ESBLs, *i.e.* for *bla*<sub>CTX-M-1-like</sub>, *bla*<sub>CTX-M-9-like</sub>, and *bla*<sub>CTX-M-25-like</sub>; and plasmid-mediated AmpCs, *i.e.*, *bla*<sub>CMY</sub>, *bla*<sub>DHA</sub>, and *bla*<sub>ACC</sub> [14,15].

### 4. Statistical analysis

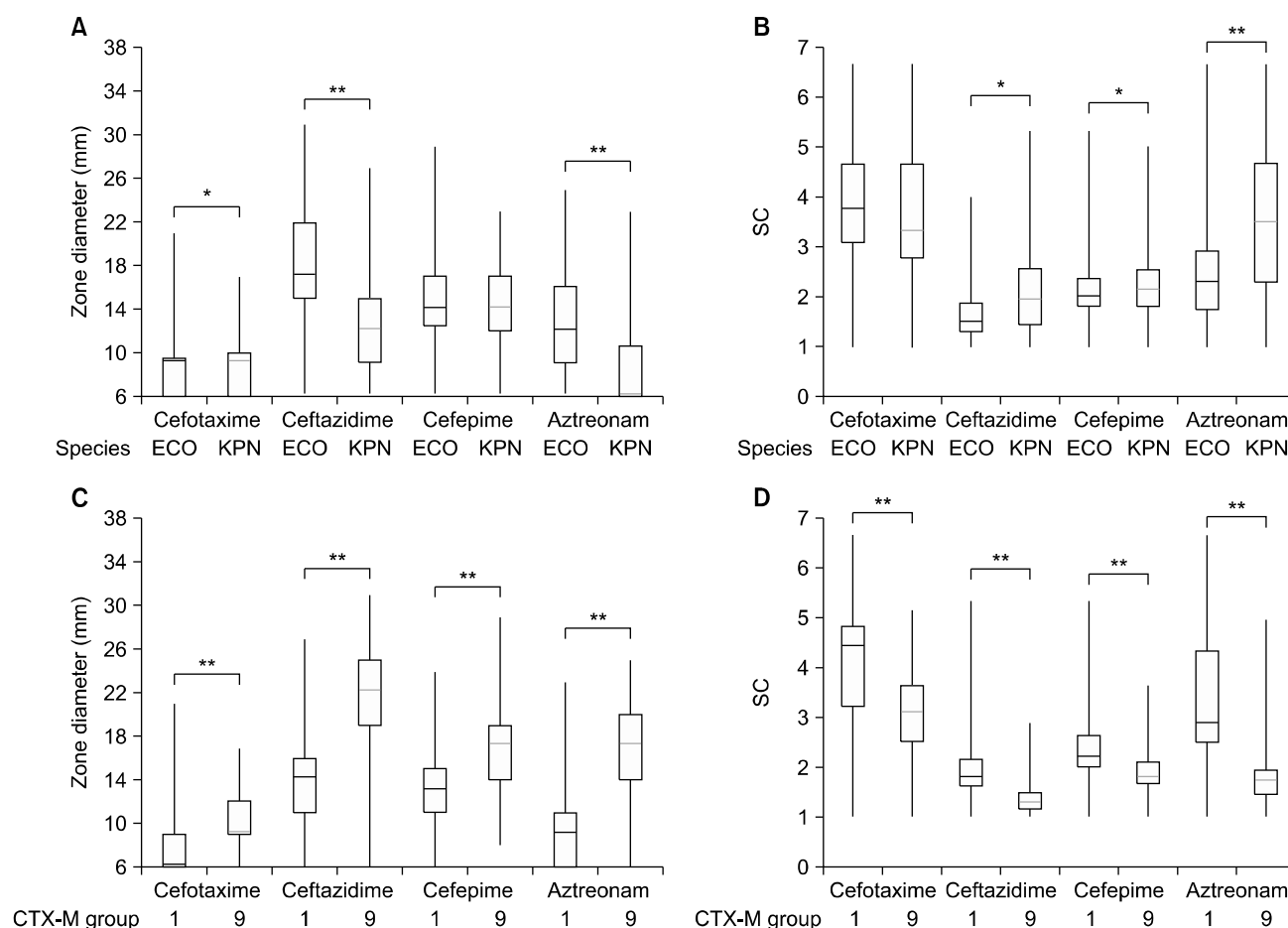
Differences existed between groups were calculated by chi-square or *t*-test [16] and the correlation coefficient (*r*) for simple linear regression was calculated using Pearson's correla-

