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*f*T>MIC Targets May Vary for Cephalosporins Within the *Enterobacteriaceae* Group

Karen E Bowker,[1] Alan R Noel,[1] Jane Ambler,[2] Alasdair P MacGowan[1]

[1]BCARE, North Bristol NHS Trust and University of Bristol, Southmead Hospital, Bristol, UK, [2]AstraZeneca Pharmaceutical LP, Waltham, MA, USA

Contact Information:
Karen Bowker
Dept of Microbiology
Lime Walk Building
Southmead Hospital
Westbury on Trym, Bristol, BS10 5NB
Tel: +44 0117 3234187
Email: Karen.Bowker@nbt.nhs.uk

Abstract

Background: Pre-clinical *in vitro* and *in vivo* pharmacodynamic (PD) models are often employed to determine pharmacodynamic index targets for *Enterobacteriaceae*; however, in most cases only *E. coli* or *Klebsiella* spp strains are tested. Many clinical infections are due to other species of *Enterobacteriaceae* and it may not be accurate to assume these species behave in a similar way to *E. coli* or *Klebsiella* spp. We tested this assumption using ceftaroline to assess how representative the PD of *E. coli* was for other *Enterobacteriaceae*.

Methods: A dilutional *in vitro* PK model of infection was used to perform ceftaroline dose ranging studies (up to 11 exposures per strain) against *E. coli* (n=4); *K. pneumoniae* (n=4), *P. mirabilis* (n=4), *Citrobacter koseri* (n=2) and *S. marcescens* (n=1). *f*T>MIC for static -1, -2 and -3 log drops in initial viable count were determined for each strain.

	*f*T>MIC for a 24 h effect			
	Static	**-1 log**	**-2 log**	**-3 log**
E. coli (n=4)	35 ± 6	37 ± 7	38 ± 8	40 ± 10
K. pneumoniae (n=4)	36 ± 8	44 ± 9	52 ± 13	85 ± 15
P. mirablis (n=4)	39 ± 26	40 ± 26	41 ± 26	61 ± 15
C. koseri (n=2)	47	49	52	54
S. marcescens (n=1)	64	66	70	>100
All *Enterobacteriaceae* (n=15)	40 ± 16	43 ± 16	47 ± 17	65 ± 23

Results: The *f*T>MIC for the various antibacterial effect endpoints is shown on the Table. For all strains, the 24 h static effect was related to a *f*T>MIC of 40 ± 16% and -1 log drop to 43 ± 16%. The 24 h static and -1 log *f*T>MIC values are similar for *E. coli*, *K. pneumoniae* and *P. mirabilis*, however, much greater strain variability in the *f*T>MIC value is present among *P. mirabilis* strains tested compared to the other species. *Citrobacter* and *Serratia* had a somewhat longer 24 h static *f*T>MIC values than *E. coli*. *f*T>MIC associated with -3 log drop in count were smaller for *E. coli*, *P. mirabilis* and *Citrobacter* but notably longer for *K. pneumoniae* and *Serratia*.

Conclusion: The *f*T>MIC for all strains for a static effect was 40% and -1 log drop 43%. *f*T>MIC targets determined using *E. coli* only and extrapolated to all *Enterobacteriaceae* may be misleading as some species appear to have greater strain-to-strain variation in *f*T>MIC targets while others show differences in 24 h static or cidal *f*T>MIC targets.

