PK/PD ASSESSMENT OF FOSFOMYCIN IN SYNTHETIC HUMAN URINE COMPARED TO POOLED HUMAN URINE IN A DYNAMIC *IN VITRO* BLADDER INFECTION MODEL

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BACKGROUND

Little is known of the impact of the bladder environment on fosfomycin activity, nor how to best simulate this *in vitro*. In a dynamic bladder infection *in vitro* model, we compare laboratory media to pooled human urine and synthetic alternatives to test which best resembles the *in vivo* enironment.

MEDIA SELECTION

Using human urine is impractical for *in vitro* testing due to its highly variable chemical make-up and the many logistical challenges in it's ethically considered collection, sterilisation and timely use before deterioration. Media tested included:

— Mueller-Hinton broth (MHB)
— MHB with 25 mg/L glucose-6-phosphate (MHB + G6P)
— Female midstream urine (MSU, randomly pooled)
— Female 24-hour collected urine (24U, pooled by equal volume)
— Artificial urine medium (AUM, Brooks *et al*.1997)[1]
— Synthetic human urine (SHU, Ipe *et al*. 2016)[2]

CHEMICAL INGREDIENTS FOR SYNTHETHIC URINE ALTERNATIVES

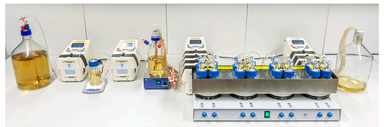
Chemical		g / L	
		Synthetic human urine (Ipe et al[2])	Artificial human urine (Brooks et al[1])
Sodium chloride	NaCl	5.844	5.2
Sodium sulfate	Na2SO4	2.4147	3.2 (decahydrate)
Urea	Urea	16.8168	10
Potassium chloride	KCl	2.8329	—
Calcium chloride	CaCl2	0.4439	0.37 (dihydrate)
Creatinine	Creatinine	1.0181	0.8
Citric acid trisodium salt dihydrate	Na3C6H5O7	1.9999	—
Ammonium chloride	NH4Cl	1.0698	1.3
Magnesium sulfate	MgSO4	0.3852	0.49 (heptahydrate)
Sodium oxalate	Na2C2O4	0.0241	—
Sodium phosphate monobasic	NaH2PO4	0.5616	—
Sodium phosphate dibasic	Na2HPO4	0.9227	—
Potassium dihydrogen phosphate	KH2PO4	2.1774	0.95
Uric acid	C5H4N4O3	0.1009	0.07
Sodium bicarbonate	NaHCO3	1.1341	2.1
Magnesium chloride hexahydrate	MgCl2·6H2O	0.6506	—
Lactic acid	C3H6O3	0.0991	1.0
Ferrous sulfate heptahydrate	FeSO4·7H2O	0.0014	0.0012
20% (w/v) casamino acids	—	0.1 % (v/v)	—
Citric acid	C6H8O7	—	0.4
Di-potassium hydrogen phosphate	HK2O4P	—	1.2
Yeast extract	—	—	0.005
Peptone L37	—	—	1.0

MSU was more dilute than the 24U (pH 7.0, osmolality 260 mOsm, glucose <0.1 mmol/L; compared to pH 6.5, osmolality 468 mOsm, glucose 0.2 mmol/L). Pooled 24U sample had negligible levels of G6P (0.2 mg/L). Synthetic urine alternatives differed slightly in chemical composition and pH (AUM pH 6.5; SHU pH 5.6), however AUM precipitation limited its used. D-glucose was added to SHU to match the concentration found in the 24U.

DYNAMIC BLADDER INFECTION MODEL

Normal urodynamics was simulated, with a urine output of 60 mL/h, six voids each day, and a post-void residual volume < 50 mL. The *in vitro* model was constructed on a 1:16 scale to *in vivo*, enabling sixteen individual bladder compartments to be run in parallel, held within a water-bath at 37°C ±1°C.

DYNAMIC BLADDER *IN VITRO* INFECTION MODEL



IN VITRO METHODS

1. Susceptiblity testing

— Agar dilution: Reference susceptiblity MIC testing method using 10^4 cfu/spot of each isolate inoculated on Mueller–Hinton II agar plates (MHA; BD Diagnostics, USA) containing 25 mg/L glucose-6-phosphate (G6P; Sigma, Germany) and fosfomycin (InfectoPharm, Germany) following CLSI recommendations in a concentration range of 0.25 – 1024 mg/L. Isolates were tested in triplicate.
— Broth microdilution (BMD): MIC determined in MHB, MHB + G6P, 24U and SHU. Isolates were tested in triplicate.

2. Static time-kill assays

— The response of 8 isolates subjected to static fosfomycin concentrations were compared in MHB + G6P, 24U and SHU.

