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Oral ciprofloxacin activity against ceftriaxone-resistant *Escherichia coli* in an *in vitro* bladder infection model

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Objectives: Pharmacodynamic profiling of oral ciprofloxacin dosing for urinary tract infections caused by ceftriaxone-resistant *Escherichia coli* isolates with ciprofloxacin MIC ≥ 0.25 mg/L.

Background: Urine-specific breakpoints for ciprofloxacin do not exist. However, high urinary concentrations may promote efficacy in isolates with low-level resistance.

Methods: Ceftriaxone-resistant *E. coli* urinary isolates were screened for ciprofloxacin susceptibility. Fifteen representative strains were selected and tested using a dynamic bladder infection model. Oral ciprofloxacin dosing was simulated over 3 days (250 mg daily, 500 mg daily, 250 mg 12 hourly, 500 mg 12 hourly and 750 mg 12 hourly). The model was run for 96 h. Primary endpoint was change in bacterial density at 72 h. Secondary endpoints were follow-up change in bacterial density at 96 h and area-under-bacterial-kill-curve. Bacterial response was related to exposure (AUC_{0-24}/MIC; C_{max}/MIC). PTA was determined using Monte-Carlo simulation.

Results: Ninety-three clinical isolates demonstrated a trimodal ciprofloxacin MIC distribution (modal MICs at 0.016, 0.25 and 32 mg/L). Fifteen selected clinical isolates (ciprofloxacin MIC 0.25–512 mg/L) had a broad range of quinolone-resistance genes. Following ciprofloxacin exposure, *E. coli* ATCC 25922 (MIC 0.008 mg/L) was killed in all dosing experiments. Six isolates (MIC ≥ 16 mg/L) regrew in all experiments. Remaining isolates (MIC 0.25–8 mg/L) regrew variably after an initial period of killing, depending on simulated ciprofloxacin dose. A >95% PTA, using AUC_{0-24}/MIC targets, supported 250 mg 12 hourly for susceptible isolates (MIC ≤ 0.25 mg/L). For isolates with MIC ≤ 1 mg/L, 750 mg 12 hourly promoted 3 $\log_{10}$ kill at the end of treatment (72 h), 1 $\log_{10}$ kill at follow-up (96 h) and 90% maximal activity ($AUBKC_{0-96}$).

Conclusions: Bladder infection modelling supports oral ciprofloxacin activity against *E. coli* with low-level resistance (ciprofloxacin MIC ≤ 1 mg/L) when using high dose therapy (750 mg 12 hourly).

Introduction

Urinary tract infections (UTIs) are extremely common.[1] Severe infection accounts for 25% of emergency sepsis cases, with a 6.2% in-hospital mortality and 8.6% ICU mortality.[2] *Escherichia coli* remains the most common uropathogen. Antimicrobial resistance (AMR) and the global spread of the MDR strain *E. coli* ST131 and, more recently, *E. coli* ST1193, greatly limits empirical therapy in serious infections.[3–7] Rates of resistance have progressively increased since 2007, with an 8-fold rise in resistance genes and increased numbers of attributable deaths and disability-adjusted life years.[8–10] AMR in UTIs is the one of the leading infectious

syndromes contributing to the global burden of deaths associated with resistance.[11] Globally in 2019, UTIs caused by *E. coli* with antimicrobial resistance resulted in >26 000 deaths directly attributable to AMR and >100 000 deaths related to AMR.[12] This was predominantly in *E. coli* with resistance to third-generation cephalosporins and fluoroquinolones.

Oral ciprofloxacin has excellent bioavailability and tissue penetration and achieves high concentrations in urine compared with plasma (Table 1). It has been well studied in the treatment of infections arising from the urinary tract. The US FDA and the EMA, however, provide black box warnings about the potential harms with the use of fluoroquinolones, including tendinopathy, aortic ruptures or tears and CNS effects.[23,24] As such, ciprofloxacin is not recommended for routine use in mild or recurrent UTIs. Alternative antibiotics with a narrower-spectrum, better side-effect profile, and less microbiome disruption are recommended to be used whenever possible, such as nitrofurantoin, fosfomycin, pivmecillinam and co-trimoxazole. The EUCAST 2022 breakpoints for ciprofloxacin are 'susceptible, standard dosing regimen' (S) MIC ≤ 0.25, and 'resistant' (R) MIC > 0.5 mg/L. An MIC measurement of 0.5 mg/L is designated as an area of technical uncertainty (ATU), dealt by the laboratory (depending on type of sample, number of alternative agents, severity of infection, consultation with clinical colleagues). Breakpoints are based on standard oral dosing of 500 mg 12 hourly, with high dose defined as 750 mg 12 hourly.[25] There are no UTI-specific breakpoints. CLSI 2022 breakpoints for ciprofloxacin are 'susceptible' (S) MIC ≤ 0.25 mg/L, 'intermediate' (I) MIC = 0.5 mg/L^, 'resistant' (R) MIC ≥ 1 mg/L, where '^' indicates the potential to concentrate in the urine. Breakpoints are also based on oral 500 mg 12 hourly.[26] The decision to report 'I^' is made by each laboratory based on institution-specific guidelines and in consultation with medical personnel. A lower dose of ciprofloxacin of 250 mg 12 hourly is commonly used for uncomplicated UTIs.[27]

A recent systematic review highlights the global rising rates of fluoroquinolone-resistant *E. coli* in community-acquired uncomplicated UTIs in women, with resistance rate rising from 0.5% to 15.3% in the UK, 8.7% to 15.1% in Germany, 22.9% to 30.8% in Spain, 4% to 12% in North America, and 25% to >40% in Asia.[28] Given high urinary concentrations achieved after oral dosing, it is hypothesized that urinary isolates categorized as ciprofloxacin resistant could still be effectively treated with high-dose ciprofloxacin. We have performed pharmacodynamic profiling of different oral ciprofloxacin dosing schedules within a dynamic bladder infection *in vitro* model to assess the applicability of UTI-specific ciprofloxacin breakpoints against ceftriaxone-resistant *E. coli* urinary isolates.

Methods

Media

Cation-adjusted Mueller-Hinton II agar (MHA, BD, USA), CAMHB (BD), pooled human urine and synthetic human urine (SHU) were used. Human urine was collected and pooled from healthy female volunteers and filter sterilized (Ethics Committee approval: Project no. 27033). SHU was modified (mSHU) from a previous study[29] with addition of 0.1% v/v yeast extract (stock solution 10% w/v) to best match *E. coli* growth in urine (Table S1 and Figure S1, available as Supplementary data at *JAC* Online). SHU and mSHU were adjusted to pH 5.6.

E. coli isolates and susceptibility testing

Clinical, non-duplicate *E. coli* isolates from a urinary source that were ceftriaxone resistant were selected from a surveillance collection at a tertiary acute care hospital (Ethics Committee approval: Project no. 533/16). Isolates underwent ciprofloxacin susceptibility testing by broth microdilution (BMD).[30] Fifteen clinical isolates, and *E. coli* ATCC 25922, were selected to reflect a range of ciprofloxacin MIC values. Ciprofloxacin MICs were repeated in triplicate by BMD in CAMHB and mSHU, and tested in pooled human urine as a single replicate. WGS was performed determining ST, phylogenetic relatedness, quinolone-resistance determinants and β-lactamase genes (see Supplementary Methods).