Introduction

- Ceftaroline fosamil the pro-drug of ceftaroline (CPT) has been approved in the USA for the treatment of acute bacterial skin and skin-structure infections and community-acquired bacterial pneumonia, and for similar indications in Europe.
- As well as *in vitro* activity against *Staphylococcus aureus* and *Streptococcus pneumoniae*, CPT has cefotaxime-like *in vitro* potency and spectrum against *Enterobacteriaceae* (Mushtaq et al 2007). The CPT $MIC_{50/90}$ for ceftazidime-susceptible *Escherichia coli* is 0.06/0.5 mg/L; *Klebsiella pneumoniae* 0.12/0.25 mg/L, *Citrobacter freundii* 0.12/0.25 mg/L, *Proteus mirabilis* 0.06/4 mg/L and *Serratia marcescens* 0.5/16 mg/L.
- There are limited published pharmacodynamic (PD) data for CPT against *Enterobacteriaceae*. Using a neutropenic murine thigh model using 21 *E. coli*, and 14 *Klebsiella* spp., Housman et al, 2012, reported a *f*T>MIC of 28% for 24 h static effect and 66% for a -1 log_{10} drop in viable count; Andes and Craig (2006) previously reported similar values in their neutropenic murine thigh model.
- There are little or no published data on *f*T>MIC PD targets for *Enterobacteriaceae* species other than *E. coli* and *K. pneumoniae*, and no data on the risks of emergence of resistance (EoR) in PD models with any *Enterobacteriaceae*.
- The aim of this study was to describe the antibacterial effect (ABE) of CPT against a range of CPT-susceptible *Enterobacteriaceae* (CPT MIC values of <1 mg/L); and to define the relationship between *f*T>MIC and antibacterial effect. In addition, the relationship between *f*T>MIC and the risk of changes in population profiles was established for all *Enterobacteriaceae* strains tested.

Methods

- A dilutional *in vitro* pharmacokinetic model was used to simulate a range of concentrations of CPT designed to achieve a *f*T>MIC range of 0–100% for each strain to define the *f*T>MIC – antibacterial effect relationship. The $t_{½}$ was 2.5 h and dosing was q12h for 96 h. Between seven and 10 doses were simulated per strain. Drug concentrations of ceftaroline were determined by HPLC.
- Sixteen wild type strains of *Enterobacteriaceae* were used: four *E. coli* CPT MICs 0.045–0.75 mg/L, five *K. pneumoniae* MIC 0.12–0.75 mg/L, four *P. mirabilis* MICs 0.12–0.5 mg/L, two *Citrobacter koseri* MICs 0.12 and 0.38 mg/L and one strain of *S. marcescens* MIC 0.5 mg/L. The inoculum was 10^6 CFU/mL and experiments were conducted over 96 h. CPT MICs were determined by CLSI standard broth dilution methodology. MICs were determined in 100% MHB and at non-doubling dilutions to more accurately determine MIC values.
- ABE was measured by log change in viable count at 24 h (d24), 48 h (d48), 72 h (d72) and 96 h (d96) relative to the starting inocula (log CFU/mL). The area under the bacterial kill curve (AUBKC, log CFU/mL) was calculated using the log linear-trapezoidal rule four times 0–24 h (AUBKC 24), 0–48 h (AUBKC 48) 0–72 h (AUBKC 72) and 0–96 h (AUBKC 96).
- A sigmoid Emax curve was fitted to the data using a Boltzmann Sigmoid Emax equation using GraphPad Prism.
- EoR for each strain was assessed by changes in population analysis profiles on nutrient agar plates containing x2, x4 and x8 the CPT MIC at 0, 24, 48, 72 and 96 h. The limit of detection was 10^2 CFU/mL.