tion [16] by the parametric method, both using SPSS statistics (version 23, IBM Corp., Armonk, NY, USA). All tests were two-tailed, and *P* value of <0.05 was determined to represent statistical significance.

## RESULTS

### 1. Resistance to extended-spectrum cephalosporins and aztreonam in *E. coli* and *K. pneumoniae* clinical isolates

Of total, 31.6% (303/959) *E. coli* and 24.0% (91/379) *K. pneumoniae* were resistant to cefotaxime and 19.7% (n=189) *E. coli* and 19.5% (n=74) *K. pneumoniae* were resistant to ceftazidime (Table 1). Resistance rates for ceftazidime and aztreonam in *E. coli* were 11.0% (n=106) and 18.8% (n=180), respectively, and those in *K. pneumoniae* were 20.0% (n=76) and 21.4%



(n=81), respectively. A total of 407 isolates including 315 (32.8%) *E. coli* and 92 (24.3%) *K. pneumoniae* met criteria for further ESBL-testing [12] and 282 *E. coli* and 88 *K. pneumoniae* exhibited ESBL phenotypes by DDST.

For the DDST-positive isolates, zone diameters of cefotaxime and cefepime were equivalent by bacterial species, presenting median values of 9 mm and 14 mm, respectively, while those of ceftazidime (17 mm vs. 12 mm) and aztreonam (12 mm vs. 6 mm) were greater in *E. coli* than in *K. pneumoniae* (Fig. 1A).

## 2. CTX-M-type ESBL-producing *E. coli* and *K. pneumoniae*

Of the DDST-positive isolates, 95.4% (269/282) *E. coli* and

**Table 2.** CTX-M-type ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae*

| Beta-lactamase                  | <i>E. coli</i><br>(n=269) | <i>K. pneumoniae</i><br>(n=76) |
|---------------------------------|---------------------------|--------------------------------|
| Group 1 CTX-M producer          | 146 (54.3%)               | 65 (85.5%)                     |
| CTX-M-1                         | 2                         | -                              |
| CTX-M-3                         | 3                         | 3                              |
| CTX-M-15                        | 103                       | 43                             |
| CTX-M-15, KPC-2                 | -                         | 1                              |
| CTX-M-15, CMY-2                 | 1                         | -                              |
| CTX-M-15, DHA-1                 | -                         | 11                             |
| CTX-M-15, SHV-2, DHA-1          | -                         | 1                              |
| CTX-M-15, SHV-25                | -                         | 1                              |
| CTX-M-15, SHV-28                | -                         | 1                              |
| CTX-M-15, SHV-28, DHA-1         | -                         | 1                              |
| CTX-M-28                        | 5                         | 1                              |
| CTX-M-28, DHA-1                 | -                         | 1                              |
| CTX-M-55                        | 30                        | 1                              |
| CTX-M-79                        | 1                         | -                              |
| CTX-M-123                       | 1                         | -                              |
| Group 9 CTX-M producer          | 113 (42.0%)               | 10 (13.1%)                     |
| CTX-M-9                         | -                         | 1                              |
| CTX-M-14                        | 57                        | 4                              |
| CTX-M-14, CMY-1                 | 1                         | -                              |
| CTX-M-14, DHA-7                 | -                         | 1                              |
| CTX-M-14, SHV-27                | -                         | 1                              |
| CTX-M-17                        | 18                        | 1                              |
| CTX-M-24                        | 4                         | 1                              |
| CTX-M-24, DHA-17                | 1                         | -                              |
| CTX-M-27                        | 29                        | 1                              |
| CTX-M-98, DHA-1                 | 1                         | -                              |
| CTX-M-113                       | 1                         | -                              |
| CTX-M-129                       | 1                         | -                              |
| Group 1 and 9 CTX-M co-producer | 10 (3.7%)                 | 1 (1.3%)                       |
| CTX-M-15, CTX-M-14              | 3                         | -                              |
| CTX-M-15, CTX-M-17              | 2                         | -                              |
| CTX-M-15, CTX-M-24              | 2                         | -                              |
| CTX-M-15, CTX-M-27              | 2                         | 1                              |
| CTX-M-15, CTX-M-98              | 1                         | -                              |