Bladder infection in vitro model

A multicompartment infection model applying a continuous dilution system was used with mSHU as the liquid medium (Figure 1).[31] Sixteen bladder compartments were run in parallel. Medium was run at a continuous flow rate of 400 mL/h from fresh media reservoirs into the 'intestinal' compartment containing ciprofloxacin (Aspen Pharmacare Australia, 200 mg/100 mL; volume 300–900 mL), and then into the 'circulatory' compartment (volume 1450 mL). The volumes of these two compartments were kept static for the duration of each experiment. Medium flow into each individual bladder compartment was 25 mL/h. Normal human urodynamics was simulated. Volume in each bladder increased over time prior to an intermittent voiding schedule that reduced the volume to a residual 5 mL. First void was 2 h after starting and continued 4 hourly thereafter. Each bladder was inoculated with an *E. coli* isolate with 10 mL of 10^6 cfu/mL, providing a total bacterial count equivalent to human UTIs (i.e. $\geq 10^5$ cfu/mL in 200 mL void). Ciprofloxacin MICs were rechecked from the starting inoculum and if an isolate regrew at the completion of an experiment.

Quantification of bacterial growth

Bacterial density (cfu/mL) was first assessed under drug-free conditions over 24 h. During ciprofloxacin exposure over 3 days, bacterial density was measured at 0, 6, 24, 30, 48, 54 h and at the end of treatment (72 h). A follow-up bacterial density was measured at 96 h. Pharmacodynamic (PD) samples were collected directly from each bladder compartment. To negate antibiotic carry-over, a centrifuge/wash process was performed twice, reducing ciprofloxacin concentrations 100-fold. Bacterial loss was minimal (<0.1 $\log_{10}$ cfu/mL). Samples were serially diluted and 20 µL from each dilution plated onto MHA. Emergence of resistance was identified by plating in parallel onto MHA with 2 mg/L and 128 mg/L ciprofloxacin (every 24 h). Plates were incubated aerobically, 35°C ± 1°C for 16–20 h. Plates with ciprofloxacin were reincubated for an additional 24 h. Limit of detection was 50 cfu/mL.

Simulated ciprofloxacin urinary exposure

Human urinary ciprofloxacin concentrations after oral dosing (Table 1) were used as targets for the *in vitro* model.[13–22] Drug distribution equations were used to inform *in vitro* flow rate, volumes and ciprofloxacin dosage to achieve the target concentrations and a calculated AUC_{0-24}.[32] Dosing schedules were administered as a 3 day course. Target exposures [peak concentration (C_{max}), trough concentration (C_{min}) and calculated AUC_{0-24} on the third day of treatment were as follows: 250 mg daily (C_{max} 220 mg/L, C_{min} 1 mg/L, AUC_{0-24} 1444 mg·h/L), 500 mg daily (C_{max} 398 mg/L, C_{min} 3 mg/L, AUC_{0-24} 2969 mg·h/L), 250 mg 12 hourly (C_{max} 232 mg/L, C_{min} 30 mg/L, AUC_{0-24} 2880 mg·h/L), 500 mg 12 hourly (C_{max} 426 mg/L, C_{min} 79 mg/L, AUC_{0-24} 5937 mg·h/L), 750 mg 12 hourly (C_{max} 579 mg/L, C_{min} 154 mg/L, AUC_{0-24} 8969 mg·h/L). Greater than 90% of each administered dose was excreted by 12 h.

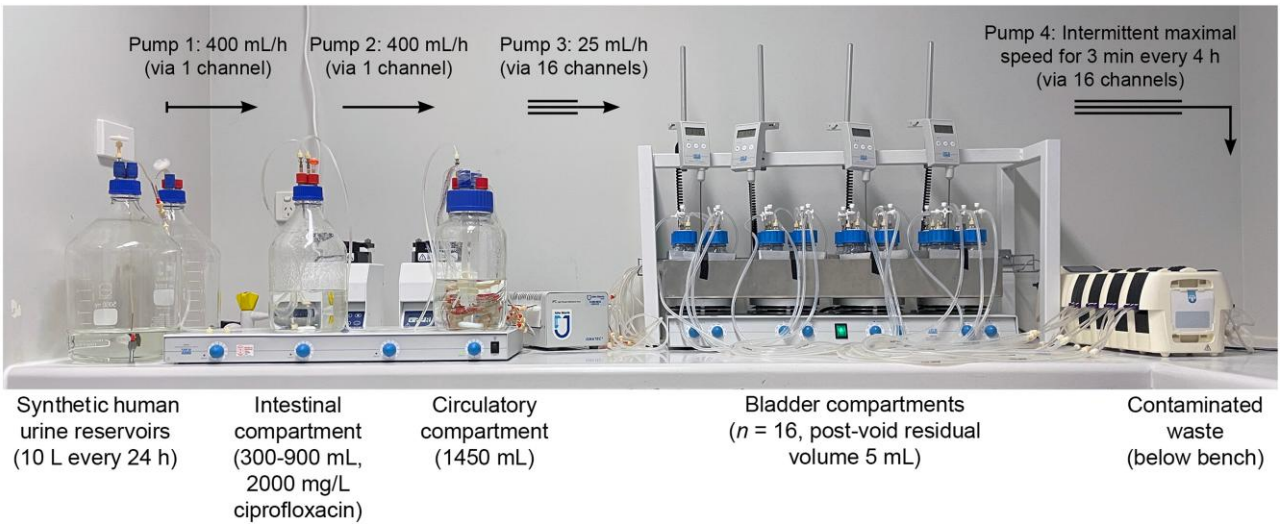

Figure 1. Bladder infection model. *In vitro* model set-up of the dynamic multicompartment dilution model used for the simulation urinary ciprofloxacin exposure following oral dosing. This figure appears in colour in the online version of *JAC* and in black and white in the print version of *JAC*.

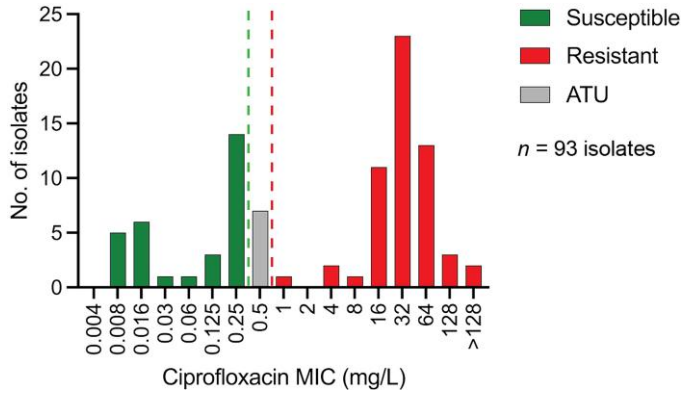

Figure 2. Ciprofloxacin MIC distribution of ceftriaxone-resistant *E. coli* urinary isolates. *E. coli* urinary isolates ($n=93$). MIC testing performed by BMD. WT isolates defined by MIC ≤ 0.064 mg/L. Susceptibility categories as per EUCAST 2022 breakpoint table. This figure appears in colour in the online version of *JAC* and in black and white in the print version of *JAC*.

