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ORIGINAL RESEARCH

Antibacterial effect evaluation of moxalactam against extended-spectrum β-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* with in vitro pharmacokinetics/pharmacodynamics simulation

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Objectives: The aim of this study was to evaluate the bactericidal effects of moxalactam (MOX), cefotaxime (CTX), and cefoperazone/sulbactam (CFZ/SBT) against extended-spectrum β-lactamase (ESBL) producing *Escherichia coli* and *Klebsiella pneumoniae*, using an in vitro pharmacokinetics (PK)/pharmacodynamics model.

Methods: Two clinical ESBL-producing strains ($bla_{CTX\text{-}M\text{-}15}$ positive *E. coli* 3376 and $bla_{CTX\text{-}M\text{-}14}$ positive *K. pneumoniae* 2689) and *E. coli* American Type Culture Collection (ATCC)25922 were used in the study. The PK Auto Simulation System 400 was used to simulate the human PK procedures after intravenous administration of different doses of MOX, CTX, and CFZ/SBT. Bacterial growth recovery time (RT) and the area between the control growth curve and bactericidal curves (IE) were employed to assess the antibacterial efficacies of all the agents.

Results: The minimum inhibitory concentrations of MOX, CTX, and CFZ/SBT against *E. coli* ATCC25922, 3376, and 2689 strains were 0.5, 0.5, 0.25; 0.06, >256, 256; and 0.5/0.5, 16/16, 32/32 mg/L. All the agents demonstrated outstanding bactericidal effects against *E. coli* ATCC25922 (RT >24 h and IE >120 $\log_{10}$ CFU/mL·h^{-1}) with simulating PK procedures, especially in the multiple dose administration models. Against ESBL producers, CTX and CFZ/SBT displayed only weak bactericidal effects, and subsequent regrowth was evident. MOX exhibited potent antibacterial activity against all the strains tested. The values of effective parameters of MOX were much higher than those of CTX and CFZ/SBT (the bacterial RTs with the 3 agents were >24, <4, and <13 h, and the IEs were >110, <10, and <60 $\log_{10}$ CFU/mL·h^{-1}, respectively).

Conclusion: MOX demonstrated excellent bactericidal effect, which is worthy of further exploration to serve as an alternative therapeutic agent against ESBL-producing Enterobacteriaceae.

Keywords: moxalactam, in vitro, pharmacokinetics/pharmacodynamics, extended-spectrum β-lactamases, *Escherichia coli, Klebsiella pneumoniae*

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Introduction

A report from the World Health Organization revealed that the resistance of *Escherichia coli* and *Klebsiella pneumoniae* to third-generation cephalosporins is spreading.[1] Extended-spectrum β-lactamases (ESBLs) confer resistance to most commonly used β-lactam agents, and often co-resistance to other antimicrobial classes, leading to the failure of clinical treatment.[2] Carbapenems are recognized as the first-line therapeutic agents to manage ESBL-producing Enterobacteriaceae infections.[2,3] However,

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the increased use of carbapenems may generate selection pressure for carbapenem-resistant isolates, resulting in their evolution and dissemination. Thus, the prudent use of antimicrobials in the clinic is crucial, as is finding alternatives of carbapenems to treat infections caused by ESBL-producing isolates and avoid exacerbation of the spreading of ESBLs.

Moxalactam (MOX) is a synthetic oxacephem β-lactam agent in which the sulfur atom in the cephalosporin nucleus is substituted by an oxygen atom, representing a 7a-methoxy-substitution.[4] MOX displays potent in vitro antibacterial activity against ESBL-producing Enterobacteriaceae and has favorable pharmacological properties, including a high serum concentration and large apparent distribution volume.[4] However, according to the descriptions of the Clinical and Laboratory Standards Institute (CLSI), ESBL-producing *E. coli* and *K. pneumoniae* should be reported as resistant to MOX irrespective of the minimum inhibitory concentration (MIC), because MOX has limited availability in many countries and clinical experience on its use in the treatment of ESBL-producing bacterial infections is scarce, and its effect is therefore controversial.[5] In the recent times, some laboratory and clinical results have indicated the efficacy of MOX against ESBL-producing bacterial infections. Matsumura et al reported that treatment of bacteremia caused by ESBL-producing *E. coli* and *K. pneumoniae* with oxacephems or carbapenems resulted in a comparable outcome.[6] Furthermore, time-kill studies and Monte Carlo simulation analysis have also demonstrated promising bactericidal activity of oxacephems against ESBL-producing Enterobacteriaceae.[7,8] Limited pharmacokinetics (PK) and pharmacodynamics (PD) data on the activity of MOX utilized in clinics emphasizes the urgent need to evaluate the appropriate dosage regimens in killing ESBL-producing isolates. Our objective was to characterize the bacterial killing effects with simulated human exposures of cefotaxime (CTX), cefoperazone/sulbactam (CFZ/SBT), and MOX against *E. coli* ATCC25922 and CTX-M-producing *E. coli* and *K. pneumoniae* to provide further evidence to support the clinical use of MOX in the treatment of ESBL-producing bacterial infections.