Results

- Tables 1 and 2 show the CPT *f*T>MIC for a static, -1 log_{10}, -2 log_{10} drop in viable count at 24 h and 96 h for *E. coli*, *K. pneumoniae* *C. koseri*, *S. marcescens* and all tested *Enterobacteriaceae* combined.
- Figures 1 and 2 show the relationships between CPT *f*T>MIC, and a static, -1 log_{10}, -2 log_{10} drop in viable count at 24, 48, 72 and 96 h for *E. coli* and *K. pneumoniae*.
- Using d24 as the ABE measure, the *f*T>MIC for a 24 h static effect for all *Enterobacteriaceae* was 39.7 ± 15.7%. The *f*T>MIC for -2 log_{10} kill was 47.1 ± 16.9%. Within this grouping, there were no clear differences in 24 h *f*T>MIC for bacteriostatic effect between *E. coli* *f*T>MIC 35.0 ± 6.3%, *K. pneumoniae* *f*T>MIC 36.1 ± 8.3% or *P. mirabilis* *f*T>MIC 39.1 ± 26.4%.
- Too few strains of *C. koseri* (2) and *S. marcescens* (1) were assessed for useful comparison. However, the strain-to-strain variation among *P. mirabilis* was markedly greater – coefficient of variation (CV) of 67.5% for static effect compared to *E. coli* CV 18% and *K. pneumoniae* CV 23.0%.
- The increased strain-to-strain variation with *P. mirabilis* was also apparent with the bactericidal end points. Although the 24 h static ABE end point was similar across species, there were clear differences between species in terms of the bactericidal end point (e.g. the *f*T>MIC to produce a -3 log_{10} change in viable count at 24 h for *E. coli* was 40.0 ± 9.6%; however for *K. pneumoniae* it was 84.8 ± 15.2%).
- In order to assess whether the marked strain-to-strain variation observed with *P. mirabilis* was reproducible, strain 45322 (low *f*T>MIC) and strain 45266 (high *f*T>MIC) were retested and confirmed (Tables 1 and 2).
- Tables 3 and 4 show EoR as shown by growth on x2, x4 and x8 CPT MIC plates for *E. coli* and *K. pneumoniae*. The *f*T>MIC most likely to produce changes in population profiles for *E. coli* (Table 3) were 21–40% at 24 h and 48 h, and 31–50% at 72 h and 96 h, these map closely to the *f*T>MIC required for bacteriostatic effect at 24, 48, 72 and 96 h.
- A similar pattern was observed with *K. pneumoniae* (Table 4) with the greatest risk of EoR occurring with *f*T>MIC values between 1–50% at 24 h and 48 h, and 1–60% at 72 h and 96 h, just below the bacteriostatic effect *f*T>MIC at 24, 48, 72 and 96 h.
- Changes in population profiles with *C. koseri* followed the same pattern, with the highest risk of population change and its size being in *f*T>MIC similar to the bacteriostatic effect (data not shown).

Table 1. *f*T>MIC relationship to antibacterial effect for CPT against *Enterobacteriaceae* after 24 h exposure

		Antibacterial effect			
Species/strain	**CPT MIC (mg/L)**	**static**	**-1 log_{10} drop**	**-2 log_{10} drop**	**-3 log_{10} drop**
E. coli 44966	0.045	26.2	26.2	26.8	27.2
E. coli 44917	0.19	38.2	42.3	46.3	50.4
E. coli 44852	0.75	40.6	41.3	41.3	40.8
E. coli 44913	0.75	34.9	36.9	38.9	41.6
All *E. coli*		**35.0 ± 6.3**	**36.8 ± 7.1**	**38.3 ± 8.3**	**40.0 ± 9.6**
K. pneumoniae 43489	0.12	42.9	51.0	59.1	84.4
K. pneumoniae 45059	0.19	44.0	49.0	53.7	68.9
K. pneumoniae 45645	0.25	27.5	28.2	29.5	70.5
K. pneumoniae 43739	0.38	26.8	43.0	63.8	>100
K. pneumoniae 38345	0.75	39.9	46.6	55.2	>100
All *K. pneumoniae*		**36.1 ± 8.3**	**43.6 ± 9.1**	**52.3 ± 13.3**	**84.8 ± 15.2**
E. coli and *K. pneumoniae* combined		35.7 ± 7.1	40.6 ± 8.5	46.1 ± 13.0	64.9 ± 26.6
C. koseri 45277	0.12	60.2	61.9	63.5	62.7
C. koseri 45661	0.38	33.5	36.7	40.5	45.7
All *C. koseri*		**46.9**	**49.3**	**52.0**	**54.2**
P. mirabilis 45967	0.12	27.3	28.6	30.6	31.8
P. mirabilis 45416	0.38	45.0	45.6	46.3	47.0
P. mirabilis 45322 Exp1	0.5	<5*	<5*	<5*	<5*
P. mirabilis 45322 Exp2		11.4	12.8	14.1	–
P. mirabilis 45266 Exp1		72.9	73.6	74.1	74.9
P. mirabilis 45266 Exp2		>75	>75	>75	>75
All *P. mirabilis*		**39.1 ± 26.4**	**40.1 ± 26.0**	**41.3 ± 25.5**	**61.1 ± 14.7**
S. marcescens 44135	0.5	64.4	66.4	69.7	>100
Citrobacter, *Proteus* and *Serratia* spp. combined		45.0 ± 22.2	46.5 ± 22.1	48.4 ± 22.0	65.8 ± 20.4
All *Enterobacteriaceae*		**39.7 ± 15.7**	**43.2 ± 15.6**	**47.1 ± 16.9**	**65.0 ± 23.5**