Measurement of ciprofloxacin concentrations

Representative pharmacokinetic (PK) samples were collected from three bladder compartments at C_{max} and C_{min} each day of dosing, and at the completion of each experiment. All 16 bladder compartments were sampled at the C_{max} on the third day. Interday and intercompartment variability was assessed by the average relative standard deviation. Samples were filtered, stored at −80°C and batched for testing. Ciprofloxacin concentrations were measured by a UHPLC with fluorometric detection (UHPLC-Fl) method on a Nexera2 liquid chromatograph connected to an RF-20Axs fluorescence detector (Shimadzu, Kyoto, Japan). Calibration range was 0.1 to 1000 mg/L. The precision (6.9%, 6.4%, 4.3% and 1.4%) and accuracy (0.9%, 5.0%, 10.%5 and −2.2% at 3, 30, 300 and 1000 mg/L) met FDA guidance.[33] Ciprofloxacin was stable when incubated in SHU, with 0.2%, 1.9% and 4.6% reduction in the measured concentration at 24, 48 and 72 h, respectively. Linear regression and Bland–Altman plots quantified the accuracy and bias of the measured concentrations compared to target.

PK/PD analysis

Primary endpoint was end-of-treatment (72 h) change in bacterial density. Secondary endpoints were change in bacterial density at follow-up (96 h), and total bacterial response measured by the area under the bacterial kill curve ($AUBKC_{0-96}$). The relationship between ciprofloxacin exposure (AUC_{0-24}/MIC and C_{max}/MIC) and bacterial response was assessed using an E_{max} non-linear regression model. No constraints were applied, unless indicated. Goodness of fit was assessed by visual inspection, residuals analysis and R^2. Exposures required for stasis, 1, 2 and 3 $\log_{10}$ kill, and 50% (EI_{50}) and 90% (EI_{90}) of the maximum effect was determined. This analysis was repeated using MIC measurements by BMD in mSHU. Monte Carlo simulations (MCS), using the 'normal random number generator' for 5000 patients (Microsoft® Excel for Mac, v16.63) was used to determine PTA for each ciprofloxacin dosing regimen, with ±50% allowance for variability in human urinary concentrations. Protein binding in urine is minimal. All data were analysed using GraphPad Prism (version 9.4.1 macOS).

Results

E. coli isolates

Ninety-three ceftriaxone-resistant *E. coli* clinical urinary isolates were selected for ciprofloxacin MIC testing. A trimodal MIC distribution was observed with modal MICs at 0.016, 0.25 and 32 mg/L (Figure 2). Most isolates (86%) demonstrated a ciprofloxacin MIC higher than the epidemiological cut-off (ECOFF) of 0.064 mg/L.[34] Clinical isolates selected for additional testing had ciprofloxacin MICs ranging from 0.25 to 512 mg/L (Table 2). Compared with standard testing in CAMHB, ciprofloxacin MIC measurements were, on average, 4 (±1) $\log_2$ dilutions higher when tested in mSHU and in pooled human urine. Isolates reflected a diverse range of STs and were not members of a transmission cluster (>45 SNPs between all five *E. coli* ST131). All isolates with ciprofloxacin MIC ≥ 4 mg/L had *parC* S80I mutation (Table 2). Sequence reads were deposited in the NCBI Sequence Read Archive (Table S2).

Table 1. Oral ciprofloxacin urinary pharmacokinetics

Year	Subjects	*n*	Dose	Time (h)	Mean (mg/L)	Min	Max	% CV	Recovery in urine	Method	Ref
1984	Healthy volunteers (all male)	12	250 mg q12; 7 days (13 doses)	0–2	—[a]	72	840	—	—	HPLC	13
				6–12	44.7±25 to 68.7±45	—	—	56 to 77%			
1984	Healthy volunteers (all male)	9	250, 500, 750 mg q12; 7 days	0–2 (250 mg)	205 to 261	—	—	—	0–12 h (250 mg): 41%±7% to 47%±19%	Bioassay[b]	14
				2–4	147 to 229						
				4–8	90 to 101						
				8–12	32 to 34						
				0–2 (500 mg)	255 to 518	—	—	—	0–12 h (500 mg): 33%±9% to 37%±10%		
				2–4	321 to 448						
				4–8	117 to 199						
				8–12	26 to 82						
				0–2 (750 mg)	243 to 846	—	—	—	0–12 h (750 mg): 31%±7% to 38%±8%		
				2–4	544 to 704						
				4–8	169 to 360						
				8–12	55 to 151						
1985	Healthy volunteers (50% female)	12	50 mg, 100 mg, 750 mg; single dose	0–3, 3–6, 6–12 & 12–24	—[a]	—	—	—	0–24 h (50 mg): 36%±10% 0–24 h (100 mg): 35%±8% 0–24 h (750 mg): 33%±5%	Bioassay[c]	15
1985	Healthy volunteers (50% female)	10	250 mg q12; 4 days (7 doses)	—[d]	—[a]	—	—	—	0–12 h: 38%±6% to 46%±6% (bioassay); 26%±5% to 30%±9% (HPLC)	Bioassay[e] and HPLC[f]	16
1985	Healthy volunteers (all male)	18	500 mg; single dose	0–2	464	28	1847	98%	0–12 h: 45%±12%	HPLC	17
				2–4	304	60	1219	95%	0–24 h: 51%±12.2%		
				4–8	189	31	731	93%			
				8–12	111	19	128	25%			
1986	Healthy volunteers (all male)	12	250, 500, 750, 1000 mg; single dose	0–2 (250 mg)	190[g]	—	—	—	0–48 h (250 mg): 36%±12%	Bioassay[b]	18
				2–4	180						
				4–8	90						
				8–12	40						
				0–2 (500 mg)	340[g]	—	—	—	0–48 h (500 mg): 44%±7.5%		
				2–4	400						
				4–8	220						
				8–12	120						
				0–2 (750 mg)	460[g]	—	—	—	0–48 h (750 mg): 40%±12.2%		
				2–4	530						
				4–8	280						
				8–12	200						
				0–2 (1000 mg)	390[g]	—	—	—	0–48 h (1000 mg): 29%±10%		
				2–4	400						
				4–8	240						
				8–12	130						
1994	Healthy volunteers (all male)	6	750 mg; single dose	0–4, 4–8, 8–12, 12–24	—[a],h	—	—	—	0–12 h: 41% (bioassay); 36% (HPLC)	Bioassay[e] and HPLC[f]	19
2003	Healthy volunteers (50% female)	12	500 mg; single dose	0–6	407	23	733	44%	0–12 h: 37%±10%	HPLC	20
				6–12	47	19	76	30%			

2004	Healthy volunteers (50% female)	12	500 mg; single dose	0–6	368	100	1000	61%		0–12 h: 32% ± 10%	HPLC	[21]
				6–12	77	30	179	48%				
2006	Healthy volunteers (50% female)	14	500 mg; single dose	0–6	268	130	967	78%		0–12 h: 34% ± 8%	HPLC	[22]
				6–12	60	25	76	21%				

[a]Average urinary concentrations not provided.
[b]*Klebsiella pneumoniae* ATCC 10031.
[c]*K. pneumoniae* ATCC 10031 for concentrations <0.15 mg/L and *Bacillus subtilis* ATCC 6633 for concentrations >0.15 mg/L.
[d]Urine collected at seven time intervals after the first and seventh doses. Average urinary concentrations not provided.
[e]*E. coli* 4004.
[f]Bioassay results higher than HPLC, supporting the assumption that microbiologically active metabolites are excreted renally.
[g]Average urinary concentrations were only presented in graphical form; measurements are approximated from the figure.
[h]Authors state that urinary concentrations were >26 mg/L from all 12–24 urine samples.