Materials and methods

Bacterial strains and media

Three isolates ($bla_{CTX\text{-}M\text{-}15}$ positive *E. coli* 3376 isolated from urine, $bla_{CTX\text{-}M\text{-}14}$ positive *K. pneumoniae* 2689 isolated from sputum, and *E. coli* ATCC25922) were investigated in this study. Organisms were grown, cultured, and quantified using Mueller–Hinton agar (MHA; #1900515, OXOID) and Mueller–Hinton broth (#1765821, OXOID).

In vitro susceptibility testing

MICs for all organisms were determined using the broth microdilution method, and breakpoints for resistance were defined in accordance with the CLSI.[5] *E. coli* ATCC25922 and *K. pneumoniae* ATCC700603 were used as quality controls in antimicrobial susceptibility testing. It is noteworthy that the breakpoint of CFZ/SBT is consistent with the CLSI interpreted breakpoint for CFZ alone.[9]

PK parameters employed in the study

The simulated human serum concentrations of CTX, CFZ/SBT, and MOX after single intravenous (IV) administration were based on PK data from previous studies (Table S1).[4,10–12] In the study, a 2-compartment PK model of the agents was used for all experiments. The regimens for CTX 2 g once daily (2 g qd), 2 g every 8 h (2 g q8h); CFZ/SBT 2 g once daily (2 g qd), thrice daily (2 g q8h); and MOX 1 g once daily (1 g qd), twice daily (1 g q12h), thrice daily (1 g q8h), 2 g once daily (2 g qd), twice daily (2 g q12h), and thrice daily (2 g q8h) after IV administration were simulated (Figure S1).

In vitro PK/PD simulation model and antibacterial activity measurements

The in vitro PK Auto Simulation System 400 (PASS-400; Dainippon Seiki, Kyoto, Japan) utilized in this study has been described previously.[13,14] Briefly, the apparatus consisted of a central unit, growth media chamber, and waste container connected by 3-way pipelines and syringe pumps to form a closed system. The central unit contained a magnetic stirrer for homogeneous mixing, and the entire unit was placed in a shaking waterbath at 37°C. Broth medium and drug solution were pumped into the central unit via a computer-controlled peristaltic pump, while growth medium was simultaneously removed through an exit port that flowed into the waste container.

Inoculum preparation included making a bacterial suspension of ~10^8 CFU/mL from fresh subcultures of the tested isolate (incubated at 37°C overnight) with 0.9% normal saline. The inoculum (1 mL) was injected into 100 mL broth medium in the model to achieve the starting inoculum of ~10^6 CFU/mL. Once inoculated, the strain was grown to log phase over 30 min, and 1.5 mL was collected for CFU determination at predetermined time points (0, 2, 4, 6, 10, 14, 18, 20, and 24 h). Each sample was centrifuged, washed, and resuspended in normal saline thrice to avoid drug carryover effect and serially diluted in 0.9% normal saline before plating on MHA plates. Bacterial clones were counted after 18 h incubation. The limit of clone detection was 30 CFU/mL.

In addition, each experiment was performed in triplicate to assure reproducibility.

Quantitative evaluation of the antimicrobial effect

The PD parameters, including Maximum Kill Down (MKD, the difference between the minimum bacterial count and the initial count during the experiment), the bacterial counting difference between time 0 and 24 h ($\Delta\log N_{24}$), bacterial growth recovery time (RT, the time from exposure to the antibiotic till the moment when the bacterial count again reaches its initial level), the time required for microbial initial count to be reduced by 90%, 99%, 99.9% ($T_{99.9\%}$), and the time during which the concentration of the drug was over the MIC (%T> MIC) were analyzed by PASS 400 Analyze Bactericidal Activity software (Figure S2).[15] The area between the control growth curve and bactericidal curves (IE) was employed as the integral parameters for evaluating antimicrobial effect; this is preferable to other PD parameters.[15] IE was calculated by the trapezoidal rule using the program GraphPad Prism 6.

Results

MICs of 3 agents against the strains

Table 1 shows the MICs for CTX, CFZ, CFZ/SBT, and MOX against *E. coli* ATCC25922, 3376, and 2689. CTX-M-producing isolates 3376 and 2689 were highly resistant to CTX and CFZ, while MOX resulted in low MICs (0.5 mg/L and 0.25 mg/L, respectively). In addition, MICs for CFZ/SBT against 3376 and 2689 were 16/16 mg/L and 32/32 mg/L, respectively.

Antibacterial effects in PK/PD simulations

After inoculating in antibiotic-free broth, the tested strains increased from ~6 $\log_{10}$ CFU/mL to ~9 $\log_{10}$ CFU/mL at 24 h in the model. All tested antibiotics exhibited a rapid bactericidal efficacy against *E. coli* ATCC25922, defined as a reduction of >4 $\log_{10}$ CFU/mL of MKD 4 h after initiation of treatments, and inhibited bacterial growth (RT >24 h) in the experimental duration (Figure 1A and B; Table 2). Notwithstanding the bacterial regrowth observed in all PK-simulated producers of CTX, CFZ/SBT, and MOX, multiple dosing (q8h and q12h) could inhibit the regrowth for a longer period of time than once-daily dosage regimens (Tables 2 and 3).