Table 2. *f*T >MIC relationship to antibacterial effect for CPT against *Enterobacteriaceae* after 96 h exposure

		Antibacterial effect			
Species/strain	**CPT MIC (mg/L)**	**static**	**-1 log_{10} drop**	**-2 log_{10} drop**	**-3 log_{10} drop**
E. coli 44966	0.045	26.6	27.0	27.5	27.2
E. coli 44917	0.19	58.0	58.7	59.9	60.8
E. coli 44852	0.75	45.3	45.3	45.3	66.6
E. coli 44913	0.75	62.5	63.3	62.3	65.6
All *E. coli*		**48.1 ± 16.1**	**48.6 ± 16.4**	**48.8 ± 16.6**	**55.1 ± 18.8**
K. pneumoniae 43489	0.12	68.8	76.1	82.2	91.1
K. pneumoniae 45059	0.19	75.1	75.1	75.1	>100
K. pneumoniae 45645	0.25	–	–	–	–
K. pneumoniae 43739	0.38	74.9	79.6	>100	>100
K. pneumoniae 38345	0.75	49.3	58.8	>100	>100
All *K. pneumoniae*		**67.0 ± 12.2**	**72.4 ± 9.2**	–	–
E. coli and *K. pneumoniae* combined		57.6 ± 16.6	60.5 ± 17.7	58.7 ± 19.9 (n=6)	62.3 ± 22.9 (n=5)
C. koseri 45277	0.12	82.6	88.6	93.3	96.8
C. koseri 45661	0.38	53.7	54.4	55.0	58.2
All *C. koseri*		**68.2**	**71.5**	**74.2**	**77.5**
P. mirabilis 45967	0.12	32.0	34.6	37.3	39.4
P. mirabilis 45416	0.38	75.8	78.5	81.9	86.6
P. mirabilis 45322 Exp1	0.5	<5	<5	<5	<5
P. mirabilis 45322 Exp2		14.1	14.8	15.4	16.2
P. mirabilis 45266 Exp1	0.5	–	–	–	–
P. mirabilis 45266 Exp2		–	–	–	–
All *P. mirabilis*		**42.5 ± 27.5**	**42.6 ± 32.6**	**44.9 ± 33.9**	**47.4 ± 35.9**
S. marcescens 44135	0.5	77.2	94.6	99.3	>100
Citrobacter, *Proteus* and *Serratia* spp. combined		47.4 ± 26.4	60.9 ± 31.8	63.7 ± 33.4	59.4 ± 33.2
All *Enterobacteriaceae*		**57.6 ± 18.0**	**60.7 ± 23.6**	**61.2 ± 23.4 (n=12)**	**60.8 ± 26.9 (n=10)**

Figure 1. Relationship between *f*T>MIC for CPT and its antibacterial effect against *E. coli* measured over 96 h