Simulated ciprofloxacin exposure in the bladder infection model

Ciprofloxacin concentrations closely matched target values with the linear regression slope 0.90 (R^2 = 0.9758) and bias −1.9% (95% CI: −35.2% to 31.5%) (Figures S2 and S3). Intercompartment variation in C_{max} across all 16 bladders on the third day treatment was 3.3% ± 0.8%. Interday variation in C_{max} in the representative sampled bladders was 7.6% ± 5.9%. All ciprofloxacin concentrations measured at follow-up (96 h) were <0.27 mg/L.

Post-exposure growth response in the bladder infection model

E. coli ATCC 25922 (ciprofloxacin MIC 0.008 mg/L) was eradicated in all dosing regimens. The six clinical isolates with ciprofloxacin MIC ≥ 16 mg/L had near maximal regrowth at 72 h (>1.9 $\Delta\log_{10}$ cfu/mL) in all dosing regimens (Table 3 and Figure 3). For the remaining nine clinical isolates (ciprofloxacin MIC 0.25–8 mg/L), after an initial period of killing, regrowth at 72 h (>0 $\Delta\log_{10}$ cfu/mL) was: three isolates (114, 132, 104 with MICs 4–8 mg/L) after 250 mg and 500 mg daily; one isolate (104 with MIC 8 mg/L) after 250 mg and 500 mg 12 hourly; none after 750 mg 12 hourly. At follow-up (96 h) regrowth in these nine isolates was: seven isolates (057, 017, 016, 019, 114, 132, 104 with MICs 0.5–8 mg/L) after 250 mg daily; five isolates (014, 016, 114, 132, 104 with MICs 0.5–8 mg/L) after 500 mg daily; three isolates (114, 132, 104) after 250 mg and 500 mg 12 hourly; one isolate (114 with MIC 4 mg/L) after 750 mg 12 hourly.

Emergence of resistance

Ciprofloxacin MIC testing of post-exposure growth from drug-free MHA did not identify any isolate with >2 $\log_2$ dilution rise MIC compared with the starting inoculum (Table S3). Only isolate 019 (MIC 0.5 to 2 mg/L after 250 mg 12 hourly) and isolate 127 (MIC 32 to 128 mg/L after 750 mg 12 hourly) had appreciable MIC rises. Emergence of resistance was also not detected on the bacterial density measurements on MHA supplemented with 2 and 128 mg/L ciprofloxacin. MHA with 2 mg/L ciprofloxacin suppressed all isolates with ciprofloxacin MIC < 2 mg/L. MHA with 128 mg/L ciprofloxacin suppressed all isolates with ciprofloxacin MIC 2–64 mg/L, except 127 (ciprofloxacin MIC 64 mg/L), which had low-level growth at 96 h (2–3 $\log_{10}$ cfu/mL) following 250 mg daily, 250 mg 12 hourly and 500 mg 12 hourly, with a ciprofloxacin MIC of 128–256 mg/L.

PK/PD analysis

The E_{max} model well described the PK/PD relationship for the primary endpoint ($\Delta\log_{10}$ cfu/mL at 72 h) with AUC_{0-24}/MIC EI_{50} = 887 (R^2 0.9062) and C_{max}/MIC EI_{50} = 76 (R^2 0.8766) (Figure 4). Target PK/PD ranged from 684 AUC_{0-24}/MIC and 56 C_{max}/MIC for stasis to 1521 AUC_{0-24}/MIC and 147 C_{max}/MIC for 3 $\log_{10}$ kill (Table 4). The PK/PD relationships for the secondary endpoints ($\Delta\log_{10}$ cfu/mL at 96 h, and $AUBKC_{0-96}$) are presented in Table 3. The best goodness of fit for data was generated using the $AUBKC_{0-96}$ endpoint, with an AUC_{0-24}/MIC EI_{50} = 692 (R^2 0.9307) and C_{max}/MIC EI_{50} = 57 (R^2 0.9082). With MIC values measured by BMD in mSHU, the AUC_{0-24}/MIC_{mSHU} targets for

Table 2. Ceftriaxone-resistant *E. coli* isolates tested in the bladder infection model

Isolate #	Ciprofloxacin MIC, mg/L (range)			ST	QRDRs	Plasmid-mediated quinolone resistance	Multidrug- efflux-pump regulator	β-Lactamase
	CAMHB	mSHU	Urine					
25922	0.008 (0.008)	0.125 (0.125)	0.0625	73	—	—	—	—
057	0.25 (0.25)	8 (8)	4	131	gyrA_S83L parE_I529L	—	—	CTX-M-27
017	0.5 (0.25–0.5)	8 (8)	8	219	—	qnrS1	—	CTX-M-15
014	0.5 (0.25–0.5)	8 (8)	4	38	gyrA_S83L	—	—	CTX-M-14
015	0.5 (0.5)	8 (8)	8	131	gyrA_S83L parC_A108V parE_I529L	—	—	CTX-M-27
016	0.5 (0.5)	8 (8)	8	131	gyrA_S83L parE_I529L parE_S458A	—	—	CTX-M-14
019	1 (1)	16 (16)	8	10320	gyrA_S83L parE_S458A	—	—	CTX-M-15
114	4 (4–8)	256 (256)	64	2599	gyrA_S83L gyrA_D87N parC_S80I	—	—	CTX-M-15
132	8 (4–8)	64 (64)	128	648	gyrA_S83L parC_S80I parE_S458A	qnrS1	—	CTX-M-65
104	8 (8)	256 (256)	256	95	gyrA_S83L gyrA_D87N parC_S80I	—	marR_S3N	CTX-M-55
093	16 (16)	512 (512)	512	1193	gyrA_S83L gyrA_D87N parC_S80I parE_L416F	qnrS1[a]	marR_S3N	CTX-M-14
124	32 (32)	512 (512)	>512	131	gyrA_S83L gyrA_D87N parC_S80I parC_E84V parE_I529L	—	—	CTX-M-15
096	32 (32)	512 (512)	>512	410	gyrA_S83L gyrA_D87N parC_S80I parE_S458A	—	—	CTX-M-15 CMY-42
127	64 (64)	>512 (>512)	>512	457	gyrA_S83L gyrA_D87Y parC_S80I parE_S458A	—	—	CMY-2
139	128 (128)	512 (512)	>512	131	gyrA_S83L gyrA_D87N parC_S80I parC_E84V parE_I529L	—	—	CTX-M-15
087	512 (512)	>512 (>512)	>512	1193	gyrA_S83L gyrA_D87N parC_S80I parE_L416F	qepA8[b]	marR_S3N	CTX-M-15, CMY-2

Ciprofloxacin MIC testing in CAMHB and mSHU was performed in triplicate by BMD using ciprofloxacin HCl (Sigma–Aldrich USA, PHR1044); median (range) reported. Testing in pooled human urine was performed as a single replicate using the parental formulation of ciprofloxacin (Aspen Pharmacare Australia). Testing performed in CAMHB and mSHU. QRDRs include mutations DNA gyrase (*gyr*) and topoisomerase IV (*par*) genes.

[a]Partial genomic sequence obtained (104/657 bases missing from assembly).

[b]Partial genomic sequence obtained (286/1542 bases missing from assembly). The ATCC 25922 strain matched the publicly available sequence.