3. Dynamic bladder infection *in vitro* model

— Sixteen clinical isolates were tested (8 *E. coli*, 4 *E. cloacae*, 4 *K. pneumoniae*), each added to a bladder compartment, at an inoculum of 10^7 cfu, providing an equivalent total number of bacteria expected in human infections (i.e. 10^5 cfu/mL in an average 250 mL void).
— Fosfomycin ('Fomicyt', InfectoPharm, Germany) was used to generate average urinary PK exposures following absorption of a single 3g oral dose (C_{max} 1984 mg/L, T_{max} 7.5h, $AUC_{0\text{-}24}$ 30938 mg.h/L),[3] with *in vitro* concentrations validated by LC-MS/MS.
— Pathogen kill/resistance was assessed over 72-hours by quantitative cultures on drug-free and fosfomycin-containing MHA (64 mg/L, 512 mg/L).
— Growth capacity in SHU and fosfomycin heteroresistance was determined by running an 18-hour drug-free dynamic incubation in the bladder infection model.

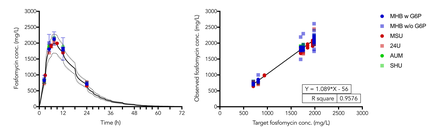
DYNAMIC BLADDER INFECTION *IN VITRO* MODEL RESULTS

BASELINE BACTERIAL STRAIN SUSCEPTIBILITY AND PHARMACODYNAMIC OUTCOME

Isolate details					Baseline fosfomycin susceptibility					Pharmacodynamic outcome in the bladder infection in vitro model					
					Broth microdilution median MIC, (mg/L)					Standard laboratory media	Pooled human urine		Synthetic urine alternative		
Species	Strain no.	Source	ESBL	Agar dilution median MIC, (mg/L)[a]	MHB with G6P	MHB without G6P	Pooled human urine[b]	Synthetic human urine	HLR subpopulation (%)	MHB with G6P	MHB without G6P	Midstream collection	24-hour collection	Artificial urine medium[c]	Synthetic human urine
E. coli	41	urine	yes	≤ 0.25	0.5	32	16	16	—	killed	killed	killed	2.5 $\log_{10}$ (no HLR)	killed	killed
	11	urine	yes	0.5	1	16	8	16	—	killed	killed	killed	killed	killed	killed
	39	urine	yes	0.5	2	32	8	64	—	killed	4.7 $\log_{10}$ (no HLR)	killed	killed	killed	4.0 $\log_{10}$ (no HLR)
	12620	rectal	yes	2	4	16	4	16	—	killed	killed	killed	killed	killed	killed
	1016	urine	yes	16	64	>1024	512	512	1E-03	9.5 $\log_{10}$ (HLR +++)	9.0 $\log_{10}$ (HLR +)	9.0 $\log_{10}$ (HLR +++)	8.7 $\log_{10}$ (HLR +++)	8.3 $\log_{10}$ (HLR +)	6.5 $\log_{10}$ (HLR +)
	1231	urine	yes	32	>1024	>1024	256	1024	4E-04	9.5 $\log_{10}$ (HLR +++)	9.3 $\log_{10}$ (HLR +)	8.9 $\log_{10}$ (HLR +++)	8.8 $\log_{10}$ (HLR +++)	8.5 $\log_{10}$ (HLR +)	8.3 $\log_{10}$ (HLR +)
	4807	rectal	yes	32	64	64	16	insuff.	—	killed	killed	killed	killed	killed	killed
	4757	rectal	yes	64	128	128	16	32	—	killed	killed	killed	killed	killed	killed
E. cloacae	35166	blood	no	0.5	1	32	8	16	—	killed	2.0 $\log_{10}$ (no HLR)	killed	killed	killed	killed
	94	n.a.	yes	1	2	8	4	4	—	killed	killed	killed	3.8 log10 (no HLR)	4.5 log10 (no HLR)	4.7 $\log_{10}$ (no HLR)
	21	rectal	yes	8	32	256	256	256	7E-06	9.5 $\log_{10}$ (HLR +++)	9.8 $\log_{10}$ (HLR +++)	9.3 $\log_{10}$ (HLR +)	8.7 $\log_{10}$ (HLR +)	9.3 $\log_{10}$ (HLR +)	9.0 $\log_{10}$ (HLR +)
	32	n.a.	yes	32	64	512	1024	512	1E-04	9.5 $\log_{10}$ (HLR +++)	9.5 $\log_{10}$ (HLR +++)	9.4 $\log_{10}$ (HLR +)	8.8 $\log_{10}$ (HLR +)	9.0 $\log_{10}$ (HLR +)	9.0 $\log_{10}$ (HLR +)
K. pneumoniae	34672	blood	no	2	8	512	256	512	4E-05	9.3 $\log_{10}$ (HLR +++)	9.5 $\log_{10}$ (HLR +++)	9.1 $\log_{10}$ (HLR +)	8.7 $\log_{10}$ (HLR +)	8.7 $\log_{10}$ (no HLR)	8.5 $\log_{10}$ (HLR +)
	31865	blood	no	2	8	256	64	256	8E-05	9.4 $\log_{10}$ (HLR ++)	9.5 $\log_{10}$ (HLR +++)	8.9 $\log_{10}$ (HLR +)	8.9 $\log_{10}$ (HLR +++)	8.7 $\log_{10}$ (no HLR)	8.5 $\log_{10}$ (HLR +)
	55	sputum	yes	4	16	1024	128	256	2E-04	6.9 $\log_{10}$ (no HLR)	9.5 $\log_{10}$ (HLR +++)	9.0 $\log_{10}$ (HLR +)	9.0 $\log_{10}$ (HLR +)	8.4 $\log_{10}$ (HLR +)	9.2 $\log_{10}$ (HLR +)
	52	urine	yes	16	32	> 1024	256	1024	7E-04	9.2 $\log_{10}$ (HLR +++)	9.7 $\log_{10}$ (HLR +++)	8.8 $\log_{10}$ (HLR +)	8.7 $\log_{10}$ (HLR +)	8.2 $\log_{10}$ (HLR +)	8.8 $\log_{10}$ (HLR +)
E. coli	ATCC 25922	no	no	1	1	64	8	32	—	Not tested in the in vitro model					

