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Antimicrobial activity of cephamycins and β-lactam/β-lactamase inhibitors against ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae* under standard and high bacterial inocula

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This study investigated the in vitro antimicrobial activity of cephamycins and novel β-lactam/β-lactamase inhibitor combinations, against extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* and *Klebsiella pneumoniae* isolates. ESBL-producing blood isolates were obtained between January and May 2023. The antibiotic susceptibility of the isolates was determined by broth microdilution method using standard (10^5 CFU/mL) and high (10^7 CFU/mL) inoculum sizes. Two randomly selected ESBL-producing isolates were subjected to time-kill assays for cephamycin. More than 80% of the isolates exhibited susceptibility to cefoxitin, cefmetazole, flomoxef, ceftazidime/avibactam, cefepime/enmetazobactam and imipenem/relebactam. Ceftolozane/tazobactam demonstrated in vitro efficacy against 62.1% of the ESBL-producing isolates. At the higher inoculum size, cefoxitin, cefmetazole, flomoxef, ceftolozane/tazobactam, ceftazidime/avibactam, cefepime/enmetazobactam, and imipenem/relebactam demonstrated in vitro efficacy against 48.3%, 75.9%, 70.0%, 0%, 82.8%, 100%, 89.7% of the ESBL-producing isolates. The frequencies of the inoculum effect with cefoxitin (3.7%), cefmetazole (14.8%), flomoxef (88.9%), ceftolozane/tazobactam (96.0%), ceftazidime/avibactam (34.5%), cefepime/enmetazobactam (10.3%), and imipenem/relebactam (10.3%) were identified. With standard inoculum, cephamycins reduced bacterial growth within 2–24 h. However, high inoculum resulted in regrowth after 12 h of cephamycin exposure. Cephamycins demonstrated high in vitro activity against ESBL-producing isolates at standard inoculum sizes. However, the in vitro time-kill experiments revealed that the efficacy of cephamycins is not fully maintained at high inoculum sizes.

Keywords Extended-spectrum β-lactamase-producing enterobacterales, Cephamycin, Inoculum effect, Cefoxitin, Cefmetazole, Flomoxef

The emergence and dissemination of extended-spectrum β-lactamase (ESBL)-producing Enterobacterales have led to increased reliance on carbapenems as a treatment option, raising concerns about the rising carbapenem consumption and its potential consequences[1]. Therefore, carbapenem-sparing antimicrobial options are needed not only to treat ESBL-producing isolates, but also to reduce the rapid emergence of carbapenem resistant pathogens[1].

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Cephamycins, a subgroup of second-generation cephalosporins, include cefotetan, cefoxitin, cefmetazole, and flomoxef. These antibiotics, along with temocillin and β-lactam/β-lactamase inhibitor combinations, are stable to hydrolysis by ESBLs[1,2]. The stability of cephamycins to ESBL hydrolysis is attributed to their unique molecular structure, which includes a 7-α-methoxy group. This structural feature provides steric hindrance, making it difficult for ESBLs to hydrolyse the β-lactam ring[3]. While the minimum inhibitory concentrations (MICs) of β-lactam/β-lactamase inhibitor combinations can increase as the bacterial inoculum size increases[4], cephamycins demonstrate in vitro antibacterial efficacy even against high inoculum concentrations[5]. Understanding the impact of inoculum size on antibiotic efficacy is crucial for optimizing treatment strategies and predicting clinical outcomes. Moreover, the increasing prevalence of ESBL-producing organisms in both hospital and community settings has raised concerns about the limited treatment options available[6]. This situation has renewed interest in older antibiotics like cephamycins as potential alternatives to carbapenems. However, the clinical efficacy of cephamycins against ESBL-producing organisms remains a subject of debate, with some studies reporting favourable outcomes[7] and others highlighting potential limitations[8].

Therefore, we aimed to evaluate the in vitro activities of cephamycins versus comparator agents, including recently approved β-lactam/β-lactamase inhibitor combinations, against ESBL-producing isolates obtained from blood cultures. Additionally, we investigated the impact of high inoculum sizes on minimum inhibitory concentrations (MICs) to identify which antibiotics retain their effectiveness against ESBL-producing bacteria, regardless of the bacterial load.