86.4% (76/88) *K. pneumoniae* were identified as CTX-M ESBL producers by PCR (Table 2). In both enterobacterial species, the group 1 CTX-M ESBL was more identified than the group 9: 146:113 in *E. coli* and 65:10 in *K. pneumoniae*. Ten *E. coli* and one *K. pneumoniae* co-produced both groups of CTX-M ESBL. Co-carrying  $\beta$ -lactamase genes were identified in 6.7% (23/345) CTX-M ESBL producers, including 25.0% (19/76) *K. pneumoniae* and 1.5% (4/269) *E. coli*: *bla*<sub>KPC-2</sub> in one *K. pneumoniae*, *bla*<sub>SHV</sub> for ESBL in three *K. pneumoniae*, both *bla*<sub>SHV</sub> for ESBL and *bla*<sub>DHA</sub> in two *K. pneumoniae*, and *bla*<sub>DHA</sub> in 13 *K. pneumoniae*, and either of *bla*<sub>CMY</sub> or *bla*<sub>DHA</sub> in four *E. coli*. The CTX-M-negative isolates with ESBL-phenotype included one KPC-2-producing *K. pneumoniae* and three SHV-12-producing *K. pneumoniae* isolates and the rest 21 isolates were negative for the known ESBLs.

The 222 CTX-M group 1 ESBLs identified were mainly composed with CTX-M-15 (78.4%, n=174) and CTX-M-55 (14.0%, n=31) and CTX-M-28 (3.2%, n=7), CTX-M-3 (2.7%, n=6), CTX-M-1 (0.9%, n=2), CTX-M-79 (0.5%, n=1), and CTX-M-123 (0.5%, n=1) were followed. The CTX-M group 9 ESBLs mostly comprised CTX-M-14 (50.0%, 67/134), CTX-M-27 (24.6%, n=33), CTX-M-17 (15.7%, n=21), and CTX-M-24 (6.0%, n=8) and, as minor, CTX-M-98 (1.5%, n=2), CTX-M-9 (0.7%, n=1), CTX-M-113 (0.7%, n=1), and CTX-M-129 (0.7%, n=1) were included in the group. Of the 174 CTX-M-15 producers, eleven isolates co-produced group 9 CTX-M ESBLs, such as CTX-M-14 (n=3), CTX-M-27 (n=3), CTX-M-17 (n=2), CTX-M-24 (n=2), and CTX-M-98 (n=1).

## 3. Resistance phenotypes conferred by CTX-M ESBL

By the group of CTX-M enzymes, the zone diameters by the disks containing extended-spectrum cephalosporins or monobactams were smaller in group 1 than those in group 9 exhibiting the median values of 6 mm vs. 9 mm for cefotaxime, 14 mm vs. 22 mm for ceftazidime, 13 mm vs. 17 mm for cefepime, and 9 mm vs. 17 mm for aztreonam (Fig. 1C). The zone diameters of 11 *Enterobacteriaceae* producing both group 1 and group 9 CTX-M ESBLs were close to those in group 1 CTX-M ESBL producers rather than those in the group 9 producer: median values at 6 mm for cefotaxime, 12 mm for ceftazidime, 11 mm for cefepime, and 9 mm for aztreonam. Co-production of either SHV ESBL or AmpC in *K. pneumoniae* retained the inhibition zone diameters: median values of zone diameter at 9 mm for cefotaxime, 10 mm for ceftazidime, 14 mm for cefepime, and 6 mm for aztreonam in SHV ESBL co-producers and 9 mm for

cefotaxime, 11 mm for ceftazidime, 14 mm for cefepime, and 6 mm for aztreonam in AmpC co-producers. The KPC-2 carbapenemase co-producer exhibited the reduced inhibition zones, 6 mm for cefotaxime, 9 mm for ceftazidime, 9 mm for cefepime, and 6 mm for aztreonam.

