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In vitro activity of flomoxef against extended-spectrum β-lactamase–producing *Escherichia coli* and *Klebsiella pneumoniae* in Korea

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ABSTRACT

To find an alternative regimen for the treatment of extended-spectrum β-lactamase (EBSL)–producing Enterobacteriaceae infections, we examined the in vitro activity of flomoxef against *Escherichia coli* and *Klebsiella pneumoniae* having CTX-M-1 group and/or CTX-M-9 group ESBLs. Boronic acid disk methods and polymerase chain reaction amplification were used to detect for ESBL, and AmpC β-lactamase and AmpC β-lactamase co-producers were excluded. Minimum inhibitory concentrations (MICs) were determined for flomoxef by broth microdilution. One hundred seventy-six isolates (*E. coli*, $n = 93$ and *K. pneumoniae*, $n = 83$) were analyzed for susceptibility test. A total of 94.3% (166/176) of isolates were susceptible to flomoxef (MIC_{50}/MIC_{90} were 0.5/8 μg/mL); 98.9% of the ESBL-producing *E. coli* (MIC_{50}/MIC_{90} were 1/4 μg/mL) and 89.2% of the ESBL-producing *K. pneumoniae* (MIC_{50}/MIC_{90} were 0.5/16 μg/mL) were susceptible to flomoxef. Flomoxef has good in vitro activity against ESBL-producing *E. coli* and *K. pneumoniae* and could be considered as an alternative for infections caused by these organisms.

1. Introduction

Extended-spectrum β-lactamases (ESBLs) confers resistance to most commonly used β-lactam antimicrobial agents and often to other classes of antibiotics concurrently, thus limiting the choice of antibiotic to carbapenems. Following the increased use of carbapenems, carbapenem-resistant Enterobacteriaceae (CRE) emerged and spread rapidly worldwide (Chia et al., 2010). CRE is a serious threat to public health, as infection with these organisms can be fatal, with mortality rates as high as 40–50% (Patel et al., 2008). Consequently, there has been much effort to reduce the use of carbapenems, as this would reduce the selection pressure for CRE. In this respect, possible alternative regimens to carbapenems for treating ESBL infections without compromising treatment outcomes have been frequently evaluated in observational studies. The candidates are β-lactam and β-lactamase inhibitor combinations, cefepime, quinolones, and cephamycins. Piperacillin–tazobactam is to date the most studied alternative, and its efficacy seems to be not inferior to that of carbapenems, at least in mild to moderate infections (Tamma and Rodriguez-Bano, 2017).

Cephamycins (e.g., cefotetan, cefoxitin, cefmetazole and flomoxef), which have a methoxy group at the 7-alpha position of their cephalosporin nuclei, have been reported to be highly active against ESBL-producing organism in vitro (Jacoby and Carreras, 1990), but they have not been evaluated as much as β-lactam and β-lactamase inhibitors in clinical studies. This is because there were early reports of a few cases of in vivo resistance during treatment with cephamycins (Pangon et al., 1989; Siu et al., 1999). Therefore, the use of this drug to treat ESBL-producers was somewhat discouraged. Flomoxef is unique among cephamycins in having a difluoromethylthio-acetamido group at position 7, which improves its in vitro activity against ESBL-producing Enterobacteriaceae (Bauernfeind et al., 1991; Jacoby and Carreras, 1990). Since Lee et al. firstly reported in vivo activity of flomoxef against ESBL-producers, a few clinical studies have evaluated the clinical efficacy of cephamycins, mainly flomoxef, as alternatives to carbapenems for the treatment of ESBL infections (Doi et al., 2013; Fukuchi et al., 2016; Lee et al., 2006, 2015b; Matsumura et al., 2015; Yang et al., 2012). Along with these clinical studies, the in vitro activity of flomoxef against clinical ESBL-producing isolates has been reevaluated in Japan, China, and Taiwan (Matsumura et al., 2016; Yang et al., 2015a, 2015b).