Materials and methods
Bacterial isolates
Forty-eight *E. coli* and 68 *K. pneumoniae* clinical isolates were obtained from the Korean National Culture Collection for Pathogens (KNUH-NCCP). These isolates were originally collected from three Korean hospitals (Kyungpook National University Chilgok Hospital (Daegu), Kyungpook National University Hospital (Daegu), and Samsung Changwon Hospital (Changwon)) between January 2023 and May 2023. The thirty isolates exhibiting an ESBL phenotype by automated antimicrobial susceptibility testing (VITEK 2) were selected for further analysis. The phenotypic confirmatory test for ESBL production was performed by the disk diffusion method according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI)[9]. Briefly, disks containing cefotaxime (30 μg) and ceftazidime (30 μg), with and without clavulanic acid (10 μg), were placed on Mueller–Hinton agar plates inoculated with the test organism. After overnight incubation at 35 °C, an increase of ≥ 5 mm in the zone diameter for either antimicrobial agent tested in combination with clavulanic acid versus its zone when tested alone confirmed the ESBL phenotype[9]. The Xpert Carba-R assay is a qualitative in vitro real-time PCR assay designed to detect five carbapenemase gene families: bla_{IMP}, bla_{VIM}, bla_{OXA-48}, bla_{NDM}, and bla_{KPC}, according to the manufacturer's instructions. The following ESBL-related genes were tested: TEM-type, CTX-M-1 group, CTX-M-9 group, and SHV-type, which were amplified by PCR from clinical isolates with primers as described in a previous study[10]. All PCR products were examined using 2% agarose gel electrophoresis.

Ethical approval
This study was conducted in agreement with the principles of the Declaration of Helsinki and was approved by the ethics committee of the Daegu Joint Institutional Review Board (DGIRB 2024-03-001). Informed consent was waived by DGIRB because this study utilized bacterial isolates from the KNUH-NCCP repository without any direct handling of human samples or patient data. All the experiments were performed in accordance with relevant guidelines and regulations.

Antimicrobial susceptibility testing
The MICs of ertapenem, imipenem, amikacin, ceftazidime, aztreonam, cefepime, cefotaxime, piperacillin/tazobactam, and trimethoprim/sulfamethoxazole were determined using automated antimicrobial susceptibility testing (VITEK 2), while the MIC of cefoxitin, cefmetazole, flomoxef, colistin, and the recently approved β-lactam/β-lactamase inhibitor combinations ceftazidime/avibactam, ceftolozane/tazobactam, cefepime/enmetazobactam and imipenem/relebactam were determined using the microdilution method at standard 10^5 CFU/mL or high 10^7 CFU/mL inoculum sizes. Susceptibility was interpreted according to the CLSI breakpoints[9]. There is no MIC breakpoint available for flomoxef from CLSI or EUCAST. Following current clinical practice in countries where flomoxef is available, we used the CLSI latamoxef (moxalactam) breakpoints[9]: susceptible at ≤ 8 mg/L, intermediate at 16 mg/L, and resistant at ≥ 32 mg/L. However, it should be noted that recent data suggests these breakpoints may not be adequate for high-burden severe infections such as bacteremia[11]. For cefepime/enmetazobactam, the FDA susceptibility breakpoint (≤ 8/8 mg/L) was applied. An inoculum effect was defined as an ≥ eightfold increase in the MIC at the higher inoculum size. *E. coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 served as quality control strains.

In vitro time-kill studies
For the time-kill curves, two ESBL-producing *E. coli* isolates that were susceptible to cephamycins were chosen. The time-kill experiments for cefoxitin, cefmetazole, and flomoxef were conducted according to a previously described method with slight modifications[12]. Briefly, colonies from overnight cultures were suspended in LB broth and adjusted to a 0.5 McFarland standard (approximately 1.5×10^8 CFU/mL). The suspensions were then diluted to achieve final concentrations of 5×10^5 CFU/mL and 5×10^7 CFU/mL in a total volume of 10 mL, and these concentrations were confirmed by dilution plating. Cefoxitin, cefmetazole, and flomoxef were added to the prepared bacterial suspensions to final concentrations of 16 μg/mL, 4 μg/mL, and 1 μg/mL, respectively, each corresponding to 2 × MIC. Antibiotic-free cultures served as growth controls. All cultures were incubated at 35 °C with continuous shaking. Viable cell counts were determined at 0, 2, 4, 8, 12, and 24 h by plating

serial dilutions on blood agar plates. Following overnight incubation at 35 °C, colony-forming units (CFU) were enumerated. The detection limit was established at 10 CFU/mL. Time-kill curves were generated by plotting $\log_{10}$ CFU/mL against time. Bactericidal activity was defined as a $\geq$ 3-$\log_{10}$ reduction in CFU/mL relative to the initial inoculum. All experiments were performed in duplicate. When discrepancies greater than 0.5 log10 CFU/mL were observed between paired results, the experiments were repeated in duplicate until consistent results (within 0.5 log10 CFU/mL variation) were obtained.