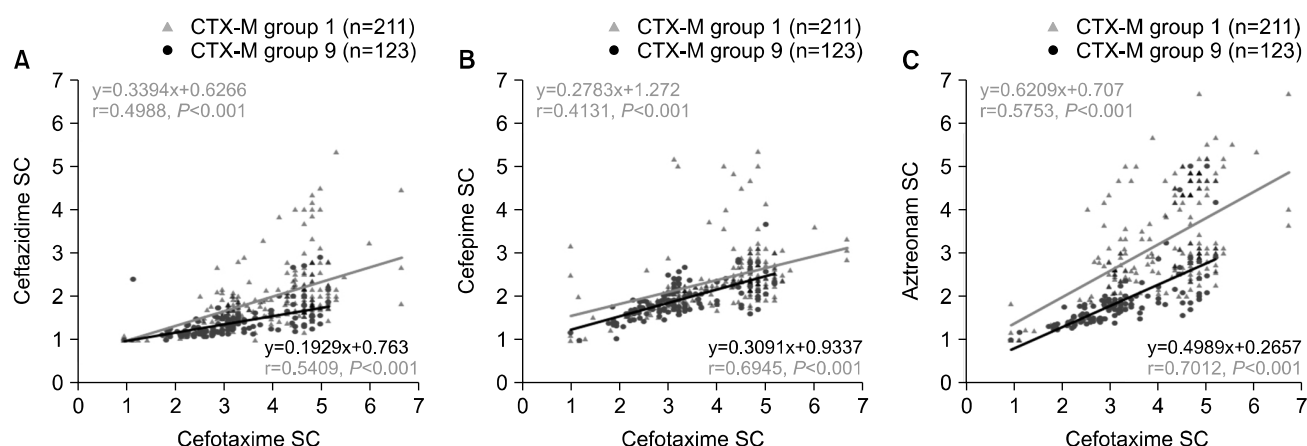
The synergistic effect with CA was evaluated using SC values (Fig. 1B-D). The cefotaxime SC was the greatest (median, 3.66) and followed by those of aztreonam (2.50), cefepime (2.14), and ceftazidime (1.63). The median SC for cefotaxime was greater in *E. coli* than in *K. pneumoniae* (3.78:3.33), while those of the other three drugs were less in *E. coli*, 1.56:2.00 for ceftazidime, 2.08:2.19 for cefepime, and 2.36:3.50 for aztreonam. By the group of CTX-M ESBLs, the SCs were always greater in the group 1 CTX-M ESBL compared to those in the group 9: 4.44:3.11 for cefotaxime, 1.83:1.30 for ceftazidime, 2.23:1.83 for cefepime, and 2.91:1.75 for aztreonam.

The group 9 CTX-M producers displayed stronger linear relationships between cefotaxime SC and either SCs of ceftazidime, cefepime, and aztreonam (Fig. 2) compared to the group 1 CTX-M ESBL producers having correlation coefficients ( $r$ ): 0.5409:0.4988 for ceftazidime SC, 0.6945:0.4131 for cefepime SC, and 0.7012:0.5753 for aztreonam SC. Slopes were calculated by comparing the SCs for ceftazidime, cefepime, and aztreonam with the cefotaxime SC. The group 1 CTX-M ESBL producers exhibited steeper slopes than the group 9 producers for ceftazidime SC (0.3394:0.1929) and for aztreonam SC (0.6209:0.4989), while no slope difference between the two groups was observed for cefepime SC (0.2783:0.3091).