Although the presence of SBT can lower the MICs of CFZ against 3376 and 2689 from >256 mg/L to 16/16 mg/L and 32/32 mg/L, respectively, the shorter value of %T > MIC achieved with CFZ/SBT (2 g qd) resulted in weak bactericidal effects, and the bacterial count was similar to that of controls at 24 h, which was similar to that observed with CTX (2 g qd) (Table 3; Figure 1C–F). Of note, the multiple dosage regimen of CFZ/SBT (2 g q8h) also showed limited bactericidal effects and were followed by rapid regrowth against susceptible 3376 and intermediate 2689 strains. In contrast, MOX still exhibited stable antibacterial activity against both 3376 and 2689 strains, as observed in *E. coli* ATCC25922. The MKD and RT of MOX against ESBL-producing isolates were similar to those against *E. coli* ATCC25922. The multiple dosage regimens achieved larger IE than once-daily dosage regimens, especially in the simulations with 2 g q8h, resulting in >145 $\log_{10}$ CFU/mL·h^{-1}, >20 h of $T_{99.9\%}$, and >3 log10 reduction in CFU/mL at 24 h against 3376 and 2689 strains (Table 2).

The relationships between %T > MIC and IE are shown in Figure 2; the %T > MIC value of 50% seemed to be the lowest requirement for bacterial inhibition. The regimens of MOX (1 g q12h, 1 g q8h, 2 g q12h, and 2 g q8h) achieved 100% of %T > MIC against *E. coli* ATCC25922, 3376, and 2689, and the IE of these simulated regimens were all over 140 $\log_{10}$ CFU/mL·h^{-1}. For the simulated regimens of CTX and CFZ/SBT, the shorter value of %T > MIC against 3376 and 2689 resulted in smaller IE (<10 and <60 $\log_{10}$ CFU/mL·h^{-1}, respectively), being significantly lower than the IE of MOX-simulated regimens (Table 3; Figure 2). Notably, the IE for CFZ/SBT and MOX against *E. coli* ATCC25922 was larger than that for tested drugs against 3376 and 2678, when %T > MIC achieved similar values, especially in the value of 50%. The 70% value of %T > MIC was responsible for the promising bacteriological eradication of 3376 and 2689.

Table 1 MICs of antibiotics against ESBL-producing bacteria and *Escherichia coli* ATCC25922

Bacteria	MIC (mg/L)				ESBL type
	CTX	CFZ	CFZ/SBT (1:1)	MOX	
E. coli 3376	>256	>256	16/16	0.5	CTX-M-15
Klebsiella pneumoniae 2689	256	>256	32/32	0.25	CTX-M-14
E. coli ATCC25922	0.06	0.5	0.5	0.5	/
K. pneumoniae ATCC700603	256	>256	8/8	4	SHV-18

Note: "/" Indicates no ESBL enzymes.
Abbreviations: ATCC, American Type Culture Collection; CFZ, cefoperazone; CTX, cefotaxime; ESBL, extended-spectrum β-lactamase; MIC, minimum inhibitory concentration; MOX, moxalactam; SBT, sulbactam.