According to the Clinical and Laboratory Standards Institute (CLSI), ESBL-producing *E. coli* and *K. pneumoniae* should be reported as resistant to moxalactam regardless of MIC value, and the cutoff for flomoxef is not currently determined. This is because moxalactam and flomoxef have been used mainly in Asia, and data about their pharmacokinetics (PK) and pharmacodynamics (PD) in ESBL infections are scarce even though moxalactam and flomoxef have potent in vitro activity against

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ESBL-producing isolates (Bauernfeind et al., 1991; Carmine et al., 1983; Jacoby and Carreras, 1990). However, recent PK/PD analysis has revealed that flomoxef and moxalactam have bactericidal activity against ESBL-producing isolates (Huang et al., 2018; Ito et al., 2013, 2014). This suggests that moxalactam and flomoxef may be promising alternatives in treatment of ESBL infections.

Flomoxef has been available in South Korea since 1990s and has been used in intra-abdominal infections and gynecologic infections, but its in vitro activity against ESBL-producers has not been investigated. Since CRE is an urgent problem in South Korea, as it is worldwide (Jeong et al., 2016), potential alternative options need to be studied to reduce carbapenem use. In view of this, we have evaluated the in vitro activity of flomoxef against ESBL-producing Enterobacteriaceae.

2. Materials and methods

2.1. Collection of ESBL-positive isolates

This study was conducted at 3 university-affiliated hospitals (Kangnam Sacred Heart Hospital, Hallym University Sacred Heart Hospital, and Wonju Severance Christian Hospital) in South Korea. Bacteria were identified in the respective microbiology laboratories using ViTek-2 (bioMérieux) or Microscan (Siemens Healthcare). Nonduplicated clinical ESBL-positive *Klebsiella pneumoniae* (ESBL-KP) and ESBL-positive *Escherichia coli* (ESBL-EC) isolates were collected between June and July 2016 and sent to a reference laboratory (Kangnam Sacred Heart Hospital) for further investigation.

2.2. Detection of ESBL and AmpC β-lactamase

ESBL phenotyping was performed using ceftazidime–clavulanate and/or cefotaxime–clavulanate in combination with boronic acid versus ceftazidime and/or cefotaxime containing boronic acid. A ≥5-mm increase in zone diameter was considered positive. AmpC β-lactamase production was tested by cefoxitin and/or cefotetan containing boronic acid versus cefoxitin and/or cefotetan; a ≥5-mm increase in zone diameter was considered positive, as described (Song et al., 2007). Isolates not susceptible to cefotaxime were evaluated for having ESBL genes, and polymerase chain reaction (PCR) amplification was performed for the $bla_{\text{CTX-M-1 group}}$ and $bla_{\text{CTX-M-9 group}}$ as described (Abdalhamid et al., 2004), but sequencing to differentiate the specific subtypes was not carried out. Among the cefotaxime-nonsusceptible isolates, those nonsusceptible to cefoxitin were further tested to detect AmpC β-lactamase by performing PCR for bla_{DHA} and bla_{CMY} family. ESBL and AmpC β-lactamase co-producers were excluded from the final susceptibility analysis.

2.3. Antimicrobial susceptibility tests

Minimum inhibitory concentrations (MICs) of flomoxef, cefotetan, and cefoxitin were measured by the broth microdilution method in accordance with CLSI (2015). Cefotetan and cefoxitin were purchased from a commercial source (Sigma, St. Louis, MO), and flomoxef was obtained from its manufacturer (Ildong Pharmaceutical, Korea).

Etests were used to determine the MICs of amoxicillin–clavulanate, cefotaxime, ceftazidime, cefepime, aztreonam, and imipenem (bioMerieux, Marcy I'Etoile, France), and imipenem-resistant isolates were excluded from further susceptibility tests. The MICs of other antibiotics (piperacillin–tazobactam, quinolones, trimethoprim–sulfamethoxazole, and amikacin) were provided from the automated systems in each hospital. Antimicrobial susceptibility was interpreted based on the CLSI (2015). Since no breakpoints for flomoxef have been published by the CLSI, the CLSI breakpoints for moxalactam were used instead (susceptible at MIC ≤8 μg/mL, intermediate at MIC 16–32 μg/mL, and resistant at MIC ≥64 μg/mL) as was done in previous studies (Matsumura et al., 2016; Yang et al., 2015a, 2015b) for comparison purposes only.