## DISCUSSION

Continuing increase in CTX-M-type ESBL-producing *Enterobacteriaceae*, mainly led by the predominant CTX-M-15 of group 1 and CTX-M-14 of group 9 [17], triggers more usage of last options for the treatment resulting in vicious antimicrobial resistance. In South Korea, cefotaxime resistance reached at 38% for *E. coli* and at 35% for *K. pneumoniae* clinical isolates in 2015 [18] and it was associated with dominance of both CTX-M-15 and -14 since the mid-2010s [19]. The enterobacterial blood isolate collection of seven-month-period in 2017 presented cefotaxime resistance rates at 31.6% for *E. coli* and at 24.0% for *K. pneumoniae*, which were less than those in 2015, probably due to the differed specimens and surveillance systems. The dominant type of group 1 was CTX-M-15 (78.4%) and that of group 9 was CTX-M-14 (50.0%).

The rate of human carriages of CTX-M producers are increasing [17] and the dissemination is issuing not only among human beings but also for animals [20,21] and environments [22]. The resistance rates for cefotaxime (5.0%) and cefepime (1.4%) in *E. coli* from food animals [23] and in *Enterobacteriaceae* from edible vegetables (10.1% for cefotaxime and 1.1% for cefepime) [24] were comparably lower than those in clinical isolates. However, the prevalence of CTX-M ESBLs (78.6% of cefotaxime-resistant *E. coli* from food animals and 73.7% of cefotaxime-resistant *Enterobacteriaceae* from vegetables) and the dominant types of the enzyme (CTX-M-14 and CTX-M-15) corresponded to those of clinical isolates, highlighting the



**Fig. 2.** Correlation between synergy with clavulanic acid (SC) of cefotaxime and ceftazidime SC (A), cefepime SC (B), and aztreonam SC (C). The SC was calculated by dividing the enlarged zone diameter near the clavulanic acid disk by inhibition zone diameter of each drug. The correlation coefficient ( $r$ ) was calculated using Pearson's correlation [16] by the parametric method using SPSS statistics and the significance ( $P$ ) was determined by a two-tailed method using the correlation value and the sample size. Each dot indicates one strain, gray triangles and black dots indicate strains producing CTX-M group 1 and group 9, respectively.

One-Health concepts.

Inhibition zone sizes of the tested drugs were always smaller for the group 1 CTX-M ESBL than those for the group 9 confirming that the group 1 enzyme confers higher level of resistance to the drugs than the group 9 [25]. As well, the SCs, indicating the hydrolytic activity inhibited by CA, were greater for the tested drugs in the group 1 CTX-M ESBL compared to those in the group 9 supporting that the group 1 enzymes hydrolyzed more the drugs than those belonging to the group 9. It is noteworthy that the ceftazidime SC in group 9 CTX-M ESBL is close to 1, agreeing the point that ceftazidime resistance, which has been used in practice as an indicator of ESBL producers, frequently leads to fail recognizing CTX-M-producers [6]. The higher prevalence of CTX-M ESBLs, which is the case of this study, may elevate the false-negative error rates [26]. Masked synergistic activity with CA by induced AmpC [10] was not observed in this study, doubtlessly because intrinsic AmpC producing enterobacterial species, including *Enterobacter* spp., *Serratia marcescens*, and *Citrobacter freundii*, were not included in the study.

The SCs for ceftazidime and aztreonam compared with that for cefotaxime pronounced well the differed range of substrates by group of CTX-M ESBL. The linear correlation of SCs for ceftazidime and aztreonam with that for cefotaxime in group 1 CTX-M ESBL presented steeper slope than those in group 9 CTX-M ESBL verifying the hydrolytic activity of the group 9 restricted to cefotaxime, while the group 1 has expanded range of the substrate. It correlates well to the biochemical properties demonstrated by kinetic constants [27,28].

Bacterial host itself seemed having little impact on the level of resistance to the tested drugs. CTX-M-producing *K. pneumoniae* presented higher levels of resistance to ceftazidime and aztreonam compared to *E. coli*. Since ceftazidime and aztreonam presented greater differences in the group 1 CTX-M compared to cefotaxime and cefepime, it is likely that the higher proportion of group 1 CTX-M in *K. pneumoniae* than in *E. coli* led the difference. The SCs differed by the bacterial species were spotted in aztreonam, which is also explained by the predominance of group 1 CTX-M ESBL in *K. pneumoniae*.

