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In Vitro Activities of 21 Antimicrobial Agents Alone and in Combination with Aminoglycosides or Fluoroquinolones against Extended-Spectrum-β-Lactamase-Producing *Escherichia coli* Isolates Causing Bacteremia

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We evaluated the *in vitro* activity of various antimicrobials alone and in combination against 291 extended-spectrum-β-lactamase-producing *Escherichia coli* (ESBL-EC) isolates causing bacteremia in South Korean hospitals. Ceftazidime, cefepime, and piperacillin-tazobactam in combination with amikacin showed greater activity than found in combination with ciprofloxacin. In settings with a high prevalence of ESBL-producing pathogens, combination aminoglycoside antimicrobial therapy, especially with amikacin, may be considered for empirical therapy against suspected Gram-negative sepsis as a carbapenem-saving strategy.

The emergence of extended-spectrum-β-lactamase-producing *Escherichia coli* (ESBL-EC) in the community, particularly those producing CTX-M β-lactamase enzymes, is one of the most significant epidemiological changes in infectious diseases during recent decades (1). ESBL-EC strains have narrow treatment options that are limited to a small number of antibiotics because coresistance to different classes of antimicrobials is frequent. Because of the multidrug resistance of ESBL-EC strains and the emergence of carbapenem-resistant *Enterobacteriaceae*, therapeutic options for the treatment of ESBL-EC infections have become limited. For the treatment of severe infections caused by ESBL-EC, carbapenems are generally considered the mainstay for antimicrobial therapy; however, the emergence of resistance is a matter of concern due to the widespread use of carbapenems (2–4). The initial purpose of combination therapy is to broaden the empirical coverage provided by two antimicrobial agents with different activity spectra (5). Advantages include the theoretical possibility of minimizing the emergence of antimicrobial resistance and potential synergistic interactions (6). The purpose of the current study was to analyze nationwide data on the susceptibilities of ESBL-EC isolates causing bacteremia and to improve empirical approaches to therapy for these serious infections.

As a part of the multicenter surveillance study on bacteremia from March 2012 to December 2013, a total of 291 ESBL-EC blood isolates were collected from seven hospitals in various regions of South Korea: Samsung Medical Center (Seoul), Kyunghee University Medical center (Seoul), Keimyung University Dongsan Medical Center (Daegu), Daegu Fatima Hospital (Daegu), Samsung Changwon Hospital (Changwon), Changwon Fatima Hospital (Changwon), and Chungnam National University Hospital (Daejeon). Isolates were maintained in brain heart infusion broth (BD Diagnostics, Sparks, MD) with 50% glycerol and stored at −70°C until use. Isolates were subcultured a minimum of three times prior to experimentation. All antibiotics except fosfomycin were tested by broth microdilution for antimicrobial susceptibility. The MIC of fosfomycin was determined by the agar dilution method using agar medium supplemented with 25 mg/liter of glucose-6-phosphate. MICs were interpreted with category designations according to the criteria of the Clinical and Laboratory Standards Institute (CLSI). *E. coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853) were used as the control strains. According to the European Committee on Antimicrobial Susceptibility Testing (EUCAST), susceptible and resistant MIC breakpoints for tigecycline are ≤1 mg/liter and >2 mg/liter, respectively; the CLSI has yet to set values.

The results of the antimicrobial susceptibility testing of each antimicrobial agent were used to evaluate the *in vitro* activity of antimicrobial combinations. If the isolate was susceptible to either one of two antimicrobial agents taken together, the isolate was considered susceptible to the antimicrobial combination. For instance, if the isolate was resistant to ceftazidime but susceptible to amikacin, the isolate was considered susceptible to the antimicrobial combination. ESBL activity was confirmed via a double-disk synergy test (ceftazidime, cefotaxime, and aztreonam MICs of ≥2 mg/liter) using BD BBL Sensi-Discs (BD Diagnostics, Sparks, MD). ESBL-related genes, including bla_{TEM}, bla_{SHV}, and bla_{CTX-M}, were amplified by PCR as described previously (7, 8). Statistical analysis was performed using SPSS for Windows (version 11.5 software; SPSS, Inc., Chicago, IL).

Results of the *in vitro* activity of 21 antimicrobial agents against

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TABLE 1 Results of antimicrobial susceptibility testing of ESBL-EC isolates