Results

Antimicrobial susceptibility of *E. coli* and *K. pneumoniae* blood isolates

Among the 116 blood isolates of *E. coli* and *K. pneumoniae*, more than 80% exhibited susceptibility to ertapenem (90.5%), imipenem (90.5%), amikacin (96.6%), gentamicin (83.6), piperacillin/tazobactam (80.2%), and cefoxitin (87.1%). All carbapenem resistance was found exclusively in *K. pneumoniae* isolates. Among these 11 carbapenem-resistant *K. pneumoniae* isolates, 10 were confirmed to produce KPC carbapenemase, while one isolate was a non-carbapenemase-producing strain that exhibited resistance through other mechanisms including ESBL production. All the 48 *E. coli* isolates were susceptible to ertapenem, imipenem, amikacin, and piperacillin/tazobactam, while 47 (97.9%) and 39 (81.3%) isolates were susceptible to cefoxitin and gentamicin, respectively. *K. pneumoniae* isolates demonstrated susceptibility to ertapenem (83.8%), imipenem (83.8%), amikacin (94.1%), gentamicin (85.3%), cefoxitin (79.4%), and piperacillin/tazobactam (66.2%). The susceptibility rates of *E. coli* isolates were 68.8%, 72.9%, 70.8%, 68.8%, 62.5%, and 66.7% for cefotaxime, ceftazidime, cefepime, aztreonam, amoxicillin/clavulanic acid, and trimethoprim/sulfamethoxazole, respectively. Among *K. pneumoniae* isolates, 61.8% (cefotaxime), 63.2% (ceftazidime), 72.1% (cefepime), 64.7% (aztreonam), 61.8% (amoxicillin/clavulanic acid), and 75.0% (trimethoprim/sulfamethoxazole) were susceptible to antibiotics (Supplement Table 1).

Among the isolates, 30 (15 *E. coli* and 15 *K. pneumoniae*) exhibited the ESBL-positive phenotype. One *K. pneumoniae* isolate demonstrated both the ESBL-positive phenotype and carbapenem resistance. This isolate was excluded from further analysis, resulting in a final study population of 29 ESBL-producing isolates.

In vitro activity of antimicrobial agents against ESBL-producing isolates

The susceptibility of the 29 ESBL-producing isolates to nineteen antimicrobial agents is presented in Table 1. More than 80% of the isolates exhibited susceptibility to ertapenem, imipenem, amikacin, cefoxitin, cefmetazole, flomoxef, ceftazidime/avibactam, cefepime/enmetazobactam and imipenem/relebactam. Ceftolozane/tazobactam demonstrated in vitro efficacy against 62.1% (18/29) of the ESBL-producing isolates. Interestingly, *E. coli* isolates showed a higher susceptibility rate of 93.3% (14/15) compared to *K. pneumoniae* isolates, which had a lower susceptibility rate of 28.6% (4/14).

Antimicrobial susceptibility of ESBL-producing isolates to cephamycins and new β-lactam/β-lactamase inhibitor combinations with different inoculum sizes

Tables 2 and 3 shows the antimicrobial susceptibility of ESBL blood isolates to three cephamycins and three new β-lactam/β-lactamase inhibitor combinations with standard (10^5 CFU/mL) or high (10^7 CFU/mL) inoculum size. The most ESBLs belonged to the CTX-M-1 group. At the higher inoculum size, cefoxitin, cefmetazole, flomoxef, ceftolozane/tazobactam, ceftazidime/avibactam, cefepime/enmetazobactam, and imipenem/relebactam demonstrated in vitro efficacy against 48.3% (n = 14), 75.9% (n = 22), 70.0% (n = 20), 0% (n = 0), 82.8% (n = 24), 100% (n = 29), 89.7% (n = 26) of the ESBL-producing isolates. The frequencies of the inoculum effect with cefoxitin (3.7%, 1/27), cefmetazole (14.8%, 4/27), flomoxef (88.9%, 24/27), ceftolozane/tazobactam (96.0%, 24/25), ceftazidime/avibactam (34.5%, 10/29), cefepime/enmetazobactam (10.3%, 3/29), and imipenem/relebactam (10.3%, 3/29) were identified.

Killing effects of cefoxitin, cefmetazole, and flomoxef on ESBL blood isolates

For the time-kill curves, two ESBL-producing *E. coli* isolates that were susceptible to cephamycins were chosen. The curves were generated using standard (5×10^5 CFU/mL) and high (5×10^7 CFU/mL) inocula. Figure 1 depicts the impact of cephamycins at concentrations corresponding to $2 \times$ MIC. When the standard inoculum was used, cephamycins reduced bacterial growth within 2–24 h. However, one isolate (ECO-2) exhibited regrowth at 24 h under cefoxitin treatment (Fig. 1A). In contrast, when high inocula was employed, both isolates demonstrated regrowth after 12 h of exposure to cephamycins (Fig. 1B, 1D).