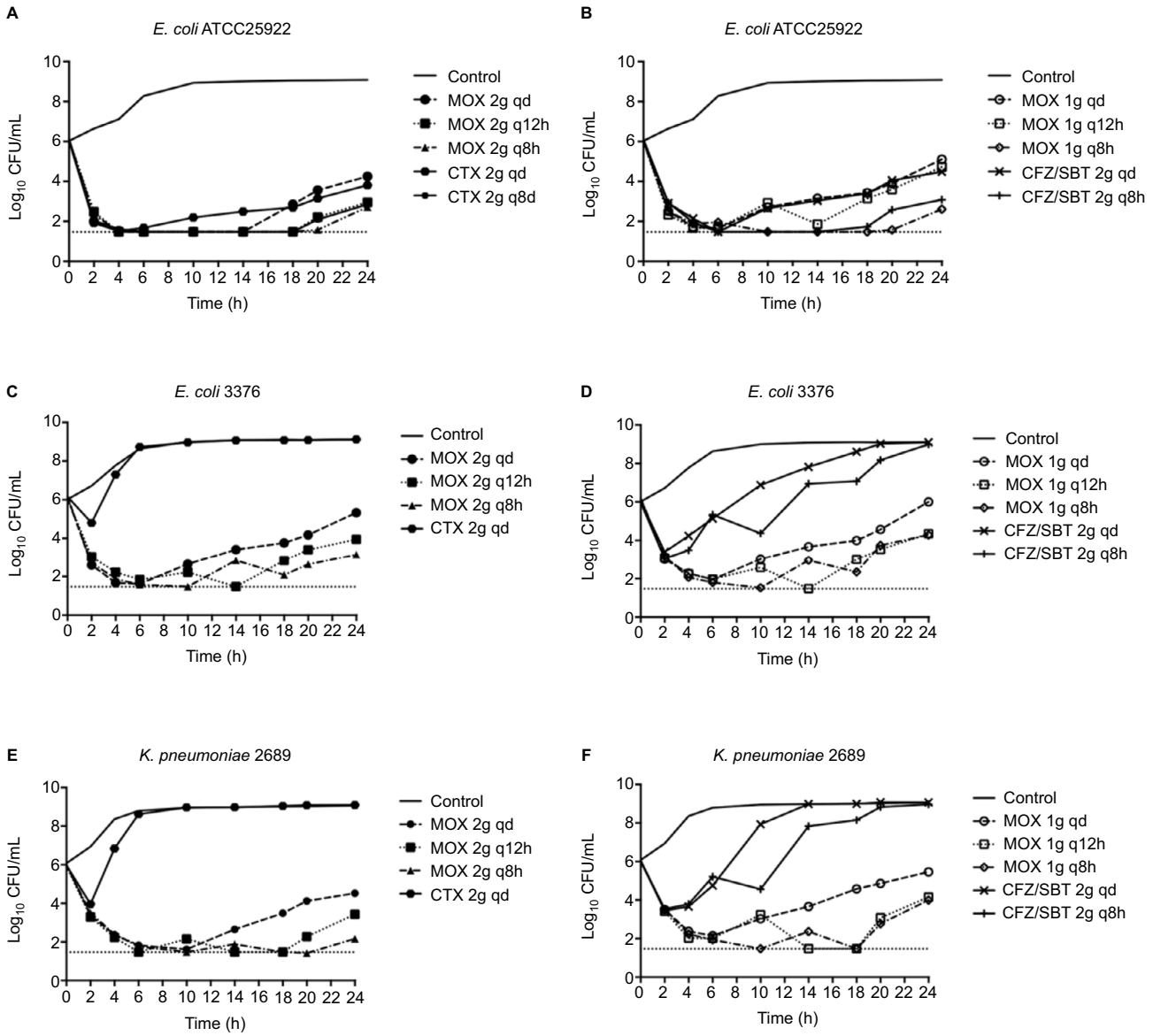

Figure 1 In vitro dynamic time-kill curves using human exposures of moxalactam, cefotaxime, and cefoperazone/sulbactam against *Escherichia coli* ATCC25922 and CTX-M-producing *E. coli* and *Klebsiella pneumoniae*.

Notes: (**A**) and (**B**) show simulated dosing regimens against *E. coli* ATCC25922; (**C**) and (**D**) show simulated dosing regimens against *E. coli* 3376; (**E**) and (**F**) show simulated dosing regimens against *K. pneumoniae* 2689. The dotted lines indicate the regimens of MOX. The solid lines indicate the regimens of CTX and CFZ/SBT. The lower limit of detection (broken line) was 1.47 $\log_{10}$ CFU/mL.

Abbreviations: ATCC, American Type Culture Collection; CFU, colony forming units; CFZ/SBT, cefoperazone/sulbactam; CTX, cefotaxime; MOX, moxalactam; qd, once-daily; q12h, twice-daily; q8h, thrice-daily.

Discussion

ESBL-producing Enterobacteriaceae are prevalent among community- and hospital-acquired infections.[16,17] Several studies have established that CTX-M-14 and CTX-M-15 are the most abundant ESBL enzymes worldwide in urinary tract, bloodstream, and intra-abdominal infections.[18–20] Because antibacterial drugs for treating ESBL-producing bacterial infections are limited, carbapenems have traditionally been recommended as the drug of choice in such circumstances. However, the increased usage of carbapenems has promoted the rapid evolution and dissemination of carbapenem- and/or multidrug-resistant isolates.[21] Thus, finding alternative agents for the clinical treatment of infections caused by ESBL producers is imperative to alleviate the selective pressure on carbapenem-resistant germs.

Replacement of the structural modification of the 7-hydroxyl group in MOX makes the drug resistant to hydrolysis by ESBL enzymes. Our results indicated that MICs for MOX against 3376 and 2689 strains were 0.5 mg/L and 0.25 mg/L, respectively, which was in the range of MIC val-

Table 2 Antibacterial effect of each simulated dosing regimen of the 3 antibiotics against ESBL-producing bacteria and *Escherichia coli* ATCC25922