3. Results

3.1. ESBL-producing isolates and their genotypic distribution

A total of 237 ESBL-positive isolates were collected from the study hospitals. Carbapenem-resistant isolates (imipenem MIC >1 μg/mL, $n = 24$) were excluded from the study, and the remaining 213 isolates were tested for ESBL and AmpC β-lactamases. In phenotyping tests, 191 were only positive for ESBL and 10 only for AmpC β-lactamase, while 12 were positive for both. Among the ESBL-only–positive isolates ($n = 191$), the number of isolates having CTX-M-1 and/or CTX-M-9 group βlactamase was 178. Of these, 2 had AmpC β-lactamase concurrently (CMY, $n = 2$). Finally, 176 isolates were subjected to the antibiotic susceptibility tests (*E. coli*, $n = 93$ and *K. pneumoniae*, $n = 83$). The sources of these isolates were: 89 urine, 54 respiratory secretion, 10 pus, 14 blood, 5 gastrointestinal fluid, and 4 bile fluid. Among the 22 AmpC β-lactamase–positive isolates from the phenotyping test (ESBL and AmpC β-lactamase co-producers, $n = 12$ and AmpC β-lactamase–only producers, $n = 10$), 11 isolates (*E. coli*, $n = 8$ and *K. pneumoniae*, $n = 3$) were found to carry AmpC β-lactamase gene (bla_{CMY}, $n = 6$ and bla_{DHA}, $n = 5$). The genotypic distributions of ESBL-EC and ESBL-KP were given in Table 1.

3.2. Antimicrobial susceptibilities of the ESBL-producing E. coli and K. pneumoniae

Amikacin had excellent activity (96.6% susceptible). The MICs of flomoxef against the 176 ESBL-producing isolates ranged from ≤0.125 μg/mL to 128 μg/mL (MIC_{50} = 0.5 μg/mL and MIC_{90} = 8 μg/mL). Rates of susceptibility to flomoxef and cefotetan were also high (94.3% and 91.5%), and to cefoxitin, they were moderate (76.7%). About 65% of the isolates were susceptible to amoxicillin–clavulanate and piperacillin–tazobactam. The rates of susceptibility of the ESBL-EC to all the tested antibiotics were greater than those of the ESBL-KP. Detailed data on the in vitro susceptibilities to all the tested antibiotics, and the MIC_{50} and MIC_{90} values of them are presented in Table 2.

3.3. In vitro susceptibilities to cephamycins according to the species and β-lactamase genotypes of E. coli and K. pneumoniae

In the ESBL-EC, the in vitro susceptibility rate to flomoxef was 98.9% (92/93), and MICs ranged from ≤0.125 μg/mL to 16 μg/mL (MIC_{50} = 1 μg/mL and MIC_{90} = 4 μg/mL). Of the ESBL-KP, 89.2% (74/83) were susceptible to flomoxef, and MICs ranged from ≤0.125 μg/mL to 128 μg/mL (MIC_{50} = 0.5 μg/mL and MIC_{90} = 16 μg/mL) (Table 3). Of the ESBL-producing isolates harboring $bla_{\text{CTX-M-1 group}}$, 92.8% (103/111) were susceptibility to flomoxef (MIC_{50} = 0.5 μg/mL and MIC_{90} = 8 μg/mL); of those harboring $bla_{\text{CTX-M-9 group}}$, 96.4% (53/55) were susceptible (MIC_{50} = 0.5 μg/mL and MIC_{90} = 8 μg/mL); and of those harboring both, the proportion was 100% (10/10) (MIC_{50} = 1.0 μg/mL and MIC_{90} = 2 μg/mL) (Table 4). The 2 AmpC co-producers were positive for bla_{CMY}, and MICs of flomoxef were 0.5 μg/mL and 8 μg/mL. Of the 11 isolates

Table 1
ESBL genotypes of the ESBL-producing *E. coli* and *K. pneumoniae* isolates.