Discussion

Our study revealed that cephamycins exhibited high susceptibility rates against ESBL-producing *E. coli* and *K. pneumoniae* clinical isolates (cefoxitin 82.8%, cefmetazole 93.1%, flomoxef 89.7%), with their efficacy maintained to a certain extent at high inoculum sizes. The study further showed that cefmetazole and flomoxef maintained high activity (75.9% and 70.0% susceptibility, respectively) even at high inoculum, while cefoxitin's efficacy decreased more substantially (from 80.0 to 48.3%). Among the β-lactam/β-lactamase inhibitor combinations, ceftazidime/avibactam, cefepime/enmetazobactam, and imipenem/relebactam showed the most robust activity across both inoculum sizes, maintaining over 80% susceptibility even at high inoculum. Ceftolozane/tazobactam, however, showed a dramatic loss of efficacy at high inoculum. These findings suggest that cephamycins can retain their efficacy against ESBL-producing strains even at high inoculum sizes. However, the in vitro time-kill experiments revealed that bacterial regrowth was observed after 12 h of cephamycin exposure at high inoculum size. This indicates that the efficacy of cephamycins is not fully maintained at high inoculum sizes.

A previous study of 102 MDR *K. pneumoniae* isolates revealed a high prevalence (> 90%) of resistance to most β-lactam antibiotics, with carbapenems and cephamycins being notable exceptions, as the isolates remained

Antimicrobial agent	Minimum inhibitory concentration (MIC) (µg/mL)			Number of isolates (%)			
	MIC50	MIC90	Range	Susceptible	SDD	Intermediate	Resistant
Ertapenem	≤0.25	≤0.25	≤0.25	29 (100)		0	0
Imipenem	≤0.25	≤0.25	≤0.25 to 0.5	29 (100)		0	0
Amikacin	≤4	≤4	≤4 to >32	26 (89.7)		0	3 (10.3)
Amoxicillin/clavulanic acid	16/8	>16/8	8/4 to >16/8	5 (17.2)		11 (37.9)	13 (44.8)
Ceftazidime	16	>16	≤0.5 to >16	3 (10.3)		1 (3.4)	25 (86.2)
Aztreonam	>16	>16	≤1 to >16	2 (6.9)		1 (3.4)	26 (89.7)
Cefepime	>16	>16	≤1 to >16	6 (20.7)	5 (17.2)		18 (62.1)
Cefotaxime	>4	>4	>4	0		0	29 (100)
Gentamicin	≤2	>8	≤2 to >8	19 (65.5)		0	10 (34.5)
Piperacillin/tazobactam	≤4/4	32/4	≤4/4 to >64/4	19 (65.5)	5 (17.2)		5 (17.2)
TMP/SMX	>2/38	>2/38	≤0.5/9.5 to >2/38	13 (44.8)			16 (55.2)
Colistin[b]	0.5	1	0.25 to 8			28 (96.6)	1 (3.4)
Cefoxitin[b]	8	>64	2 to >64	24 (82.8)		1 (3.4)	4 (13.8)
Cefmetazole[b]	2	8	0.5 to >64	27 (93.1)		0	2 (6.9)
Flomoxef[b,c]	0.5	16	0.25 to >64	26 (89.7)		1 (3.4)	2 (6.9)
Ceftolozane/tazobactam[b]	2/4	>64/4	0.5/4 to >64/4	18 (62.1)		2 (6.9)	9 (31.0)
Ceftazidime/avibactam[b]	0.5/4	1/4	0.0625/4 to 1/4	29 (100)			0
Cefepime/enmetazobactam[b,d]	0.12/8	0.25/8	0.06/8 to 0.5/8	29 (100)			0
Imipenem/relebactam[b]	0.125/4	0.5/4	0.0625/4 to 1/4	29 (100)		0	0

Table 1. Antimicrobial activity of antimicrobial agents against extended β-lactam-resistant *E. coli* and *K. pneumoniae* blood isolates (n = 29)[a].
MIC minimum inhibitory concentration; MIC_{50} median minimum inhibitory concentration; MIC_{90} 90th percentile minimum inhibitory concentration; *SDD* susceptible-dose dependent; *TMP/SMX* trimethoprim/sulfamethoxazole.
[a]Gray background indicates no category recommended in Clinical and Laboratory Standards Institute (CLSI) guidelines.
[b]Performed using the microdilution method.
[c]There is no MIC breakpoint available for flomoxef, and physicians in countries where flomoxef is available use the latamoxef MIC breakpoint, which is available from the CLSI. Isolates are reported as susceptible to flomoxef at a MIC of ≤ 8 mg/L; as intermediate at an MIC of 16 mg/L and as resistant at an MIC ≥ 32 mg/L.
[d]FDA susceptibility breakpoint (≤ 8/8 mg/liter) was applied.