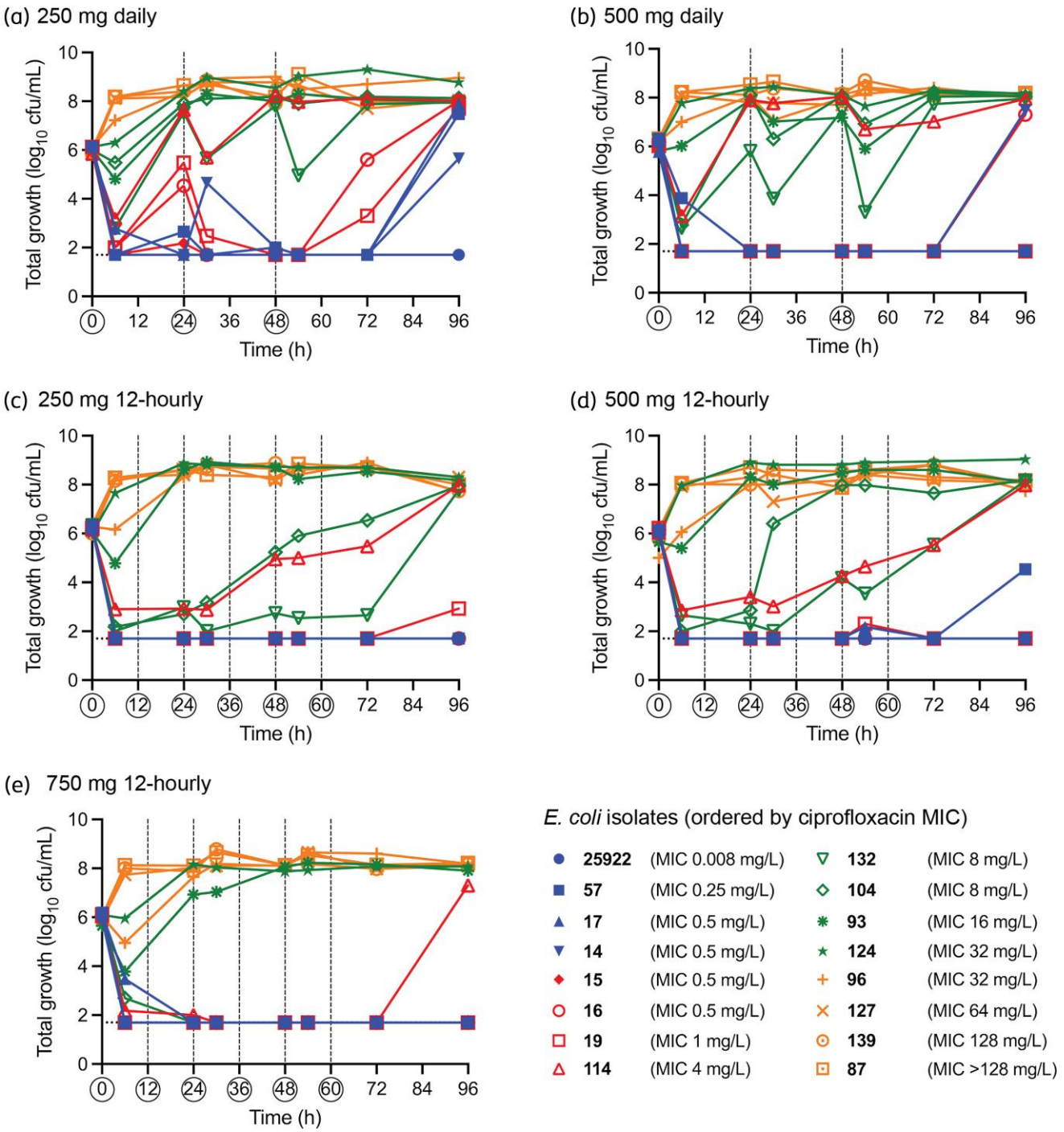

Figure 3. Growth response following urinary ciprofloxacin exposure over 96 h incubation in the bladder infection model following the five dosing regimens (a–e). Sixteen *E. coli* isolates tested, each with a unique symbol. Limit of detection was considered to be 50 cfu/mL. Circled times on the *x*-axis indicate the time when repeat doses of ciprofloxacin were added to the *in vitro* model. This figure appears in colour in the online version of *JAC* and in black and white in the print version of *JAC*.

$\Delta\log_{10}$ cfu/mL at 72 h ranged from 27 for stasis to 72 for 3 $\log_{10}$ kill. Similarly, the total bacterial response ($AUBKC_{0-96}$) had the best goodness of fit (R^2 0.9207), with the AUC_{0-24}/MIC_{mSHU} EI_{50} = 30, and EI_{90} = 100 (Table S4 and Figure S4).

Monte Carlo simulation

PTA applying AUC_{0-24}/MIC targets for the primary endpoint (72 h $\Delta\log_{10}$ cfu/mL) for each oral ciprofloxacin dosing regimen is graphed overlying *E. coli* ciprofloxacin MIC distributions