Dosage regimen		MKD (Log_{10}CFU/mL)	$\Delta logN_{24}$ (Log_{10}CFU/mL)	RT (h)	$T_{90\%}$ (h)	$T_{99\%}$ (h)	$T_{99.9\%}$ (h)	IE (Log_{10}CFU/mL h^{-1})
25922	MOX (1g qd)	−4.4	−0.9	>24	23.1	19.3	10.0	127.6
	MOX (1g q12h)	−4.4	−1.3	>24	>23.5	20.7	16.1	134.8
	MOX (1g q8h)	−4.7	−3.5	>24	>23.5	>23.0	>22.5	155.8
	MOX (2g qd)	−4.7	−1.8	>24	>23.6	21.5	17.2	145.7
	MOX (2g q12h)	−4.8	−3.2	>24	>23.5	>23.0	>22.5	155.4
	MOX (2g q8h)	−4.6	−3.3	>24	>23.5	23.1	>22.5	158.1
	CTX (2g qd)	−4.6	−2.2	>24	>23.6	>23.2	18.2	140.8
	CTX (2g q8h)	−4.8	−3.2	>24	>23.6	>23.2	>22.7	156.6
	CFZ/SBT (2g qd)	−4.6	−1.5	>24	>23.5	18.7	10.0	128.0
	CFZ/SBT (2g q8h)	−4.7	−3	>24	>23.5	>22.9	>22.1	151.5
3376	MOX (1g qd)	−4.2	−0.2	>24	21.1	17.5	8.5	118.9
	MOX (1g q12h)	−4.7	−1.9	>24	>23.5	22.1	16.7	138.7
	MOX (1g q8h)	−4.8	−1.9	>24	>23.4	19.7	17.3	139.2
	MOX (2g qd)	−4.6	−0.8	>24	23.0	19.0	10.6	127.7
	MOX (2g q12h)	−4.6	−2.1	>24	>23.4	>22.8	16.6	142.4
	MOX (2g q8h)	−4.8	−2.9	>24	>23.5	>22.9	20.6	148.2
	CTX (2g qd)	−1.4	3.0	3.2	1.6	NA	NA	45.0
	CFZ/SBT (2g qd)	−2.7	3.1	8.0	5.1	2.4	NA	39.4
	CFZ/SBT (2g q8h)	−3.2	3.0	12.6	4.9	3.3	1.1	62.0
2689	MOX (1g qd)	−3.9	−0.5	>24	20.3	14.0	7.3	116.8
	MOX (1g q12h)	−5.1	−1.9	>24	>23.3	21.0	6.3	143.2
	MOX (1g q8h)	−4.7	−2	>24	>23.3	>22.5	17.9	147.6
	MOX (2g qd)	−4.5	−1.5	>24	>23.3	18.1	13.2	135.8
	MOX (2g q12h)	−4.7	−2.5	>24	>23.3	>22.6	19.5	153.0
	MOX (2g q8h)	−4.6	−3.8	>24	>23.3	>22.5	>21.3	157.5
	CTX (2g qd)	−2.0	3.1	3.5	2.3	0.4	NA	10.5
	CFZ/SBT (2g qd)	−2.8	3.0	7.6	3.8	3.5	NA	32.4
	CFZ/SBT (2g q8h)	−2.7	3.0	12.0	4.9	2.8	NA	52.3

Note: Negative numbers indicate reduction in CFU from 0 h.

Abbreviations: CFU, colony forming units; CTX, cefotaxime; CFZ/SBT, cefoperazone/sulbactam; ESBL, extended-spectrum β-lactamase; IE, the area between the control growth and antibacterial killing curves; $\Delta log\ N_{24}$, the bacterial count difference between time 0 and 24 h; MOX, moxalactam; NA, not applicable; MKD, maximum kill down; qd, once-daily; q12h, twice-daily; q8h, thrice-daily; RT, bacterial growth recovery time; $T_{90\%}$, $T_{99\%}$, and $T_{99.9\%}$, the time required for microbial initial count to be reduced by 90%, 99%, and 99.9%, respectively.

Table 3 %T > MIC values for MOX, CTX, and CFZ/SBT treatment regimens

Bacteria	MOX (1g qd) (%)	MOX (1g q12h) (%)	MOX (1g q8h) (%)	MOX (2g qd) (%)	MOX (2g q12h) (%)
Escherichia coli ATCC25922	76.60	100	100	85.10	100
Klebsiella pneumoniae 2689	87.38	100	100	95.63	100
E. coli 3376	76.60	100	100	85.10	100
Bacteria	**MOX (2g q8h) (%)**	**CTX (2g qd) (%)**	**CTX (2g q8h) (%)**	**CFZ/SBT (2g qd) (%)**	**CFZ/SBT (2g q8h) (%)**
E. coli ATCC25922	100	56.13	100	48.83	100.00
K. pneumoniae 2689	100	0	0	10.42	31.79
E. coli 3376	100	0	0	16.63	50.38

Abbreviations: ATCC, American Type Culture Collection; CTX, cefotaxime; CFZ, cefoperazone; MIC, minimum inhibitory concentration; MOX, moxalactam; SBT, sulbactam; %T > MIC, the time during which the concentration of the drug remained above the MIC; qd, once-daily; q12h, twice-daily; q8h, thrice-daily.

ues for 90% of organisms (MIC_{90}) for MOX against ESBL-producing *E. coli* and *K. pneumoniae* (0.5 and 0.25 mg/L, respectively) reported in a previous epidemiology antimicrobial surveillance study in China.[22] MICs for CTX and CFZ/SBT were much higher than for MOX against 3376 and 2689. Due to the powerful antibacterial activity in vitro and favorable pharmacology of MOX (longer $t_{1/2}$, higher C_{max}, and larger area under the curve), its clinical value is worthy of exploration.