ESBL gene	Occurrence, *n* (%)		
	Total ($n = 176$)	*E. coli* ($n = 93$)	*K. pneumoniae* ($n = 83$)
$bla_{\text{CTX-M-1 group}}$	111 (63.1)	48 (51.6)	63 (75.9)
$bla_{\text{CTX-M-9 group}}$	55 (31.3)	38 (40.9)	17 (20.5)
$bla_{\text{CTX-M-1 group}}$ and $bla_{\text{CTX-M-9 group}}$	10 (5.7)	7 (7.5)	3 (3.6)

Table 2
In vitro activities of antimicrobial agents against ESBL-producing *E. coli* and *K. pneumoniae*.

Antimicrobial	All isolates (n = 176)					*E. coli* (n = 93)					*K. pneumoniae* (n = 83)				
	S%	I%	R%	MIC_{50}	MIC_{90}	S%	I%	R%	MIC_{50}	MIC_{90}	S%	I%	R%	MIC_{50}	MIC_{90}
Flomoxef[a]	94.3	5.1	0.5	0.5	8	98.9	1.1	0	1	4	89.2	9.6	1.2	0.5	16
Cefotetan	91.5	4.0	4.5	2	16	94.6	2.2	3.2	2	8	88.0	6.0	6.0	2	32
Cefoxitin	76.7	13.1	10.2	8	16	81.7	12.9	5.4	4	16	71.1	13.3	15.7	8	64
Amoxicillin/clavulanate	66.5	30.1	3.4	8	16	66.7	31.2	2.2	8	16	66.3	28.9	4.8	8	16
Piperacillin/tazobactam	63.1	13.1	23.9	16	≥128	80.6	14.0	5.4	8	64	43.4	12.0	44.6	64	≥128
Cefotaxime	0	0	100.0	>32	>32	0	0	100.0	>32	>32	0	0	100.0	>32	>32
Ceftazidime	34.7	16.5	48.9	8	>256	40.9	24.7	34.4	8	64	27.7	7.2	65.1	16	>256
Cefepime	14.8	35.2	50.0	8	64	20.4	38.7	40.9	8	64	8.4	31.3	60.2	16	128
Aztreonam	18.8	6.3	75.0	64	>256	29.0	7.5	63.4	32	>256	7.2	4.8	88.0	128	>256
Imipenem	100	0	0	0.125	0.25	100.0	0	0	0.125	0.25	100.0	0	0	0.125	0.25
Ciprofloxacin[b]	14.6	1.5	83.8	≥4	≥4	19.4		80.6	≥4	≥4	8.4	2.4	71.1	≥4	≥4
Levofloxacin[c]	25.7	8.0	66.4	≥8	≥8	28.3	5.0	66.7	≥8	≥8	14.5	7.2	42.2	≥8	≥8
Trimethoprim/sulfamethoxazole[c]	38.1		62.0	80	≥320	50.5		49.5	80	≥320	25.3		74.7	80	≥320
Amikacin	96.6		3.4	8	16	97.8		2.2	4	16	95.2		4.8	16	64

R = resistant; S = susceptible; I = intermediate; $MIC_{50/90}$ = minimum inhibitory concentrations at which 50% and 90% of isolates were inhibited.
[a] The CLSI breakpoints of moxalactam (S ≤ 8 μg/mL, R ≥ 64 μg/mL for Enterobacteriaceae) were applied for MIC interpretation of flomoxef.
[b] Ciprofloxacin susceptibility data available for 130 isolates: 62 in *E. coli* and 68 in *K. pneumoniae*.
[c] Levofloxacin susceptibility available for 113 isolates: 60 in *E. coli* and 53 in *K. pneumoniae*.

carrying only AmpC β-lactamase (bla_{CMY}, n = 5 and bla_{DHA}, n = 6), 91% (10/11) were resistant to flomoxef, with the MICs ranging from 0.5 μg/mL to 125 μg/mL (MIC_{50} = 64 μg/mL and MIC_{90} = 256 μg/mL).