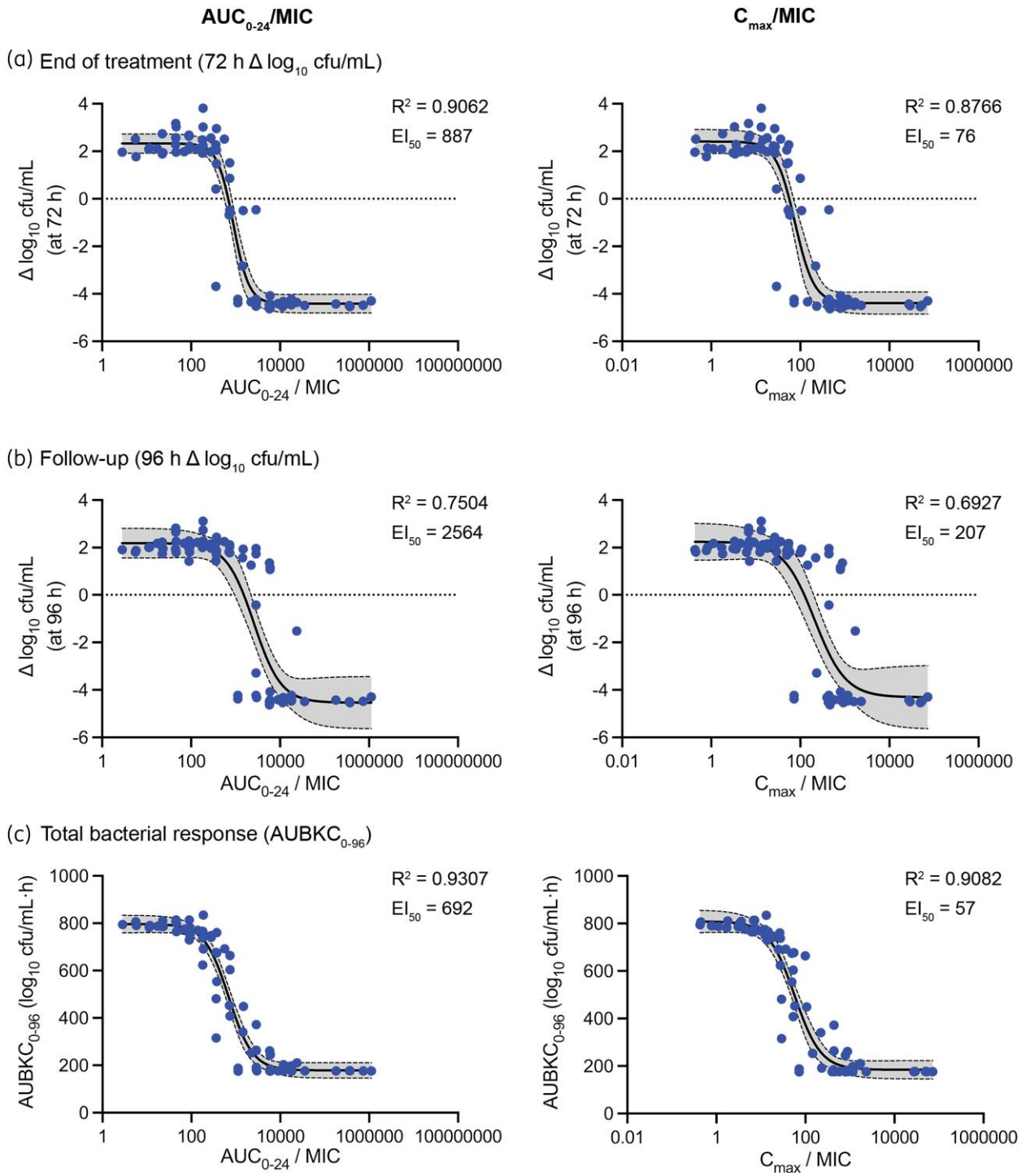

Figure 4. Exposure–response relationships. E_{max} models detailing the relationship between exposure [AUC_{0-24}/MIC (left) and C_{max}/MIC (right)] and the change in bacterial density, measured at (a) 72 h and (b) 96 h, and the total bacterial response (c) $AUBKC_{0-96}$. This figure appears in colour in the online version of *JAC* and in black and white in the print version of *JAC*.

(Figure 5). A >95% PTA for a 3 log_{10} kill effect following 250 mg daily, 500 mg daily, 250 mg 12 hourly, 500 mg 12 hourly and 750 mg 12 hourly was found for isolates with ciprofloxacin MICs of ≤0.25, 0.5, 0.5, 1 and 1 mg/L. A >95% PTA for stasis was found for isolates with ciprofloxacin MICs of ≤0.5, 1, 1, 2 and 4 mg/L for each dosing regimen, respectively (Table S5).

Secondary endpoints [follow-up (96 h); total bacterial response ($AUBKC_{0-96}$)] are presented in Tables S6 and S7 and

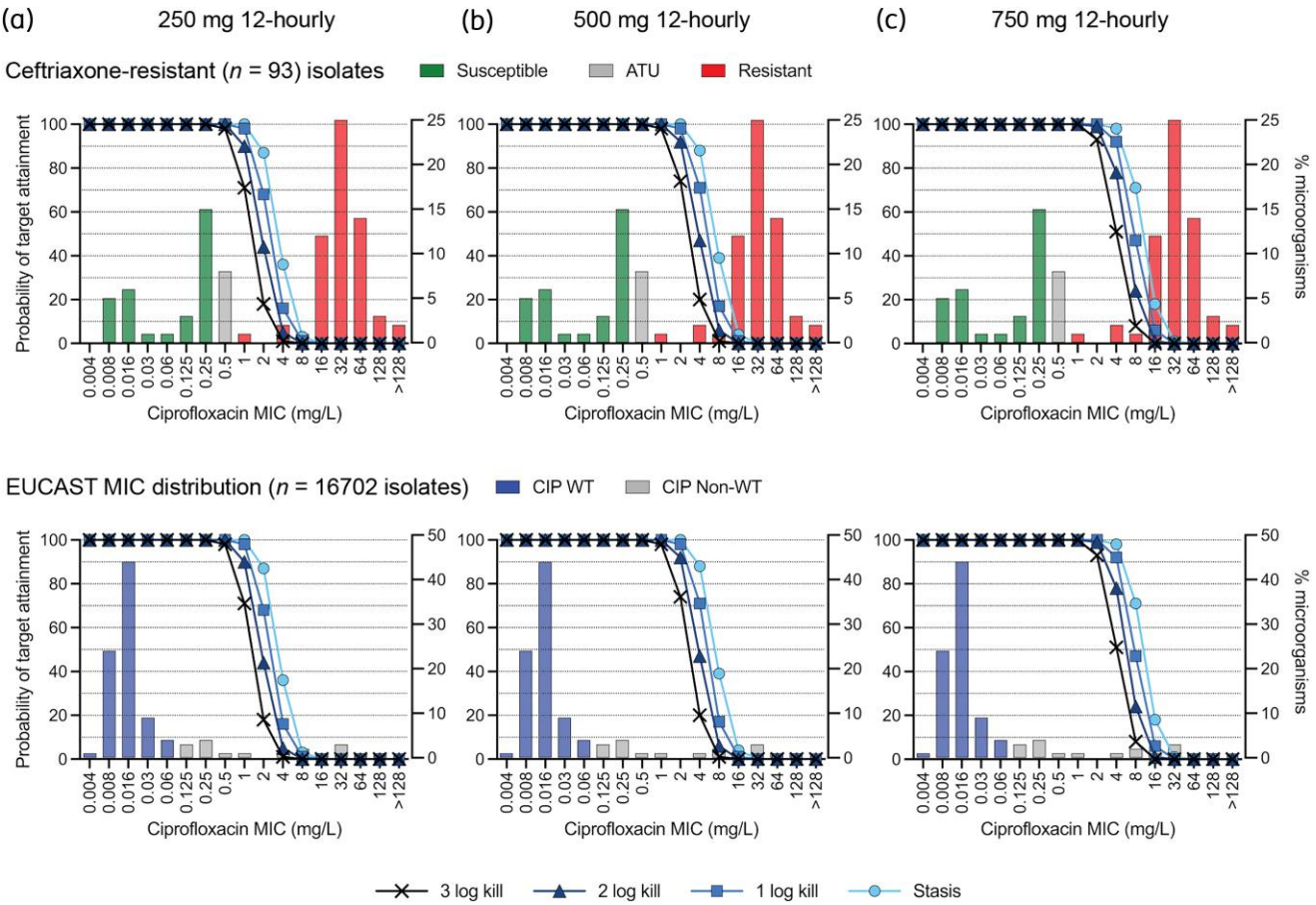

Figure 5. End-of-treatment (72 h) Monte Carlo simulation. PTA of stasis and log kill at 72 h for 12 hourly dosing of oral ciprofloxacin at (a) 250, (b) 500, and (c) 750 mg. Graphs overlying the ciprofloxacin MIC distribution from the 93 ceftriaxone-resistant *E. coli* clinical isolates (top row) and the EUCAST MIC distribution (bottom row). PTA values are shown in Table S3. This figure appears in colour in the online version of *JAC* and in black and white in the print version of *JAC*.

Figures S5 and S6. A ciprofloxacin MIC ≤ 1 mg/L allowed for >95% PTA for a 1 $\log_{10}$ kill at follow-up (96 h) and 90% maximal efficacy with high (750 mg 12 hourly) dosing. For standard dosing (500 mg 12 hourly) and low dosing (250 mg 12 hourly), PK/PD breakpoints are MIC ≤ 0.5 and ≤ 0.25 mg/L for 1 $\log_{10}$ kill at 96 h, and MIC ≤ 0.5 and ≤ 0.25 mg/L for 90% maximal efficacy for $AUBKC_{0–96}$.