susceptible to these agents[13]. In that investigation, cephamycins including cefoxitin and cefotetan showed better efficacy (40.2% and 68.6%, respectively) compared to carbapenems (23.5 to 34.3%) against *K. pneumoniae* isolates[13]. Our findings indicated that over 80% of ESBL-producing *E. coli* and *K. pneumoniae* isolates were susceptible to cephamycins (cefoxitin (82.8%), cefmetazole (93.1%), and flomoxef (89.7%)). Flomoxef had a lower MIC50 value (0.5 µg/mL) compared to cefoxitin (8 µg/mL) and cefmetazole (2 µg/mL). In addition, flomoxef maintained its activity against 92.8% of the isolates, even with a high inoculum size, despite showing an inoculum effect of 88.9%. These results are consistent with studies conducted in China, Japan, and Korea, which demonstrated the potent activity of flomoxef against ESBL-producing isolates[14–16]. Additionally, flomoxef exhibited excellent activity against ESBL-producing bacteria, including an isolate with an MIC eight times the MIC90 value[17].

Ceftazidime-avibactam, cefepime/enmetazobactam and imipenem-relebactam also exhibited potent activity inhibiting 100% of ESBL-isolates at the standard inoculum, supporting their potential to preserve carbapenem efficacy. However, inoculum effects abrogating susceptibility were noteworthy for ceftolozane/tazobactam against most isolates, especially *K. pneumoniae* with SHV-type enzymes. This result is consistent with that ceftolozane/tazobactam inhibits 92.9% of the isolates harbouring CTX-M group but exhibited limited activity against isolates carrying SHV group (61.1%)[18]. This contrasts with the superior efficacy of cephamycins like flomoxef, which maintained activity against 86.7% isolates even at high inoculum despite exhibiting an inoculum effect in 88.9%. These findings suggest cephamycins could be advantageous over ceftolozane/tazobactam for treating high burden ESBL infections not amenable to carbapenem therapy. On the other hand, ceftazidime/avibactam, cefepime/enmetazobactam or imipenem/relebactam may be an option for the treatment of infections caused by ESBL-producing organisms but should be used judiciously to preserve its activity against carbapenem-resistant organisms.

Notwithstanding the efficacy if cephamycin against ESBL-producing organisms demonstrated in this paper, cephamycin has several limitations to use in the clinical setting. Currently, there are no established MIC breakpoints for flomoxef. Lee et al. observed comparable mortality rates between flomoxef and carbapenems when the flomoxef MIC was ≤ 1 mg/L, but flomoxef performed worse than carbapenem at MICs of 4–8 mg/L[8]. In our study, when considering a susceptibility threshold of 1 mg/L, the percentage of isolates susceptible

| Isolate no. | Sex/age | Type of β-lactamase | Species | MIC (mg/L) | | | | | | | | |
| | | | | Cefoxitin | | | Cefmetazole | | | Flomoxef[a] | | |
				10^5 CFU/mL	10^7 CFU/mL	Increase in MIC	10^5 CFU/mL	10^7 CFU/mL	Increase in MIC	10^5 CFU/mL	10^7 CFU/mL	Increase in MIC
1	M/74	CTX-M group 1,TEM	ECO	8	16	2	2	8	4	0.5	16	32
2	F/64	CTX-M group 1,TEM	ECO	8	8	1	2	8	4	0.25	4	16
3	M/86	CTX-M group 1	ECO	8	8	1	2	8	4	0.25	4	16
4	F/71	CTX-M group 1	ECO	4	16	4	2	8	4	0.25	8	32
5	F/84	CTX-M group 9	ECO	4	8	2	2	8	4	0.25	8	32
6	F/73	CTX-M group 1,TEM	ECO	8	16	2	4	4	1	0.5	32	64
7	F/62	CTX-M group 1	ECO	4	16	4	1	32	32	0.25	32	128
8	F/88	CTX-M group 9,TEM	ECO	4	8	2	2	4	2	0.5	8	16
9	F/90	CTX-M group 1	ECO	4	8	2	4	4	1	0.25	16	64
10	M/76	CTX-M group 1, CTX-M group 9	ECO	8	16	2	8	32	4	1	8	8
11	F/71	CTX-M group 1	ECO	2	16	8	2	64	32	0.25	4	16
12	M/65	CTX-M group 1, CTX-M group 9	ECO	8	32	4	4	8	2	0.5	16	32
13	F/84	CTX-M group 1	ECO	8	8	1	4	8	2	0.5	4	8
14	M/83	CTX-M group 1	ECO	8	8	1	4	8	2	0.25	8	32
15	F/90	CTX-M group 1, CTX-M group 9	ECO	4	8	2	2	8	4	0.5	16	32
16	F/64	CTX-M group 1, SHV	KPN	4	8	2	1	2	2	0.25	4	16
17	F/49	CTX-M group 1, CTX-M group 9,TEM, SHV	KPN	16	32	2	2	4	2	0.5	4	8
18	M/48	CTX-M group 1,TEM, SHV	KPN	4	8	2	2	2	1	1	4	4
19	F/84	CTX-M group 1,TEM, SHV	KPN	>64	128	1	4	256	64	16	32	2
20	M/62	CTX-M group 1,SHV	KPN	4	16	4	2	4	2	0.5	8	16
21	F/82	CTX-M group 1,TEM, SHV	KPN	8	16	2	2	4	2	0.25	4	16
22	M/72	CTX-M group 1,TEM, SHV	KPN	4	8	2	2	4	2	0.25	4	16
23	M/81	CTX-M group 1,SHV	KPN	>64	>512	ND	>64	>256	ND	>64	>128	ND
24	M/75	CTX-M group 1,SHV	KPN	>64	>512	ND	>64	>256	ND	>64	>128	ND
25	M/72	CTX-M group 1,TEM, SHV	KPN	4	16	4	1	4	4	0.25	4	16
26	M/62	CTX-M group 9,SHV	KPN	2	2	1	0.5	1	2	0.5	4	8
27	M/84	CTX-M group 1,SHV	KPN	8	8	1	1	4	4	0.5	4	8
28	F/63	CTX-M group 1,TEM, SHV	KPN	8	8	1	2	4	2	0.25	4	16
29	F/80	CTX-M group 1,TEM, SHV	KPN	32	32	1	4	32	8	2	8	4