4. Discussion

This study has shown that flomoxef has excellent in vitro activity against ESBL-EC and ESBL-KP and compared other possible alternatives to carbapenems for treating ESBL infections. The overall susceptibility rates of flomoxef were 94.3% (98.9% in ESBL-EC and 89.2% in ESBL-KP), which were similar to those seen in previous studies. Yang et al. investigated the in vitro susceptibility of 320 ESBL-producing isolates to flomoxef in China, and over 95% of them (97.4% in ESBL-EC, 98.4% in ESBL-KP, and 98.8% in ESBL-producing *Proteus mirabilis*) were susceptible to flomoxef (Yang et al., 2015b). Flomoxef also had excellent in vitro activity against ESBL-producing isolates in Japan (99.5%, 392/394) (Matsumura et al., 2016).

Despite the good in vitro activity of cephamycin against ESBL-producing isolates and its resistance to hydrolysis, it has not been one of the recommended options for ESBL infections because of concerns about acquired resistance. After cefoxitin treatment, initially cefoxitin-susceptible ESBL-KP isolate was found to have become resistant with decreased porin protein expression, and the infection was uncontrolled (Pangon et al., 1989). Similarly, Lee et al. first observed in vitro and in vivo activity of flomoxef against ESBL-KP, but subsequently, he reported porin mutation and concomitant AmpC β-lactamase acquisition in ESBL-KP after exposure to flomoxef, resulting in resistance to flomoxef. Moreover, this isolate had also become resistant to ertapenem (Lee et al., 2007). Recently, the authors reported that 2 ESBL-KP isolates responsible for recurrent bacteremia carried porin mutations and/or AmpC β-lactamase after flomoxef had been used against the initial bacteremia (Lee et al., 2015a).

With regard to clinical efficacy, several studies have compared treatment outcomes between cephamycins (mainly flomoxef or cefmetazole) and carbapenem since an early small study (flomoxef group, n = 7; carbapenem group, n = 20) obtained comparable clinical outcomes for those 2 agents in ESBL-KP bacteremia (Lee et al., 2006). In a larger study by the same author, the efficacies of flomoxef (n = 58) and ertapenem (n = 188) in cefotaxime-resistant Enterobacteriaceae bacteremia did not differ (Lee et al., 2018). Matsumura et al. studied the effects of cefmetazole and flomoxef versus carbapenem in ESBL-EC, and the cephamycins were not inferior to carbapenem (Matsumura et al., 2015). Treatment outcomes for cefmetazole in urinary tract infections and bacteremias due to ESBL-producing Enterobacteriaceae seemed to be similar with those of carbapenem (Doi et al., 2013; Fukuchi et al., 2016). Also, cefoxitin was tested in France as an alternative regimen for urinary tract infections caused by ESBL-producing Enterobacteriaceae, and it gave results comparable to carbapenem (Pilmis et al., 2014). However, there are also contradictory data. In the largest study to date (132 in the flomoxef arm and 257 in the carbapenem arm), flomoxef was inferior to carbapenem in ESBL-producing bacteremia (30-day mortality in flomoxef, 28.8%; carbapenem, 12.8%), and in propensity score-matched analysis, flomoxef therapy for the ESBL-producing isolates with MICs of 2–8 μg/mL for flomoxef was associated with poorer outcomes than carbapenem therapy (30-day mortality in flomoxef, 38.4%; carbapenem, 18.6%) (Lee et al., 2015b). And recently, the use of flomoxef was reported to be associated with recurrent bacteremia caused by ESBL-EC or ESBL-KP (Lee et al., 2015a).

These mixed results may have been due to differences in severity of disease and in the distribution of clinical syndromes. In addition, differences in inclusion criteria could have had an effect. Specifically, whether AmpC β-lactamase co-producers were included or not may have influenced the clinical outcomes. The large clinical study mentioned above only established ESBL status according to the CLSI guidelines, not by PCR for ESBL genes (Lee et al., 2015b). And another study included cefotaxime-resistant Enterobacteriaceae without any confirmation test for ESBL status (Lee et al., 2018). Some AmpC β-lactamase co-producers may have been included in those studies, and they may have been treated by cephamycins only based on the susceptibility data, resulting in treatment failures or recurrence. All the clinical studies were retrospective observational studies, and the

Table 3
MIC distribution of flomoxef in all ESBL-producing isolates, ESBL-producing *E. coli*, and ESBL-producing *K. pneumoniae*.