Discussion

PK/PD analysis and MCS support the *in vitro* efficacy of high-dose ciprofloxacin (750 mg orally 12 hourly) against *E. coli* urinary isolates with a ciprofloxacin MIC ≤ 1 mg/L for a 3 $\log_{10}$ kill at the end of 3 days of treatment, 1 $\log_{10}$ kill at follow-up (at 96 h), and 90% maximal activity ($AUBKC_{0–96}$). Standard-dose ciprofloxacin (500 mg 12 hourly) similarly achieved 3 $\log_{10}$ kill at 72 h at MIC ≤ 1 mg/L, whereas 1 $\log_{10}$ kill at 96 h and 90% maximal activity required a lower MIC of ≤0.5 mg/L. Low-dose ciprofloxacin (250 mg 12 hourly, or 500 mg daily) achieved 3 $\log_{10}$ kill at 72 h at MIC ≤ 0.5 mg/L, 1 $\log_{10}$ kill at 96 h and 90% maximal activity at MIC ≤ 0.25 mg/L. These data support current ciprofloxacin dosing practices for UTIs caused by *E. coli* categorized as susceptible (MIC ≤ 0.25 mg/L), and the potential to expand activity with high-dose therapy for urinary isolates with low-level ciprofloxacin resistance (MIC ≤ 1 mg/L).

Expanding ciprofloxacin susceptibility can provide an oral antimicrobial option for select groups of patients, such as: UTIs caused by MDR bacteria; oral step-down following initial IV therapy; infections following urinary tract instrumentation; infections in males; catheter-associated UTIs; and after consultation with clinical colleagues. However, given restrictions on the use of ciprofloxacin by the EMA and FDA, any change to ciprofloxacin breakpoints would need to ensure that ciprofloxacin is not promoted for uncomplicated and recurrent UTIs. Using cascade (or selective) reporting provides a valuable antimicrobial stewardship strategy for the reporting laboratory.[35,36]

Established ciprofloxacin target plasma PK/PD ratios are *f*AUC/MIC ratio of 140 for 2 $\log_{10}$ kill in a neutropenic thigh model and *f*AUC/MIC ratio of 87.5 for clinical efficacy in hospital-acquired pneumonia.[37] Despite PK data recording high ciprofloxacin concentrations in urine, neither animal nor clinical PK/PD targets exist for UTIs. The UTI PK/PD targets determined in the present study

Table 3. Change in bacterial density at 72 and 96 h following oral ciprofloxacin dosing simulations

		End of treatment (72 h Δcfu/mL)					Follow-up (96 h Δcfu/mL)				
Isolate #	CIP MIC (mg/L)	250 mg daily	500 mg daily	250 mg q12	500 mg q12	750 mg q12	250 mg daily	500 mg daily	250 mg q12	500 mg q12	750 mg q12
25922	0.008	—	—	—	—	—	—	—	—	—	—
057	0.25	—	—	—	—	—	1.35	—	—	−1.52	—
017	0.5	—	—	—	—	—	1.74	—	—	—	—
014	0.5	—	—	—	—	—	−0.43	1.17	—	—	—
015	0.5	—	—	—	—	—	—	—	—	—	—
016	0.5	−0.46	—	—	—	—	1.96	1.08	—	—	—
019	1	−2.83	—	—	—	—	1.57	—	−3.29	—	—
114	4	2.27	0.86	−0.68	−0.50	—	2.18	1.79	1.82	1.94	1.26
132	8	1.92	1.47	−3.69	−0.48	—	2.00	1.69	1.44	2.12	—
104	8	2.19	2.05	0.41	1.51	—	2.13	1.87	1.82	2.04	—
093	16	2.08	2.23	2.48	2.95	2.51	1.98	2.27	2.12	2.44	2.25
124	32	3.17	2.20	2.68	3.02	1.98	2.65	2.06	2.28	3.11	1.98
096	32	2.57	2.21	2.64	3.81	2.56	2.82	1.81	1.43	2.74	2.15
127	64	1.89	1.96	2.29	2.10	2.10	2.23	1.80	1.94	2.00	2.22
139	128	2.09	1.94	2.74	3.03	1.98	2.00	1.96	1.74	2.26	2.18
087	512	1.96	1.77	2.51	2.14	2.10	1.91	1.88	1.81	2.01	2.18

CIP, ciprofloxacin. CIP MIC as determined by BMD in CAMHB. — indicates that growth was not detected. Limit of detection was considered to be 50 cfu/mL.

Table 4. Ciprofloxacin urinary PK/PD targets for different bacterial response endpoints

	AUC_{0-24}/MIC (95% CI)	C_{max}/MIC (95% CI)
End of treatment: 72 h Δlog_{10} cfu/mL		
Stasis	684 (557–821)	56 (45–71)
1 log_{10} kill	878 (730–1063)	76 (62–100)
2 log_{10} kill	1125 (933–1420)	102 (80–145)
3 log_{10} kill	1521 (1185–2025)	147 (104–222)
R^2	0.9062	0.8766
Follow-up: 96 h Δlog_{10} cfu/mL		
Stasis	1534 (949–2225)	123 (69–199)
1 log_{10} kill	2383 (1607–3306)	204 (123–313)
2 log_{10} kill	3651 (2584–5207)	338 (211–524)
3 log_{10} kill	6045 (4078–9602)	633 (374–1274)
R^2	0.7504	0.6927
Total bacterial response: $AUBKC_{0-96}$		
EI_{50}	692 (562–861)	57 (45–76)
EI_{90}	2727 (1778–4599)	253 (146–527)
R^2	0.9307	0.9082

Non-linear regression E_{max} curves described by the equation: $E = (E_{max} - E_{min}) \times EI^n/(EI^n + EI_{50}^n) + E_{min}$, where E_{max} reflects maximal growth and E_{min} reflects no growth, EI is the exposure index of AUC_{0-24}/MIC and C_{max}/MIC, EI_{50} is the exposure index required to achieve 50% of E_{max}, EI_{90} is the exposure index required to achieve 90% of E_{max}, and n is the slope of the dose–effect relationship (Hill coefficient).

were 8–16 times higher than plasma targets. This reflects a reduction in the activity of ciprofloxacin in a bladder infection model and supported by elevated MIC measurements in mSHU and pooled human urine (three to four 2-fold increase compared with testing in CAMHB). When applying ciprofloxacin MIC values measured by BMD in mSHU, the resulting urinary PK/PD targets are similar to the plasma targets in the literature. Namely, *f*AUC/MIC_{mSHU} ratio of 72 for 3 log_{10} kill at 72 h, *f*AUC/MIC_{mSHU} ratio of 149 for 1 log_{10} kill at 96 h, and *f*AUC/MIC_{mSHU} ratio of 100 for 90% maximal activity ($AUBKC_{0-96}$). These observations support the clinical correlation of the bladder infection model to predict antibiotic efficacy in UTIs. However, applying non-standardized MIC measurements limits the practical translation of the *in vitro* results. The MIC should be measured by the reference method to provide a phenotypic endpoint in a defined and standardized system as a reproducible measure of antibiotic activity against that microorganism. The value should not be extrapolated as a concentration directly comparable with *in vivo* concentrations found at the site of infection during treatment.[38]

Establishing UTI-specific PK/PD targets can inform optimized antibiotic dosing strategies. However, simulating urinary PK has greater complexity compared with plasma PK given the marked variability observed between individuals, largely due to fluid intake and voiding behaviour (Table 1). Antibiotic protein binding, which is an important consideration in plasma PK, is less of an issue when considering free-drug activity and total drug measurements in urine, given the renal excretion of unbound drug and the paucity of albumin found in urine in patients with normal renal glomerular function.[39,40] Urinary ciprofloxacin PK variability has been modelled in relation to urine output (1 versus 2.5 L/day), healthy young adults versus elderly, and circadian changes in diuresis and absorption.[41] Such variability in urinary PK is not unique to ciprofloxacin. Wijma *et al.*[42] found a 47% coefficient of variability (CV%) in urinary fosfomycin exposure (AUC_{0-48} 21284 ± 9965 mg·h/L). Similarly, Wenzler *et al.*[43] measured urinary fosfomycin concentrations at selected time periods with a CV % of 67%–84%, and a similar CV% in the volume of urine voided

(60%–73%). Informed from these studies, and those presented in Table 1, we applied a ±50% variability in the expected urinary ciprofloxacin exposure (AUC_{0-24}) in our MCS.