Table 2. Antimicrobial susceptibility of extended β-lactam-resistant *E. coli* and *K. pneumoniae* blood isolates to three cephamycins.
ECO Escherichia coli; *KPN, Klebsiella pneumoniae*; *MIC* minimum inhibitory concentration;
Red highlight indicates resistance category, blue indicates intermediate category, and black indicates sensitivity category.
Gray background indicates the presence of an inoculum effect.
[a]There is no MIC breakpoint available for flomoxef, and physicians in countries where flomoxef is available use the latamoxef MIC breakpoint, which is available from the CLSI. Isolates are reported as susceptible to flomoxef at a MIC of ≤ 8 mg/L; as intermediate at an MIC of 16 mg/L and as resistant at an MIC ≥ 32 mg/L.

to flomoxef dropped from 86.7 to 83.3%. Furthermore, a previous study discovered that cefmetazole was only effective against isolates with MICs ≤ 2 μg/mL, and the currently approved dosing might be suboptimal for isolates with higher MICs[17]. These observations emphasize the importance of establishing updated susceptibility breakpoint of cephamycins.

The findings of this study have several potential implications for clinical practice and antimicrobial stewardship. Our results suggest that cephamycins may serve as carbapenem-sparing alternatives for the treatment of certain ESBL-producing infections[19,20]. The demonstrated in vitro efficacy of cephamycins against ESBL-producing isolates supports their potential integration into antimicrobial stewardship programs, which could contribute to reducing carbapenem usage, thereby preserving the efficacy of these crucial antibiotics for infections caused by more resistant organisms[21]. Furthermore, the observed inoculum effect underscores the critical importance of considering bacterial load when selecting antimicrobial agents for severe infections. This insight may inform clinical decision-making processes, particularly in guiding the selection of appropriate empiric therapy for high-inoculum infections, such as those associated with osteomyelitis or endocarditis.