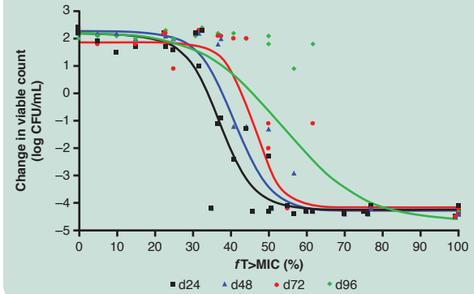

Figure 2. Relationship between *f*T>MIC for CPT and its antibacterial effect against *K. pneumoniae* measured over 96 h

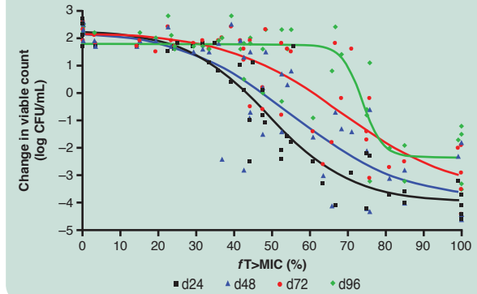

Table 3. Change in population profiles for *E. coli* over 96 h

		Growth on MICx4 plates								Growth on MICx8 plates							
		24 h		48 h		72 h		96 h		24 h		48 h		72 h		96 h	
***f*T>MIC**	**Total number of exps**	**N (%) exps >2 log growth**	**Viable count**	**N (%) exps >2 log growth**	**Viable count**	**N (%) exps >2 log growth**	**Viable count**	**N (%) exps >2 log growth**	**Viable count**	**N (%) exps >2 log growth**	**Viable count**	**N (%) exps >2 log growth**	**Viable count**	**N (%) exps >2 log growth**	**Viable count**	**N (%) exps >2 log growth**	**Viable count**
0	4	0	–	0	–	0	–	0	–	0	–	0	–	0	–	0	–
1–10	2	1 (50%)	2.9	2 (100%)	2.7	1 (50%)	5.2	1 (50%)	6.0	0	–	0	–	0	–	0	–
11–20	3	2 (67%)	3.2	2 (67%)	3.4	2 (67%)	4.7	2 (67%)	6.0	0	–	0	–	1 (33%)	2.2	1 (33%)	2.2
21–30	3	3 (100%)	4.8 ± 0.9	3 (100%)	5.2 ± 0.7	2 (67%)	4.6	2 (67%)	4.7	3 (100%)	4.3 ± 2.0	2 (67%)	5.6	3 (100%)	4.3 ± 1.6	3 (100%)	4.5 ± 1.7
31–40	5	3 (60%)	4.5 ± 1.2	3 (60%)	5.2 ± 0.7	5 (100%)	5.7 ± 2.1	5 (100%)	5.6 ± 1.9	2 (40%)	4.7	2 (40%)	5.2	4 (80%)	4.1 ± 1.6	4 (80%)	4.3 ± 1.4
41–50	5	1 (20%)	3.4	1 (20%)	4.6	3 (60%)	5.8 ± 1.9	3 (66%)	5.9 ± 2.0	3 (60%)	4.3 ± 1.5	2 (40%)	6.9	0	–	1 (20%)	2.4
51–60	4	1 (25%)	3.4	1 (25%)	4.9	1 (25%)	4.4	1 (25%)	4.5	1 (25%)	2.1	1 (25%)	2.1	0	–		
61–70	1	0	–	0	–	0	–	0	–	0	–	0	–	0	–		
71–80	4	0	–	1 (25%)	4.0	1 (25%)	4.5	1 (25%)	4.5	0	–	0	–	0	–		
81–90	0	0	–	0	–	0	–	0	–	0	–	0	–	0	–		
91–100	4	0	–	0	–	1 (25%)	4.2	1 (25%)	4.2	0	–	0	–	0	–		