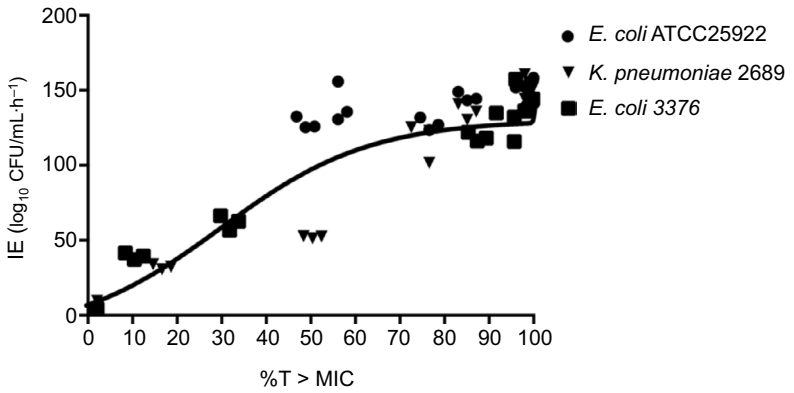

Figure 2 The relationship between %T > MIC of MOX, CTX and CFZ/SBT against the tested bacteria and IE.
Note: Three isolates are denoted by different symbols.
Abbreviations: ATCC, American Type Culture Collection; CFU, colony forming units; CFZ, cefoperazone; CTX, cefotaxime; *E. coli*, *Escherichia coli*; IE, the area between the control growth and bacterial killing and regrowth curves; *K. pneumoniae*, *Klebsiella pneumoniae*; MIC, minimum inhibitory concentration; MOX, moxalactam; SBT, sulbactam; %T > MIC, the time during which the concentration of the drug remained above the MIC.

The in vitro PK/PD simulation model can simulate the human PKs of antimicrobial agents and the results can define the optimal dosage regimens to maximize clinical efficacy. Our results showed that the simulated human exposures of tested agents exhibited sustained antibacterial killing effects against non-ESBL-producing *E. coli* ATCC25922, which was also identified in an in vitro PK/PD model using ceftazidime (2 g q8h) against susceptible strains resulting in ≥3 log_{10} CFU/mL reduction from 8 to 24 h.[23]

It is noteworthy that MOX was more significantly bactericidal than CTX and CFZ/SBT against 3376 and 2689, presumably due to its higher stability against ESBLs and the higher %T > MIC value achieved by all MOX dosing regimens. A previous study using in vitro time-kill analysis also found that flomoxef, a member of oxacephems, exhibited a rapid rate of killing (reduction of >3 log_{10} CFU/mL in the bacterial count at 6 h after application of flomoxef at 8 mg/L) against all ESBL-producing *K. pneumoniae* strains, and inhibited regrowth for at least 24 h.[7] Subsequent clinical studies showed that treatment of bacteremia or urinary tract infections caused by ESBL-producing *E. coli* and *K. pneumoniae* with oxacephems or carbapenems resulted in a similar outcome. In a multicenter retrospective study of 126 patients with bacteremia caused due to ESBL-producing *E. coli*, no difference was found in adjusted clinical success between those treated with flomoxef or cefmetazole (1 g q8h) and carbapenems (1 g q8h or 0.5 g q8h) at 30 days (empirical therapy, 100% versus 96.5%; definitive therapy, 98.4% versus 96.3%).[6] These results highlighted the promising efficacy of MOX for the treatment of infections caused by ESBL-producing isolates.

Given that %T > MIC is the optimal PD index for β-lactams, increasing the free drug concentration above the MIC should be a major aim of treatment, which was observed by higher %T > MIC and larger IE achieved for MOX (1 g q12h) compared with MOX (2 g qd). Notably, the target value of %T > MIC for treating ESBL producers was found to be more stringent than non-ESBL-producing isolates with MOX and CFZ/SBT, similar to a previous study showing reduced efficacy for piperacillin/tazobactam against ESBL-producing *K. pneumoniae* compared with non-ESBL-producing *K. pneumoniae* at identical drug exposure (Figure 2).[24] And > 70% of %T > MIC was required for the promising bacterial killing of ESBL producers in this study. Therefore, physicians should use multiple-dosing regimens and prolonged and continuous infusion of β-lactams to achieve a higher %T > MIC when treating ESBL-producing isolates with MICs that are relatively high but still within the range of susceptibility.

β-lactam/β-lactamase inhibitor combinations (BLBLIs) account for a large proportion of antibiotic consumption in China. CFZ/SBT is the second most widely used combination in hospitals, and is recommended for the treatment of infections caused by ESBL producers in clinics.[25,26] However, several disadvantages for using BLBLIs to treat ESBL-producing isolates have been reported. The size of the inoculum can affect results, as can the presence of other β-lactam resistance genes such as bla_{CMY-2} and bla_{FOX-5}, as well as decreased membrane permeability.[27–29] Moreover, PK/PD studies indicate that conventional dosing regimens for BLBLIs cannot achieve the target %T > MIC value associated with positive outcomes (especially when the MIC is at the higher end of susceptible range).[30] In the present study, CFZ/SBT failed to maintain effective killing and allowed significant regrowth to occur, as shown by the bacterial count, which was similar to untreated controls at 24 h. The %T > MIC value against *E. coli* 3376 and *K. pneumoniae* 2689 was low at all tested doses. However, CFZ/SBT (2 g q8h) also displayed

