Pharmacodynamics of Ceftolozane-Tazobactam (C/T) as Monotherapy and in Combination with Tobramycin or Fosfomycin Against *P.aeruginosa* with C/T MICs at or Above 4mg/L

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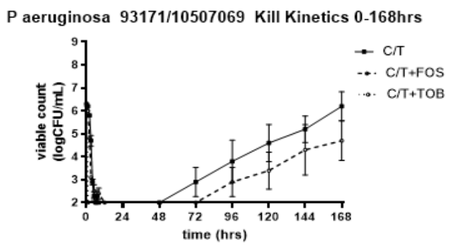
Background: Ceftolozane-tazobactam (C/T) is an emergent and increasingly used intravenous antibacterial therapy for severe *Pseudomonas aeruginosa* (Pa) infections, including multidrug resistant strains. Previously, we showed in an in vitro pharmacokinetic model of Pseudomonal infection that a free T>MIC (fT>MIC) ceftolozane-tazobactam target for 24hr and -1 log reduction in bacterial load were 25±3% and 27±4% respectively. Emergence of resistance was suppressed at fT>MC exposures of >60%. Simulation of ceftolozane-tazobactam human serum concentrations associated with doses of 2g/1g q8h for 7days showed eradication of bacterial load and suppression of emergence of resistance for *P aeruginosa* stains with MICs in the range 0.38-4mg/L. Using the same pre-clinical model, we simulated human serum concentrations of ceftolozane-tazobactam q8h for 7days as monotherapy and in combination with either tobramycin or fosfomycin. P aeruginosa strains with MICs on or above the MIC (4-64mg/L) were employed and the primary endpoints were reduction in bacterial load and emergence of resistance as measured by changes in population profiles

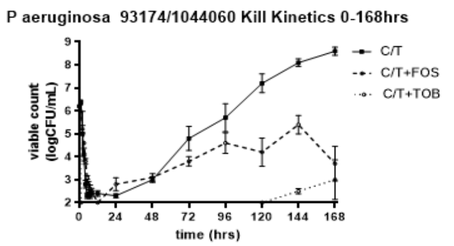
Materials and methods

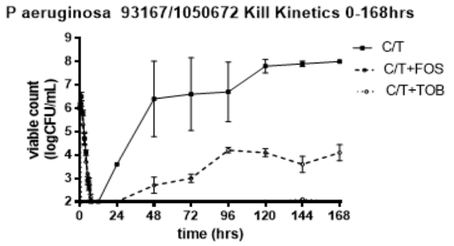
In vitro pharmacokinetic model: An *in vitro* one-compartment pharmacokinetic model was used to simulate the free-drug serum concentrations associated with standard doses of ceftolozane-tazobactam, tobramycin and fosfomycin. The apparatus, consists of a central culture chamber for infusion of the study drugs, ensuring the correct $t_{½l}$ is modelled which is connected via aluminium and silicone tubing to a reservoir containing broth. Owing to the differing serum $t_{½}$ values of ceftolozane and tazobactam (2.5 and 1 h, respectively) the model was supplemented with ceftolozane throughout each dosing period via a separate dosing chamber to achieve the required concentration–time profiles for both ceftolozane and tazobactam. The temperature was maintained at 37°C , with an initial inoculum of 10^6 cfu/mL.

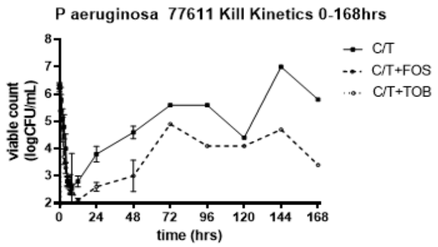
Antimicrobials: Ceftolozane and tazobactam were supplied by Merck and Co. Inc., Kenilworth, NJ, USA. Tobramycin and fosfomycin solutions were prepared according to their manufacturers' instructions.

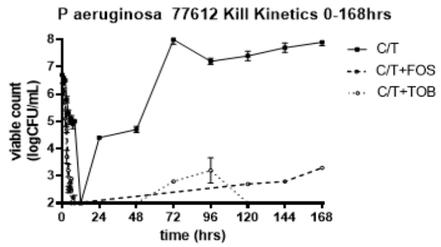
Medium: Unsupplemented Mueller–Hinton broth was used for all experiments.