Mutations associated with low- and high-level ciprofloxacin resistance are complex and varied.[44] In our 15 clinical *E. coli* isolates, ciprofloxacin MIC appeared to be related to the type and number of mutations detected. This has been similarly reported in *qnr*-containing *E. coli* with additional topoisomerase mutations and increased expression of efflux pump genes.[45] Subinhibitory antibiotic concentrations have also been reported to promote resistance via target mutations and changes in drug efflux.[46] An interesting observation from our data is the overall lack of emergence of ciprofloxacin resistance, determined by MIC testing at follow-up (96 h) and plating on MHA supplemented with 2 and 128 mg/L ciprofloxacin. We did not repeat WGS on the post-exposure growth or assess efflux expression. Possible explanations could be the down-regulation of genes prior to MIC testing, or that regrowth reflects tolerance, persistence and quiescence.[47,48]

First-line oral antimicrobials, nitrofurantoin and fosfomycin, may not be preferred agents for a variety of clinical reasons. Their activity beyond the bladder is uncertain, renal impairment impacts on nitrofurantoin,[49] and fosfomycin is not reliably active against non-*E. coli* uropathogens.[50] Alternatively, amoxicillin/clavulanate for the treatment of ESBL-producing uropathogens has been supported by several observational studies.[51,52] However, a randomized control trial comparing 3 days of amoxicillin/clavulanate with 3 days of ciprofloxacin demonstrated a higher failure rate with amoxicillin/clavulanate.[53] The potential superiority of fluoroquinolones over other agents, including β-lactams, has been reported in two systematic reviews.[54,55]

New oral antimicrobials for ESBL-producing uropathogens include: third-generation oral cephalosporins with clavulanate;[56] oral carbapenems (sulopenem non-inferior to ciprofloxacin,[57] tebipenem non-inferior to ertapenem[58,59]); omadacycline;[60] gepotidacin;[61] and oral β-lactamase inhibitors (QPX7728, ETX0282, VNRX7145 and ARX1796).[62–64] Although these agents may provide valuable future options, the complexities of licensing new agents can limit their availability, as evidenced by an FDA regulatory hurdle for tebipenem that prompted Spero Therapeutics to suspend commercialization activities.

Our dynamic *in vitro* model applies a high media flow rate and large volume shifts that mimic urodynamics and mSHU to reflect the urinary environment. The addition of yeast extract and casamino acids to the 18 chemical components included in the mSHU enables the *E. coli* uropathogens to have a similar growth rate to growth in human urine, while not expected to bind free ciprofloxacin. However, the bactericidal activity of the ciprofloxacin is decreased by several *in vivo* conditions, including the presence of cations and acidic urine pH leading to higher MICs.[65,66] Other dynamic PK/PD *in vitro* models studying ciprofloxacin against *E. coli* have either lacked urodynamic simulation,[67] or were not specific for UTIs (i.e. urinary ciprofloxacin exposures; media mimicking urine).[68,69] Where urodynamics and the urinary environment were simulated, as performed using the original UTI bladder infection *in vitro* model,[70] and a dilutional model,[71] fluoroquinolones were very effective against *E. coli* isolates.

Compared with *in vivo* studies, a mouse ascending UTI model demonstrated that isogenic *E. coli* strains with low-level ciprofloxacin resistance genes (*qnrA1*, MIC 0.19 mg/L; *qnrB19*, MIC 0.38 mg/L; *qnrS1*, MIC 0.38 mg/L) had reduced ciprofloxacin kill in urine and bladder bacterial counts compared with the WT strain (MIC 0.032 mg/L), despite ciprofloxacin reaching high urine concentrations (urinary AUC_{0-24} 2572 mg·h/L, urinary C_{max} 553 mg/L) following the dose of 0.2 mg per mouse four times daily to correspond to a human dose of 500 mg twice daily.[72] In another mouse model of ascending UTI in diabetic mice, following ciprofloxacin dose-fraction studies a plasma exposure–response relationship was found with an approximate AUC/MIC IC_{50} of 120–170.[73] A 24 h plasma AUC/MIC of ~400 associated with complete bacterial clearance in kidneys and bladder tissues.[73] Corresponding to human exposures following oral 500 mg 12 hourly, a mouse equivalent dose (196 mg/L) would be expected to provide exposures correlating with significant microbiology activity in bladder, kidney and urine and resolution of clinical symptoms (plasma AUC/MIC of 566). As urine ciprofloxacin concentrations were not measured, plasma concentrations were considered as a surrogate to evaluate therapeutic concentrations in the bladder and kidneys. This study was limited by infecting mice with the fully ciprofloxacin susceptible *E. coli* ATCC 25922 strain and assessing the response over 24 h.

There are important limitations to our *in vitro* model, including the lack of host response and bladder tissue architecture. Immunocompetent and novel 'bladder-on-a-chip' *in vitro* models have attempted to overcome some of these limitations.[74,75] Our findings are also specific to *E. coli* and may not directly translate to other uropathogens. Furthermore, it is uncertain if these data can be extrapolated to complicated UTI, or patients with renal dysfunction. This work does not examine extended-release formulations of oral ciprofloxacin that have comparable clinical outcomes,[76–78] with the benefit of daily administration, high urinary drug levels over the entire 24 h period, higher peak plasma concentrations, superior bactericidal activity and lower interpatient variability.[79]

In summary, these data support oral ciprofloxacin efficacy for *E. coli* urinary isolates with MIC ≤0.25 mg/L with the standard dose and with MIC ≤1 mg/L when dosed at 750 mg 12 hourly and applying conservative measures for bacterial response. The application of urinary-specific ciprofloxacin breakpoints should be cautiously considered in specific clinical scenarios and supported with strong antimicrobial stewardship practices.

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Transparency declarations

I.J.A. has received consultancies/advisory boards for MSD. J.M. has received research grants from Pfizer, Gilead, Astellas, MSD, F2G and Venatorx. J.A.R. has consultancies/advisory boards for MSD, QPEX, Discuva Ltd, Accelerate Diagnostics, Bayer, bioMérieux; speaking fees for MSD, bioMérieux, Pfizer; industry grants from MSD, The Medicines Company, Cardeas Pharma, bioMérieux, QPEX, Pfizer. A.Y.P. has received investigator-initiated research funding from MSD. All other authors: none to declare.

Supplementary data

Supplementary Methods, Figures S1 and S6 and Tables S1 to S7 are available as Supplementary data at *JAC* Online.

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