poor antibacterial activity against *E. coli* 3376 when %T > MIC exceeded 50%. This may be because the exposure–response relationship may be different between drug classes, species, and host immune response. Indeed, McKinnon et al suggested that patients receiving cefepime or ceftazidime for the treatment of serious infections with %T > MIC of 100% had a significantly greater chance of a clinical cure (82% versus 33%) and bacterial eradication (97% versus 44%) than patients with %T > MIC of <100%.[31] Although authors of a prospective controlled clinical trial from October 2002 to April 2005 did show a favorable efficacy for CFZ/SBT (2 g q8h) in the treatment of infections caused by ESBL-producing *E. coli* coupled with clinical treatment success rates of 71.4%, this could be because all isolates tested were susceptible to CFZ/SBT and patients were clinically stable.[32] Nevertheless, it should be noted that resistance of ESBL-producing isolates to BLBLIs is on the rise, especially in developing countries. Recent epidemiological studies in China reported high prevalence of ESBL producers in community-onset bloodstream infections and MIC_{90} values for ESBL-producing *E. coli* and *K. pneumoniae* with CFZ/SBT of 64 mg/L.[22,33] Thus, the empirical therapy of CFZ/SBT (2 g q8h) inevitably decreases the clinical efficacy, especially for severely ill patients, and this combination of agents should be used with more caution.

The application of preclinical PD studies using dynamic PK/PD models can provide more representative data for in vivo bacterial killing situation. Although our in vitro PK/PD simulation failed to account for the host immune response, the presence of a competent immune system can markedly reduce the required PD values.[34] Thus, the bacterial killing effects of MOX against ESBL producers may be more encouraging in animal experiments and clinical trials. The next step should focus on current regimens of MOX against ESBL producers with animal models to design more effective and representative therapies.

Conclusion

The need for effective therapeutic agents to combat infections caused by ESBL-producing isolates is widely known, and access to antimicrobial drugs continues to be limited. Our in vitro PK/PD simulation model demonstrated superior bactericidal activity of MOX under conventional dosing regimens against ESBL-producing *E. coli* and *K. pneumoniae*. The simulated regimen of CFZ/SBT, which is often prescribed by clinicians for the treatment of infections in China at present, showed limited bactericidal effects against ESBL producers. The findings of this study are supportive in prescribing MOX as an alternative agent against ESBL producers in clinical practice. Further studies, including in vitro tests, animal models, and clinical trials, are required to evaluate the exact efficacy of MOX as a reasonable carbapenem-sparing option for the treatment of infections caused by ESBL producers.

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Author contributions

YX, CH and BZ developed the concept and designed the experiments. CH, WY, TN, TX and JZ performed the experiments. BZ and YX gave conceptual advice. CH wrote the paper. All authors contributed toward data analysis, drafting and revising the paper and agree to be accountable for all aspects of the work.

Disclosure

The authors report no conflicts of interest in this work.