Table 4. Change in population profiles for *K. pneumoniae* over 96 h

		Growth on MICx4 plates								Growth on MICx8 plates							
		24 h		48 h		72 h		96 h		24 h		48 h		72 h		96 h	
***f*T>MIC**	**Total number of exps**	**N (%) exps >2 log growth**	**Viable count**	**N (%) exps >2 log growth**	**Viable count**	**N (%) exps >2 log growth**	**Viable count**	**N (%) exps >2 log growth**	**Viable count**	**N (%) exps >2 log growth**	**Viable count**	**N (%) exps >2 log growth**	**Viable count**	**N (%) exps >2 log growth**	**Viable count**	**N (%) exps >2 log growth**	**Viable count**
0	5	0	–	0	–	0	–	0	–	0	–	0	–	0	–	0	–
1–10	2	2 (100%)	6.9	2 (100%)	7.6	2 (100%)	8.1	2 (100%)	8.2	1 (50%)	5.7	1 (50%)	2.9	1 (50%)	4.3	1 (50%)	7.5
11–20	3	3 (100%)	6.5 ± 1.6	3 (100%)	7.6 ± 0.2	3 (100%)	7.5 ± 0.7	3 (100%)	7.6 ± 0.6	1 (33%)	5.4	1 (33%)	5.4	1 (33%)	6.9	1 (33%)	5.9
21–30	5	5 (100%)	6.0 ± 2.4	5 (100%)	6.0 ± 2.5	5 (100%)	6.4 ± 2.7	5 (100%)	6.3 ± 2.4	4 (80%)	4.2 ± 0.4	3 (60%)	4.6 ± 0.2	3 (60%)	5.8 ± 1.8	3 (60%)	6.1 ± 2.0
31–40	5	2 (40%)	6.0	3 (60%)	6.1 ± 0.8	5 (100%)	5.9 ± 2.2	5 (100%)	5.2 ± 2.1	0	–	3 (60%)	4.6 ± 0.3	3 (60%)	5.1 ± 2.3	3 (60%)	5.3 ± 1.7
41–50	8	3 (37%)	5.4 ± 0.2	3 (37%)	5.4 ± 1.9	5 (62%)	5.7 ± 1.5	5 (62%)	5.5 ± 2.2	1 (12%)	3.2	2 (25%)	5.3 ± 1.1	5 (62%)	4.0 ± 2.1	3 (37%)	7.2 ± 4.6
51–60	6	1 (17%)	5.2	1 (17%)	5.2	2 (33%)	6.7	2 (33%)	7.0	0	–	1 (17%)	3.1	2 (33%)	6.2	2 (33%)	7.0
61–70	5	0	–	0	–	2 (40%)	5.6	2 (40%)	5.0	0	–	0	–	0	–	0	–
71–80	6	0	–	0	–	0	–	0	–	0	–	0	–	0	–	1 (17%)	4.9
81–90	1	0	–	0	–	0	–	0	–	0	–	0	–	0	–	0	–
91–100	5	0	–	0	–	0	–	0	–	0	–	0	–	0	–	0	–

Conclusions

- The CPT *f*T>MIC after 24 h for all *Enterobacteriaceae* strains tested for a static effect was 40% and -1 log_{10} drop 43%.
- *f*T>MIC targets determined using *E. coli* only and extrapolated to all *Enterobacteriaceae* may be misleading, as some species appear to have greater strain-to-strain variation in *f*T>MIC targets, while others show differences in 24 h static or cidal *f*T>MIC targets.
- In future, pre-clinical assessments of PD targets for *Enterobacteriaceae* should involve testing a greater number of strains and species.

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*f*T>MIC Targets May Vary for Cephalosporins Within the *Enterobacteriaceae* Group

Karen E Bowker,[1] Alan R Noel,[1] Jane Ambler,[2] Alasdair P MacGowan[1]

[1]BCARE, North Bristol NHS Trust and University of Bristol, Southmead Hospital, Bristol, UK, [2]AstraZeneca Pharmaceutical LP, Waltham, MA, USA

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