	Cumulative number (%) of isolates with flomoxef MIC (μg/mL)								
	≤.125	0.25	0.5	1	2	4	8	16	>16
All isolates (n = 176)	29 (16.5)	54 (30.7)	92 (52.3)	124 (70.5)	144 (81.8)	156 (88.6)	166 (94.3)	175 (99.4)	176 (100)
E. coli (n = 93)	7 (7.5)	20 (21.5)	45 (48.4)	70 (75.3)	83 (89.2)	90 (96.8)	92 (98.9)	93 (100)	93 (100)
K. pneumoniae (n = 83)	22 (26.5)	34 (41.0)	47 (56.6)	54 (65.1)	61 (73.5)	66 (79.5)	74 (89.2)	82 (98.8)	83 (100)

Table 4
MIC distributions of flomoxef, cefotetan, and cefoxitin in ESBL-producing isolates with blaCTX-M-1 group and blaCTX-M-9 group.

Antimicrobials	ESBL genotype	Cumulative number (%) of isolates with flomoxef MIC (μg/mL)									S (%)[a]
		≤.125	0.25	0.5	1	2	4	8	16	>16	
Flomoxef	All isolates	29 (16.5)	54 (30.7)	92 (52.3)	124 (70.5)	144 (81.8)	156 (88.6)	166 (94.3)	175 (99.4)	176 (100)	166 (94.3)
	$bla_{\text{CTX-M-1 group}}$	23 (20.7)	36 (32.4)	59 (53.2)	75 (67.6)	89 (80.2)	97 (87.4)	103 (92.8)	111 (100)	111 (100)	103 (92.8)
	$bla_{\text{CTX-M-9 group}}$	6 (10.9)	17 (30.9)	29 (52.7)	43 (78.2)	45 (81.8)	49 (89.1)	54 (98.2)	55 (100)	55 (100)	53 (96.4)
	$bla_{\text{CTX-M-1 group}} + bla_{\text{CTX-M-9 group}}$	0 (0)	1 (10)	4 (40)	6 (60)	10 (100)	10 (100)	10 (100)	10 (100)	10 (100)	10 (100.0)
Cefotetan	All isolates	10 (5.7)	23 (13.1)	32 (18.2)	55 (31.3)	103 (58.5)	131 (74.4)	148 (84.1)	161 (91.5)	176 (100)	161 (91.5)
	$bla_{\text{CTX-M-1 group}}$	5 (4.5)	13 (11.7)	19 (17.1)	34 (30.6)	63 (56.8)	80 (72.1)	89 (80.2)	99 (89.2)	111 (100)	99 (89.2)
	$bla_{\text{CTX-M-9 group}}$	4 (7.3)	9 (16.4)	11 (20.0)	18 (32.7)	34 (61.8)	43 (78.2)	50 (90.9)	53 (96.4)	55 (100)	53 (96.4)
	$bla_{\text{CTX-M-1 group}} + bla_{\text{CTX-M-9 group}}$	0 (0)	1 (10.0)	2 (20.0)	3 (30.0)	6 (60.0)	8 (80.0)	9 (90.0)	9 (90.0)	10 (100)	9 (90.0)
Cefoxitin	All isolates	1 (0.6)	1 (0.6)	2 (1.1)	2 (1.1)	6 (3.4)	82 (46.6)	136 (77.3)	159 (90.3)	176 (100)	136 (77.3)
	$bla_{\text{CTX-M-1 group}}$	1 (0.9)	1 (0.9)	2 (1.8)	2 (1.8)	5 (4.5)	48 (43.2)	81 (73.0)	98 (88.3)	111 (100)	81 (73.0)
	$bla_{\text{CTX-M-9 group}}$	0 (0)	0 (0)	0 (0)	0 (0)	1 (1.8)	30 (54.5)	46 (83.6)	51 (92.7)	55 (100)	46 (83.6)
	$bla_{\text{CTX-M-1 group}} + bla_{\text{CTX-M-9 group}}$	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	4 (40.0)	9 (90.0)	10 (10.0)	10 (100)	8 (80.0)