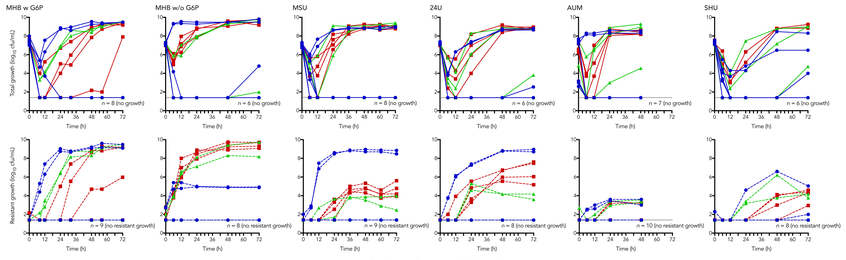
[a] performed in triplicate on Mueller-hinton agar (MHA) supplemented with 25 mg/L glucose-6-phosphate; [b] drug-free, pooled 24 h collected urine from healthy female volunteers; [c] pharmacodynamic outcome recorded at 72 h after fosfomycin administration, expect for testing in Artificial urine medium, which was made at 48 h due to media precipitation preventing the final assessment; ESBL, extended-spectrum beta-lactamase; MHB, Mueller-hinton broth; G6P, glucose-6-phosphate; HLR, high-level fosfomycin resistance (percentage calculated from the quantitative growth on MHA containing 512 mg/L fosfomycin, divided by growth on drug-free MHA); +++, HLR greater than 1%; ++, HLR from 0.01 to 1%; +, HLR less than 0.01%; insuff., indicates that the MIC was not reportable due to absence of visible growth in the drug-free control well (after 18 h incubation); — indicates not detected.

DYNAMIC FOSFOMYCIN PHARMACOKINETICS



Left: Fosfomycin concentration-time curve. Solid line represents the expected concentration time-curve from the mathematical simulation (± 15%). Right: Relationship between observed and targeted free-drug fosfomycin concentrations across all studies. Target fosfomycin concentrations were determined from the mathematical simulation.

GROWTH CURVES IN THE DIFFERENT MEDIA IN THE BLADDER INFECTION MODEL



Total growth (solid line) and resistant growth (dashed lines). Resistant growth determined by growth on Mueller-hinton agar with 512 mg/L fosfomycin. Dotted line represents the limit of detection (1.4 $\log_{10}$ cfu/mL). Testing in AUM was stopped early due to precipiation of the media within the model.

— BMD in MHB+G6P demonstrated ≥1-dilution higher MIC compared to agar dilution. Without G6P, MICs were ≥4-fold higher, except two E. coli (MIC 32 & 64mg/L) where MIC was unchanged. These isolates were killed in the dynamic model in all media.

— Fosfomycin static time-kill pharmacodynamics in pooled female urine are significantly different to testing in standard laboratory media (MHB + G6P). SHU is shown to be a good substitute for human urine, especially in those isolates that have a detectable HLR subpopulation.