#				Ceftolozane/tazobactam			Ceftazidime/avibactam			cefepime/enmetazobactam			Imipenem/relebactam		
				10^5 CFU/mL	10^7 CFU/mL	Increase in MIC	10^5 CFU/mL	10^7 CFU/mL	Increase in MIC	10^5 CFU/mL	10^7 CFU/mL	Increase in MIC	10^5 CFU/mL	10^7 CFU/mL	Increase in MIC
1	M/74	CTX-M group 1,TEM	ECO	>64:4	>256:4	ND	0.5:4	0.5:4	1	0.12:8	0.12:8	1	0.12:4	0.25:4	2
2	F/64	CTX-M group 1,TEM	ECO	2:4	16:4	8	0.25:4	1:4	4	0.06:8	0.12:8	2	0.12:4	0.25:4	2
3	M/86	CTX-M group 1	ECO	0.5:4	32:4	64	0.5:4	2:4	4	0.12:8	0.12:8	1	0.12:4	0.25:4	2
4	F/71	CTX-M group 1	ECO	1:4	64:4	64	0.25:4	64:4	256	0.06:8	0.12:8	2	0.12:4	0.25:4	2
5	F/84	CTX-M group 9	ECO	0.5:4	32:4	64	0.25:4	1:4	4	0.06:8	0.12:8	2	0.12:4	0.25:4	2
6	F/73	CTX-M group 1.TEM	ECO	2:4	32:4	16	0.25:4	4:4	16	0.06:8	0.25:8	4	0.12:4	0.5:4	4
7	F/62	CTX-M group 1	ECO	0.5:4	16:4	32	0.06:4	64:4	1024	0.06:8	0.12:8	2	0.12:4	1:4	8
8	F/88	CTX-M group 9,TEM	ECO	1:4	16:4	16	0.25:4	4:4	16	0.06:8	0.12:8	2	0.12:4	0.25:4	2
9	F/90	CTX-M group 1	ECO	1:4	32:4	32	0.25:4	8:4	32	0.12:8	0.12:8	1	0.12:4	0.25:4	2
10	M/76	CTX-M group 1, CTX-M group 9	ECO	1:4	32:4	32	0.5:4	0.5:4	1	0.25:8	0.25:8	1	0.12:4	0.25:4	2
11	F/71	CTX-M group 1	ECO	1:4	32:4	32	0.25:4	2:4	8	0.12:8	0.12:8	1	0.12:4	0.25:4	2
12	M/65	CTX-M group 1, CTX-M group 9	ECO	1:4	64:4	64	0.5:4	0.5:4	1	0.06:8	0.12:8	2	0.12:4	0.25:4	2
13	F/84	CTX-M group 1	ECO	2:4	32:4	16	0.5:4	0.5:4	1	0.12:8	0.12:8	1	0.12:4	0.25:4	2
14	M/83	CTX-M group 1	ECO	1:4	64:4	64	0.25:4	32:4	128	0.12:8	8:8	66	0.12:4	0.25:4	2
15	F/90	CTX-M group 1, CTX-M group 9	ECO	1:4	32:4	32	0.12:4	1:4	8	0.12:8	0.12:8	1	0.12:4	0.5:4	4
16	F/64	CTX-M group 1, SHV	KPN	1:4	64:4	64	0.5:4	2:4	4	0.12:8	0.12:8	1	0.25:4	1:4	4
17	F/49	CTX-M group 1, CTX-M group 9,TEM, SHV	KPN	4:4	64:4	16	0.5:4	1:4	2	0.12:8	0.25:8	2	0.25:4	0.25:4	1
18	M/48	CTX-M group 1,TEM, SHV	KPN	>64:4	>256:4	ND	1:4	4:4	4	0.12:8	0.12:8	1	0.25:4	2:4	8
19	F/84	CTX-M group 1,TEM, SHV	KPN	>64:4	>256:4	ND	0.5:4	32:4	64	0.5:8	4:8	8	0.12:4	0.5:4	4
20	M/62	CTX-M group 1,SHV	KPN	32:4	>256:4	>8	0.5:4	1:4	2	0.5:8	8:8	16	0.5:4	0.5:4	1
21	F/82	CTX-M group 1,TEM, SHV	KPN	1:4	64:4	64	0.5:4	1:4	2	0.12:8	0.25:8	2	0.5:4	2:4	4
22	M/72	CTX-M group 1,TEM, SHV	KPN	16:4	>256:4	>16	1:4	1:4	1	0.12:8	0.12:8	1	0.25:4	0.25:4	1
23	M/81	CTX-M group 1,SHV	KPN	2:4	128:4	64	0.5:4	16:4	32	0.12:8	0.12:8	1	0.25:4	1:4	4
24	M/75	CTX-M group 1,SHV	KPN	32:4	>256:4	>8	1:4	2:4	2	0.12:8	0.25:8	2	0.5:4	0.5:4	1
25	M/72	CTX-M group 1,TEM, SHV	KPN	8:4	128:4	16	0.5:4	1:4	2	0.25:8	0.25:8	1	0.12:4	0.25:4	2
26	M/62	CTX-M group 9,SHV	KPN	1:4	16:4	16	0.25:4	0.5:4	2	0.12:8	0.12:8	1	0.25:4	0.25:4	1
27	M/84	CTX-M group 1,SHV	KPN	4:4	32:4	8	0.5:4	2:4	4	0.12:8	0.12:8	1	1:4	1:4	1
28	F/63	CTX-M group 1,TEM, SHV	KPN	>64:4	>256:4	ND	0.5:4	1:4	2	0.12:8	0.12:8	1	0.12:4	4:4	32
29	F/80	CTX-M group 1,TEM, SHV	KPN	16:4	64:4	4	0.25:4	1:4	4	0.12:8	0.25:8	2	0.06:4	0.25:4	4

Table 3. Antimicrobial susceptibility of extended β-lactam-resistant *E. coli* and *K. pneumoniae* blood isolates to three β-lactam/β-lactamase inhibitor combinations.
ECO *Escherichia coli*; *KPN* *Klebsiella pneumoniae*; *MIC* minimum inhibitory concentration;
Red highlight indicates resistance category, blue indicates intermediate category, and black indicates sensitivity category.
Gray background indicates the presence of an inoculum effect.