[a] The CLSI breakpoints of moxalactam (S ≤ 8 μg/mL, R ≥ 64 μg/mL for Enterobacteriaceae) were applied for MIC interpretation of flomoxef.

treating physicians could not discriminate accurately between ESBL producers and AmpC co-producer based on the susceptibility results alone. ESBL and AmpC co-producers showed decreased susceptibility to cephamycins (Lee et al., 2010; Matsumura et al., 2016). ESBL-KP with plasmid-mediated AmpC β-lactamase (all were DHA-1) were 100% resistant to flomoxef. However, in our study, the 2 AmpC β-lactamase co-producers had MICs in the range of ≤8 μg/mL, and nearly 50% of Matsumura's isolates were susceptible to flomoxef (Matsumura et al., 2015). So, with the current cutoff value (≤8 μg/mL), clinicians would treat a substantial portion of AmpC β-lactamase co-producers with flomoxef, leading to unwanted outcomes because of the acquisition of mutations. Moreover, decreased susceptibility to carbapenem could further limit the treatment options, as described (Lee et al., 2015a). Because of this and in the light of the previous observation that the use of flomoxef for ESBL-producing bacteremias with MICs of 2–8 μg/mL was associated with poor outcomes, concerns about the cutoff for flomoxef and the use of flomoxef against ESBL-producing infections exist as others have suggested (Lee et al., 2015b). When we look into our isolates, 70% of the isolates (124/176) are still susceptible even if the cutoff is lowered to ≤1 μg/mL, and a substantial proportion of patients with infection by ESBL-producing organism could be treated with flomoxef safely. In addition, it would be more prudent to identify ESBL and AmpC β-lactamase co-producers when flomoxef is used in infections caused by ESBL-producing isolates.

PK/PD data are important for determining the cutoff values for treating clinical infections. In the PK/PD data referred to earlier, the number of ESBL-producing isolates was small ($n = 31$) and the MIC_{90} of flomoxef (0.5 μg/mL) was lower than in the clinical studies (Ito et al., 2013). In a recent clinical study, definitive flomoxef therapy appeared to be inferior to carbapenems in treating ESBL bacteremia, particularly for isolates with MICs of 2–8 μg/mL (Lee et al., 2015b). However, the isolates included in the study had higher MICs than those used in the PK/PD analysis, with 2 μg/mL being the modal value (29.0%) ($MIC_{50} = 2$ and $MIC_{90} = 8$), and this might have been responsible for the poor outcomes with flomoxef. There needs to be more PK/PD analysis to evaluate the efficacy of flomoxef in the case of isolates with higher MICs (2–8 μg/mL) and to establish appropriate cutoffs and regimens for treatment of ESBL infections.

Our study has several limitations. First, it was conducted in only 3 Korean hospitals over 2 months, and the isolates may not be representative of similar isolates worldwide. However, our data are consistent with those of previous studies, providing the first Korean data demonstrating excellent in vitro activity of flomoxef against ESBL-EC and ESBL-KP. In addition, to our knowledge, this is the first study evaluating the in vitro activity of the 3 cephamycins against clinical ESBL-producing Enterobacteriaceae. Second, we included only the CTX-M-1 group and/or CTX-M-9 group types of ESBL, so we may have missed other types of ESBL. However, most ESBLs belong to these 2 groups, and we could not find any reports of differences in vitro activities between ESBL genotypes in the literatures; therefore, the results are unlikely to have been affected by this. Also, by using PCR for AmpC β-lactamase and boronic acid disk methods, we were able to detect AmpC β-lactamase co-producers efficiently and exclude them from the study (Song et al., 2007). In addition, by excluding imipenem-resistant isolates, carbapenem-resistant Enterobacteriaceae was also excluded. As a result, only ESBL-producing isolates were investigated.

In conclusion, flomoxef has good in vitro activity against ESBL-EC and ESBL-KP and seems to be an effective alternative to carbapenem in the treatment of ESBL infections.

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