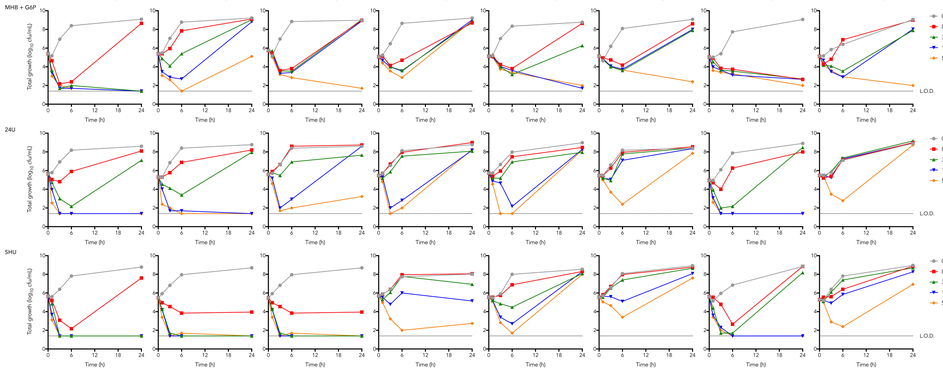
— Dynamic *in vitro* fosfomycin concentrations in the bladder infection model matched the simulation (accuracy 4.7 ±2.7%), with minimal variation (relative SD 4.4% ±3.0%).

— Overall, in all media the same 8-isolates (2 *E. coli*, 2 *E. cloacae*, 4 *K. pneumoniae*) re-grew and the same 4-isolates (4 *E. coli*) were killed. The remaining 4-isolates (2 *E. coli*, 2 *E. cloacae*) variably had minimal re-growth in urine and synthetic media.

— Emergence of high-level fosfomycin resistance (proportion >0.01%) was depended on the media (7/8 MHB+G6P; 6/10 MHB, 4/8 MSU; 5/10 24U; 0/9 AUM; 1/10 SHU).

STATIC TIME-KILL ASSAY RESULTS

COMPARATIVE PD OUTCOMES IN MHB (WITH G6P), POOLED 24H FEMALE URINE AND SYNTHETIC HUMAN URINE



Limit of detection was 1.4 $\log_{10}$ cfu/mL.

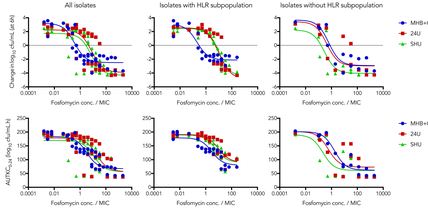
FOSFOMYCIN ACTIVITY IN URINE COMPARED TO MHB (WITH G6P) AND SHU

Strain selection	Media	Change in $\log_{10}$ cfu/mL at 6h				AUKC (0-24 h)			
		Top	IC_{50}	R^2	p-value	Top	IC_{50}	R^2	p-value
All isolates	24U	2.235	7.684	0.666	—	184	3.24	0.8297	—
	MHB+G6P	3.196	0.36	0.8202	<0.0001	178.7	19.13	0.5847	0.0414
	SHU	1.733	6.861	0.6835	0.5288	169.7	11.45	0.5165	0.5305
HLR detected	24U	2.543	10.9	0.763	—	179.1	2.944	0.7753	—
	MHB+G6P	3.05	0.2031	0.8727	<0.0001	191.1	9.46	0.7319	0.0005
	SHU	2.478	9.093	0.9101	0.971	189.7	5.873	0.7476	0.5733
HLR not detected	24U	3.439	0.4333	0.6254	—	201.4	1.748	0.9072	—
	MHB+G6P	3.674	0.6208	0.848	0.9306	201.8	0.5747	0.5373	0.7353
	SHU	2.233	0.2838	0.7152	0.2232	190.2	0.2187	0.484	0.5808

Left: Values determined from a sigmoid E_{max} non-linear regression model. Media were compared to 24 h pooled female urine to determine any significant differences in the average pharmacodynamic response.

Below: Change in $\log_{10}$ cfu/mL at 6 h, and the area under the time-kill curve over 24 h, of the time-kill assay. Sigmoid E_{max} non-linear regression line was determined for each media, with the fosfomycin exposure normalised to the baseline MIC of the pathogen tested.

PHARMCODYNAMIC RESPONSE AT 6 H AND 24 H



CONCLUSION

Synthetic human urine (SHU) serves well as a substitute for human urine to determine the efficacy of antimicrobials for the treatment of UTIs.

Emergence of fosfomycin resistance, however, appears to be restricted in both urine and synthetic alternatives, when compared to standard laboratory media.

This research highlights the importance of the make-up of the media in which antimicrobial susceptibliity and PK/PD experiments are performed.

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References

1. Brooks *et al*. Lett Appl Microbiol (1997)
2. Ipe *et al*. J Microbiol Methods (2016)
3. Abbott IJ *et al*. J Antimicrob Chemo (2018)

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