Finally, this study confirmed that the phenotype testing with drugs including 4<sup>th</sup> generation cephalosporins and monobactams is critical for screening the CTX-M-producers with better sensitivity [16]. From 0.9% to 2.9% CTX-M ESBL producers had SCs at 1 for at least one drugs, which means no obvious synergistic effect with CA was obtained for cefotaxime (n=5), cefta-

zidime (n=10), cefepime (n=3), and aztreonam (n=5). Moreover, four strains (1.2%) had both cefotaxime SC and ceftazidime SC at 1, and the simplified DDST using only the two drugs could have missed the CTX-M ESBL producers. Estimating the group of CTX-M ESBL by DDST was unsound, however evaluating the composition of CTX-M ESBL producer population would be possible somehow by using linear correlation for SCs of cefotaxime SC-ceftazidime SC and cefotaxime SC-aztreonam SC.

This study had aware imperfections: i) the restricted bacterial species, ii) only isolations from blood specimens of patients in one country, and iii) lack of other groups of CTX-M ESBLs, such as the group 2 and the group 25. However, at least, the most clinically relevant panel of ESBL producers in the world, group 1 and 9 CTX-M-producing *E. coli* and *K. pneumoniae*, were covered and the necessity of complete set of testing drugs for DDST in the medical laboratory was emphasized. The not-yet-determined ESBLs in the strains exhibiting ESBL phenotypes are going to be further studied.

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=국문초록=

## CTX-M 광범위 베타락탐 분해효소의 그룹별 내성표현형 차이

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**배경:** CTX-M 광범위 베타락탐 분해효소(CTX-M-type extended-spectrum  $\beta$ -lactamase, ESBL)를 생성하는 임상검체 분리 장세균을 대상으로 CTX-M 효소의 그룹별 내성표현형의 차이를 비교하였다.

**방법:** 2017년 1월부터 7월에 국내 8개 종합병원 환자의 혈액에서 분리된 총 1,338주의 장세균(*Escherichia coli* 959주 및 *Klebsiella pneumoniae* 379주)을 대상으로 하였다. 디스크 확산법으로 항균제 감수성을 시험하였고, 3세대 세팔로스포린계 세포타심 및 세프트지딤, 4세대 세팔로스포린계 세페뎀 및 모노박탐계 아즈트레오남과 클라불란산 간의 항균력 상승작용을 이중 디스크 확산법으로 확인하고, 확장된 억제대의 크기를 측정하였다. 내성유전형은 PCR 및 염기서열분석으로 확인하였다.

**결과:** *E. coli*의 31.6% (303/959)와 *K. pneumoniae*의 24.0% (91/379)가 세포타심에 내성이었고, *E. coli* 28.1% (269/959)와 *K. pneumoniae* 20.1% (76/379)에서 CTX-M ESBL 유전자가 검출되었다. *E. coli*와 *K. pneumoniae*에서 검출된 CTX-M 유전자의 58.0% (156/269)와 86.8% (66/76)가 그룹 1 효소였고, 46.8% (126/269)와 14.5% (11/76)는 그룹 9 효소였다. *E. coli* 10주와 *K. pneumoniae* 1주는 CTX-M 그룹 1과 9 유전자를 모두 지니고 있었다. CTX-M 그룹 1 효소를 생성하는 균주는 그룹 9 효소를 생성하는 균주보다 세포타심, 세프트지딤, 세페뎀 및 아즈트레오남 디스크에 의한 억제대가 작았으며, 클라불란산과의 상승작용에 의해 억제대가 더 크게 확장되는 양상을 보였다.

**결론:** CTX-M 그룹 1과 그룹 9 효소를 생성하는 균주는 차별되는 내성표현형을 보였다. 이중 디스크 확산법에 의한 CTX-M 효소 생성 균주의 검출 민감도를 높이기 위해서는 3세대 세팔로스포린계 세포타심과 세프트지딤 디스크뿐만 아니라 4세대 세팔로스포린계 및 모노박탐계 항균제 디스크도 함께 사용하는 것이 요구된다. [Ann Clin Microbiol 2019; 22:1-8]

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