Conclusions

Our study suggested that cephamycins represent promising alternatives against ESBL-producing bacteria based on their in vitro activity. Cephamycins exhibited persistent in vitro activity against ESBL-producing isolates irrespective of the inoculum size. However, bacterial regrowth was observed when high inoculum size was employed, and caution should be required when using cephamycins in high-inoculum infections caused by ESBL-producing Enterobacterales. Further clinical studies are needed to confirm that the observed in vitro efficacy and inoculum independence of cephamycins translate into clinical effectiveness.
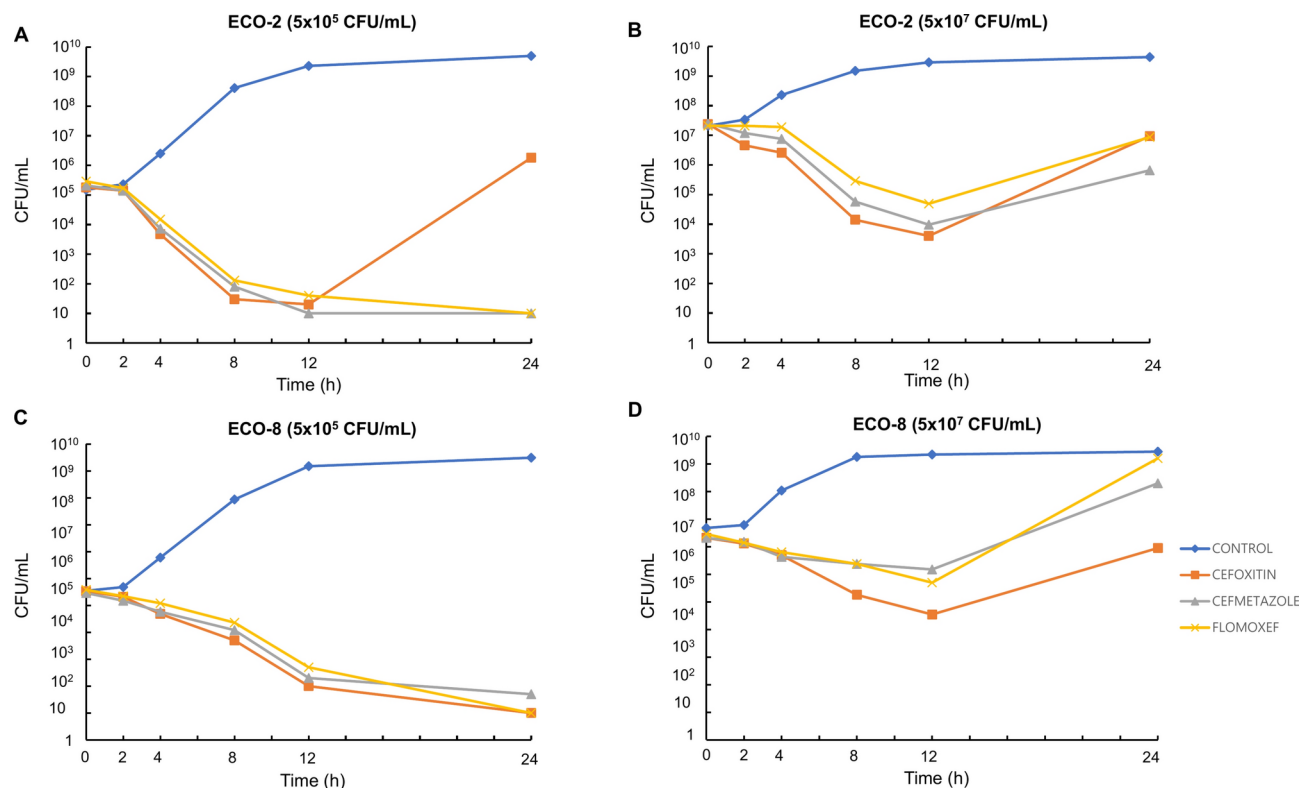
Fig. 1. Time-kill curves of two cephamycin-susceptible ESBL-producing *E. coli* exposed to cefoxitin, cefmetazole, and flomoxef. (**A**) ECO-2, inoculum 5×10^5 CFU/mL; (**B**) ECO-2, inoculum 5×10^7 CFU/mL; (**C**) ECO-8, inoculum 5×10^5 CFU/mL; (**B**) ECO-8, inoculum 5×10^7 CFU/mL. The lower limit of detection for this assay was 10 CFU/mL.

Data availability
The data of this study are available on request from the corresponding author.

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Author contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Wi YM, Choi JY, Lee DE, Jun SH, Kwon KT, Ko KS. The first draft of the manuscript was written by Wi YM and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Declarations

Competing interests

The authors declare no competing interests.

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