Antimicrobial agent(s)	MIC (mg/liter)[a]			No. (%) of nonsusceptible isolates
	50%	90%	Range	
Fosfomycin	2	4	0.25–256	13 (4.5)
Ampicillin	>256	>256	0.06–256	273 (93.8)
Ampicillin-sulbactam	64/32	>64/32	0.06/0.03–64/32	266 (91.4)
Amoxicillin-clavulanic acid	16/8	>32/16	0.03/0.015–32/16	230 (79.0)
Ticarcillin-clavulanic acid	128/2	>256/2	0.25/2–256/2	246 (84.5)
Ceftazidime	16	>64	0.06–64	166 (57.0)
Cefotaxime	>128	>128	0.12–128	265 (91.1)
Cefepime[b]	128	>128	0.12–128	207 (71.1)
Aztreonam	32	>64	0.06–64	233 (80.1)
Piperacillin-tazobactam	4/4	256/4	0.25/4–256/4	95 (32.6)
Imipenem	0.12	0.25	0.06–64	1 (0.3)
Meropenem	0.06	0.12	0.06–64	6 (2.1)
Doripenem	0.03	0.06	0.015–16	5 (1.7)
Ertapenem	0.03	0.25	0.03–32	15 (5.2)
Ciprofloxacin	32	>64	0.06–64	204 (70.1)
Amikacin	8	32	0.12–128	34 (11.7)
Gentamicin	8	>64	0.06–64	151 (51.9)
Tobramycin	8	64	0.06–64	151 (51.9)
Tigecycline	0.25	1	0.06–64	10 (3.4)
Colistin	0.25	0.5	0.06–64	
Trimethoprim-sulfamethoxazole	>32/608	>32/608	0.03/0.59–32/608	157 (54.0)

[a] 50% and 90%, MICs at which 50% and 90% of the isolates tested were inhibited.
[b] For cefepime, isolates with MICs between 4 and 8 mg/liter (susceptible-dose dependent) were considered susceptible.

291 ESBL-EC isolates are shown in Table 1. More than 80% of these isolates were nonsusceptible to ampicillin, ampicillin-sulbactam, ticarcillin-clavulanic acid, cefotaxime, and aztreonam. The proportion of nonsusceptible isolates to tigecycline, fosfomycin, and amikacin was <12%. Among the carbapenems, only one isolate was nonsusceptible to imipenem, while 5 (1.7%), 6 (2.1%), and 15 (5.2%) were nonsusceptible to doripenem, meropenem, and ertapenem, respectively. Of the CTX-M groups, CTX-M-14 (44.3%) was the most frequent type, followed by CTX-M-15 (34.7%), CTX-M-55 (6.1%), CTX-M-27 (4.4%), and CTX-M-24 (3.0%). A total of 130 isolates (44.6%) produced TEM-1 along with CTX-M-type ESBL, and 3 isolates produced SHV-12 and TEM-1.

In coresistance analysis, >80% of ESBL-EC isolates resistant to ceftazidime, cefepime, and piperacillin-tazobactam were concurrently resistant to ampicillin, ampicillin-sulbactam, amoxicillin-clavulanic acid, ticarcillin-clavulanic acid, cefotaxime, and aztreonam. In contrast, <25% of isolates resistant to ceftazidime, cefepime, and piperacillin-tazobactam were resistant to fosfomycin, amikacin, tigecycline, and the carbapenems. The proportions of isolates nonsusceptible to ceftazidime, cefepime, and piperacillin-tazobactam were 57.0%, 71.1%, and 32.6%, respectively. The distributions of ceftazidime, cefepime, and piperacillin-tazobactam MICs are shown in Fig. 1. The *in vitro* efficacy of several antimicrobial combinations was assessed with β-lactam antibiotics (ceftazidime, cefepime, and piperacillin-tazobactam) in com-

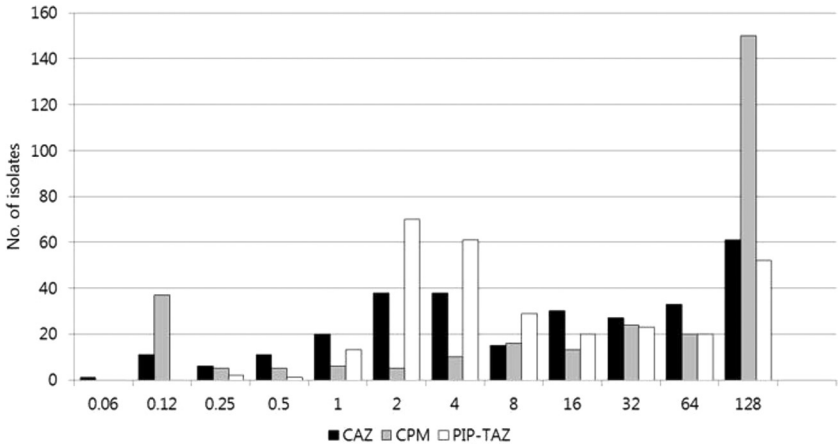

FIG 1 Distribution of MICs (mg/liter) of ceftazidime (CAZ), cefepime (CPM), and piperacillin-tazobactam (PIP-TAZ). The MIC breakpoints for resistance were as follows: ceftazidime, ≥16 mg/liter; cefepime, ≥16 mg/liter; and piperacillin-tazobactam, ≥128/4 mg/liter. For cefepime, isolates with MICs between 4 and 8 mg/liter (susceptible-dose dependent) were susceptible.