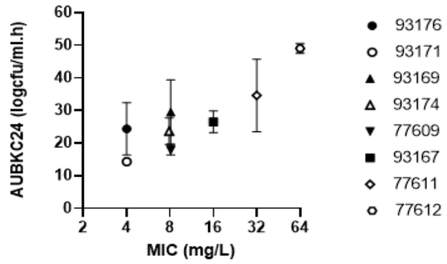


Figure 1 Antibacterial effect of simulated ceftolozane alone(C/T) and in combination with fosfomycin(C/T+FOS) or tobramycin(C/T+TOB) against P aeruginosa 93171 (C/T 4mg/L)
Figure 2 Antibacterial effect of simulated ceftolozane alone(C/T) and in combination with fosfomycin(C/T+FOS) or tobramycin(C/T+TOB) against P aeruginosa 93174 (C/T MIC 8mg/L)
Figure 3 Antibacterial effect of simulated ceftolozane alone(C/T) and in combination with fosfomycin(C/T+FOS) or tobramycin(C/T+TOB) against P aeruginosa 93167 (C/T 16mg/L)
Figure 4 Antibacterial effect of simulated ceftolozane alone(C/T) and in combination with fosfomycin(C/T+FOS) or tobramycin(C/T+TOB) against P aeruginosa 77611 (C/T 32mg/L)
Figure 5 Antibacterial effect of simulated ceftolozane alone(C/T) and in combination with fosfomycin(C/T+FOS) or tobramycin(C/T+TOB) against P aeruginosa 77612 (C/T 64mg/L
Figure 6 Ceftolozane/tazobactam MIC versus ceftolozane/tazobactam AUBKC2

Bacteria: Eight strains of *P aeruginosa* were used with ceftolozane-tazobactam MICs of 4mg/L (2 strains), 8mg/L (3 strains) and ≥16mg/L (3 strains). Fosfomycin MICs were in the range 4-1024mg/L and tobramycin MICs 0.25-8mg/L

Bacterial killing curves : Viable counts were determined using a spiral plater (Don Whitley, Shipley, UK).

Pharmacokinetics: Free-drug serum concentrations of ceftolozane-tazobactam at 2 g-1 g q8h were modelled (i.e. C_{max} at 1 h of infusion was 112 mg/L for ceftolozane and 32 mg/L for tazobactam, and the $t_{½}$ of 2.5-1hr . Fosfomycin concentrations modelled were those associated with 4 g q8h as a bolus (i.e. C_{max} 250 mg/L and $t_{½}$ 2.5 h). Tobramycin was simulated at 7mg/kg 24hrly doses (C_{max} 20mg/L, $t_{½}$ 1.0 h).

Antibiotic assays: Ceftolozane, tazobactam were measured using HPLC and tobramycin by competitive inhibition immunoassay. Fosfomycin concentrations we measured by bioassay.

Table 1 Summary of antibacterial effect for ceftolozane alone and in combination with either fosfomycin or tobramycin

Strain	Ceftolozane/ tazobactam MIC(mg/L)	Ceftolozane alone		+ Fosfomycin			+Tobramycin		
		AUBKC24	AUBKC168	MIC	AUBKC24	AUBKC168	MIC	AUBKC24	AUBKC168
93171	4	14.3±1.1	272.9±139.0	4	13.1±1.8	177.3±138.4	0.5	2.2±0.1	2.2±0.1
93176	4	24.4±8.0	632.5±172.7	8	18.7±4.1	38.2±23.9	0.25	2.2±0.1	2.2±0.1
93169	8	29.5±9.9	878.6±75.3	4	19.4±4.4	388.2±103.5	1	3.7±2.2	70.1±74.3
93174	8	23.7±4.1	744.3±51.9	4	23.0±6.7	362.1±31.6	2	2.2±0.1	487±55.7
77609	8	17.9±0.6	57.8±18.9	1024	23.0±6.6	23.0±6.6	3	10.7±3.8	40.0±28.6
93167	16	26.5±3.3	727.5±199.2	4	16.6±0.7	222.8±17.2	0.5	2.2±0.1	2.2±01
77611	32	34.6±11.1	542.6	96	19.5±4.8	319.6	2	2.4±0.5	2.4±0.5
77612	64	49.0±0.8	762.3±19.3	16	16.5±1.1	45.6	8	12.3±1.5	78.6±58.4

Table 2 Changes in ceftolozane population profiles for ceftolozane alone and in combination with either fosfomycin or tobramycin