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Supplementary materials

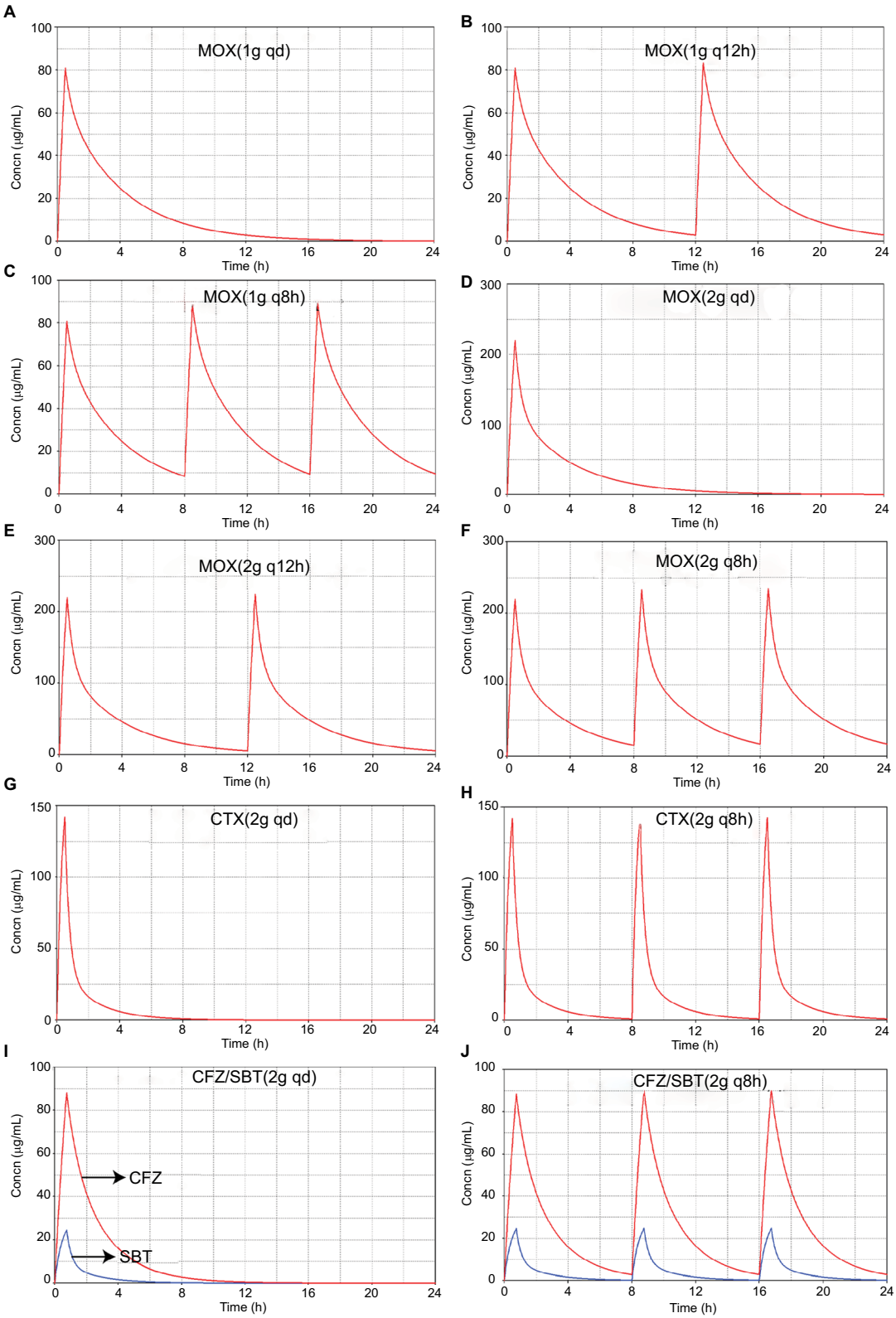

Figure S1 The simulated time–concentration curve of each dosage regimen in the in vitro Pharmacokinetics Auto Simulation System 400.

Notes: (**A**), (**B**), and (**C**) show simulated time–concentration curve of MOX at 1g qd, 1g q12h, and 1g q8h, respectively; (**D**), (**E**), and (**F**) show simulated time–concentration curve of MOX at 2g qd, 2g q12h, and 2g q8h, respectively; (**G**) and (**H**) show simulated time–concentration curve of CTX at 2g qd and 2g q8h, respectively; (**I**) and (**J**) show simulated time–concentration curve of CFZ/SBT at 2g qd and 2g q8h, respectively.

Abbreviations: CFZ, cefoperazone; CTX, cefotaxime; MOX, moxalactam; qd, once-daily; q12h, twice-daily; q8h, thrice-daily; SBT, sulbactam.

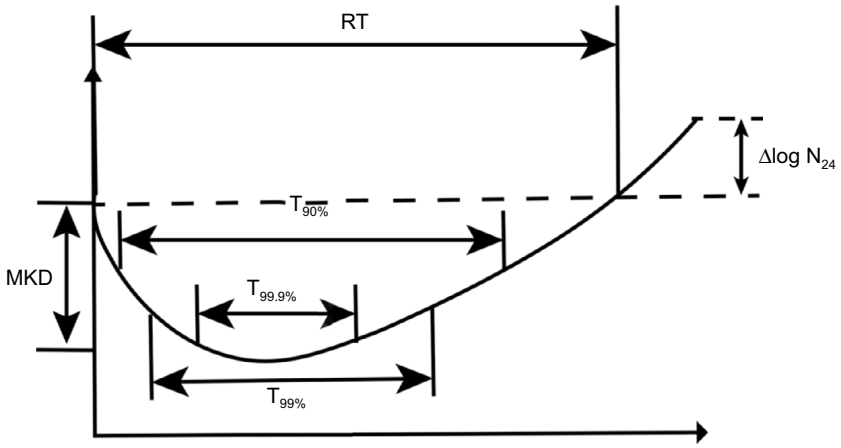

Figure S2 The sketch of pharmacodynamic parameters.
Abbreviations: MKD, the difference between the minimum bacterial count and the initial count during the experiment; $\Delta\log N_{24}$, the bacterial count difference between time 0 and 24 h; RT, bacterial growth recovery time, the time from exposure to antibiotic till the moment when the bacterial count again reaches its initial level; $T_{90\%}$, $T_{90\%}$, and $T_{99.9\%}$, the time required for microbial initial count to be reduced by 90%, 99%, 99.9%, respectively.

Table S1 The pharmacokinetic parameters employed in the study of CTX, CFZ/SBT, and MOX after intravenous infusion

Drug and dosage	Infusion time (min)	C_{max} (μ/mL)	$T_{1/2\alpha}$ (h)	$T_{1/2\beta}$ (h)	AUC (μ·h/mL)	References
CTX 2g	30	125.86	0.2	1.43	141.93	1
MOX 2g	30	219.8	0.26	2.52	536	2
MOX 1g	30	81	0.28	2.56	264	3
CFZ/SBT 2g (1:1)	45 (CFZ)	88.33	0.43	1.55	200.4	4
	45 (SBT)	24.53	0.21	1.3	32.08	4

Abbreviations: C_{max}, peak serum concentration; $T_{1/2\alpha}$, distribution half-life; $T_{1/2\beta}$, elimination half-life; AUC, area under the concentration-time curve. MOX, moxalactam; CTX, cefotaxime; CFZ, cefoperazone; SBT, sulbactam.

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