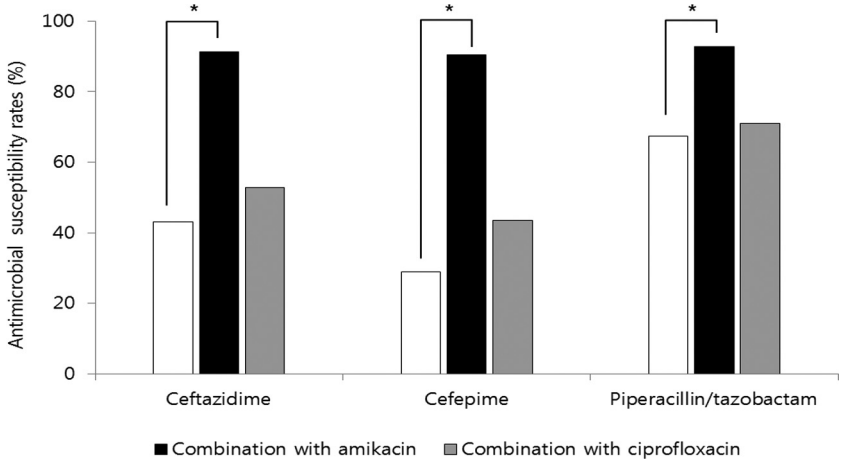

FIG 2 Susceptibility of ESBL-EC to three β-lactam antibiotics alone and in combination with amikacin and ciprofloxacin. *, $P < 0.05$.

bination with amikacin or ciprofloxacin, which are currently available in clinical practice (Fig. 2). For evaluation of the *in vitro* activity of antimicrobial combinations, if the isolate was susceptible to either of two antimicrobial agents taken together, the isolate was considered susceptible to the antimicrobial combination. Based on the antimicrobial susceptibility data, the three β-lactams (ceftazidime, cefepime, and piperacillin-tazobactam) in combination with amikacin (susceptibility rates, 91.4%, 90.4%, and 92.8%, respectively) showed greater activity than that seen in combination with ciprofloxacin (52.9%, 43.6%, and 71.1%, respectively). The combination of piperacillin-tazobactam with amikacin was the most susceptible regimen, other than the carbapenems; in particular, the rate of susceptibility to cefepime in combination with amikacin was much higher than that of cefepime alone (90.4% versus 28.9%; $P < 0.05$).

Among the antimicrobial agents other than the carbapenems included in this study, tigecycline and fosfomycin showed good activity *in vitro* against ESBL-EC. The rates of susceptibility to ceftazidime, cefotaxime, cefepime, and ciprofloxacin were quite low, similar to those in a previous study (9). Carbapenems were the most active agents against ESBL-EC, and only one isolate was nonsusceptible to imipenem. However, increased empirical use of carbapenems in response to an increased prevalence of ESBL-producing isolates may be accompanied by a rapid emergence of carbapenem resistance in other pathogens (10), rendering the genes encoding carbapenem-hydrolyzing enzymes, such as KPCs or metallo-β-lactamases, easier to spread via horizontal gene transfer (11). Therefore, alternatives to the carbapenems should be considered for empirical treatment of suspected Gram-negative sepsis whenever possible.

For infections caused by Gram-negative bacteria, antimicrobial synergy has traditionally been seen with β-lactam and aminoglycoside combination treatment, as the combination of a β-lactam and an aminoglycoside allows for different mechanisms of bacterial killing (12, 13). Our study evaluated the β-lactams ceftazidime, cefepime, and piperacillin-tazobactam in combination with amikacin or ciprofloxacin. These three β-lactams in combination with amikacin showed greater activity than when combined with ciprofloxacin because the susceptibility rate to amikacin was much higher than that to ciprofloxacin. Similarly, the combination of cefepime with amikacin increased the susceptibility rate from 28.9% to 90.4% against ESBL-EC. The combination of piperacillin-tazobactam with amikacin was the most susceptible regimen (92.8%) among the combinations, comparable to the carbapenems. Ciprofloxacin had low activity against ESBL-EC, with only 29.9% of susceptible isolates. Based on the *in vitro* susceptibility testing results, amikacin would be the most likely agent to increase the range of antimicrobial coverage against ESBL-EC.

Recent studies suggested that a survival benefit based on initial combination therapy appears to be the greatest in patients at the highest risk of death, such as those with septic shock (14, 15). Although the role of combination therapy in Gram-negative sepsis has been controversial with regard to synergistic effects, it is becoming increasingly important to achieve adequate empirical antimicrobial therapies (16).

In this study, we assessed the *in vitro* efficacy of 21 antimicrobial agents alone and in combination against ESBL-EC isolates causing bacteremia in South Korean hospitals. Our *in vitro* results indicate that the combination of β-lactam antibiotics, such as cefepime or piperacillin-tazobactam, with an aminoglycoside or a fluoroquinolone increases isolate susceptibility. In settings with a high prevalence of ESBL-producing pathogens, combination antimicrobial therapy with an aminoglycoside, especially amikacin, can be considered an empirical therapy against suspected Gram-negative sepsis, as one of the carbapenem-saving strategies.

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We declare that we have no conflicts of interest.

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