		Changes in population profiles					
Strain (ceftolozane MIC/mg/L)		24h		96hrs		168hr	
	Drugs	MICx4	MICx8	MICx4	MICx8	MICx4	MICx8
93171 (4)	Ceftolozane alone	<2	<2	<2	<2	3.6(n=2)	3.2(=2)
	Ceftolozane+fosfomycin	<2	<2	<2	<2	<2	<2
	Ceftolozane+tobramycin	<2	<2	<2	<2	<2	<2
93176 (4)	Ceftolozane alone	<2	<2	7.7(n=2)	7.2(n=2)	6.8±0.9	6.9±0.9
	Ceftolozane+fosfomycin	<2	<2	<2	<2	<2	<2
	Ceftolozane+tobramycin	<2	<2	<2	<2	<2	<2
93169 (8)	Ceftolozane alone	<2	<2	6.0±0.1	5.4±0.2	7.8±0.1	5.4±0.1
	Ceftolozane+fosfomycin	<2	<2	<2	<2	3.3±1.5	<2
	Ceftolozane+tobramycin	<2	<2	<2	<2	3.8(n=2)	<2
93174 (8)	Ceftolozane alone	<2	<2	4.2±1.6	3.5±2.0	7.3±0.3	5.7±3.0
	Ceftolozane+fosfomycin	<2	<2	<2	<2	<2	<2
	Ceftolozane+tobramycin	<2	<2	<2	<2	<2	<2
77609 (8)	Ceftolozane alone	<2	<2	<2	<2	<2	<2
	Ceftolozane+fosfomycin	<2	<2	<2	<2	<2	<2
	Ceftolozane+tobramycin	<2	<2	<2	<2	<2	<2
93167 (16)	Ceftolozane alone	8.4(n=2)	4.8(n=4)	8(n=2)	4(n=2)	8.0(n=2)	7.9(n=2)
	Ceftolozane+fosfomycin	<2	<2	<2	<2	<2	<2
	Ceftolozane+tobramycin	<2	<2	<2	<2	<2	<2
77611 (32)	Ceftolozane alone	<2	<2	<2	<2	<2	<2
	Ceftolozane+fosfomycin	<2	<2	<2	<2	<2	<2
	Ceftolozane+tobramycin	<2	<2	<2	<2	<2	<2
77612 (64)	Ceftolozane alone	4.2±0.2	3.5±0.2	6.7±1.8	6.5±1.5	79±0.1	7.2±0.9
	Ceftolozane+fosfomycin	<2	<2	2.6(n=1)	2.2(n=1)	2.8(n=1)	2.8(n=1)
	Ceftolozane+tobramycin	<2	<2	<2	<2	<2	<2

Measurement of antibacterial effects and emergence of resistance: Antibacterial effect was measured by log change in viable count over the duration of simulations (log cfu/mL). The antibacterial endpoints were log change in viable count from initial inoculum (time 0) at 24, 48, 72, 96, 120, 144 and 168 h as well as the area under the bacterial kill curve (AUBKC; log cfu/mL·h) between time 0 and 24, 48, 72, 96, 120, 144 and 168 h. AUBKC at 168 h ($AUBKC_{168}$) was the co-primary endpoint along with emergence of resistance measured by growth on 4 × MIC plates.

Results: The target and achieved concentrations of ceftolozane, tazobactam, fosfomycin and tobramycin agreed (data not shown).
Figures 1-5 show the time kill curves for *P aeruginosa* strains 93171 C-T MIC 4mg/L Figure 1, *Pa* strain 93174 C-T MIC 8mg/L Figure 2, *Pa* strain 93167 C-T MIC 16mg/L Figure 3, *Pa* strain 77611 C-T MIC 32mg/L Figure 4 and *Pa* strain 77612 C-T MIC 64mg/L Figure 5.
Figure 6-shows the AUBKC24(log CFU/mL.h)values for ceftolozane-tazobactam alone versus MIC
Table 1 provides a summary of the antibacterial effects in terms of AUBKC24 and AUBKC168 values measured in logCFU/mL.h showing the mean +/-SD
Table 2 shows the changes in the ceftolozane-tazobactam population profiles with each strain and antibiotic exposures over time up to 168 hrs. Values are absolute bacterial counts in logCFU/mL showing mean +/-SD.

Conclusions: Simulations of ceftolozane-tazobactam alone against P aeruginosa strains with MICs in the range 4-64mg/L indicate early reductions in bacterial load but with subsequent regrowth with most strains. Ceftolozane-tazobactam activity was related to MIC. As MICs increased activity in the model decreased.
Addition of fosfomycin resulted in moderate suppression of bacterial grow back compared to ceftolozane-tazobactam alone. This effect was not related to fosfomycin MIC
Addition of tobramycin resulted in a more marked suppression of bacterial load over the duration of the simulations (168h)
Addition of either fosfomycin or tobramycin to ceftolozane-tazobactam reduced C-T population profile changes seen with C-T alone indicating suppression of the risk of resistance

Summary: In a pre-clinical pharmacokinetic model addition of either fosfomycin or tobramycin to ceftolozane-tazobactam at simulated human exposures reduced *P aeruginosa* bacterial loads and reduced the risk of resistance to ceftolozane-tazobactam with *P aeruginosa* strains with MICs on or above the clinical breakpoint. Clinical studies on the effect of combination therapies on the risk of emergence of resistance are warranted