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Activity of Fosfomycin against Gram-Negative Baseline Bacterial Isolates from Patients in a Phase 3 Complicated Urinary Tract Infection Trial (ZEUS)

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Abstract

Background: ZTI-01 (fosfomycin, FOS, for injection) is an investigational antibiotic under US development to treat complicated urinary tract infections (cUTI). FOS is different than other antimicrobials in that it is thought to inhibit an early step in cell wall synthesis via covalent binding to MurA. FOS demonstrated broad *in vitro* activity against gram-negative (GN) and -positive (GP) bacteria, including organisms containing extended-spectrum β-lactamases (ESBL). In this study, we investigated the activity of FOS against GN clinical isolates from the phase 3 cUTI trial (ZEUS).

Methods: FOS was tested against 465 GN baseline clinical isolates collected in the cUTI clinical trial. Isolates were susceptibility (S) tested against comparator agents by reference broth microdilution and FOS by reference agar dilution (25 µg/mL glucose-6-phosphate supplementation). Existing FDA FOS breakpoints of ≤64 mg/L for the oral formulation were used for comparative assessments. Screen-positive ESBL *Enterobacteriaceae* isolates were characterized by whole genome sequencing and analysis for the presence of known β-lactamase genes.

Results: FOS was very active against *Enterobacteriaceae* ($MIC_{50/90}$, 1/16 mg/L; 96.4% ≤64 mg/L). For 329 *E. coli,* 100.0% were S to FOS ($MIC_{50/90}$, 1/1 mg/L), and for 62 *Klebsiella pneumoniae* the FOS $MIC_{50/90}$ values were 16/128 mg/L (87.1% ≤64 mg/L). The FOS $MIC_{50/90}$ values for 14 *Enterobacter cloacae* complex and 20 *Proteus mirabilis* were 16/128 and 2/32 mg/L, respectively. For 21 *Pseudomonas aeruginosa*, higher FOS $MIC_{50/90}$ were observed, 64/256 mg/L. Regarding the characterized ESBL-containing isolates, the FOS $MIC_{50/90}$ for 49 *E. coli* were 1/2 mg/L, for 28 *K. pneumoniae* were 16/128 mg/L, and for 3 *P. mirabilis* the MIC_{50} was 2 mg/L. The most common ESBL was CTX-M (42/49 *E. coli* and 27/28 *K. pneumoniae*), with CTX-M-15 (belonging to Group 1) the most common variant. Carbapenemase genes, NDM-1 and OXA-48, were detected in 2 *K. pneumoniae* isolates, which had FOS MICs of 8 and 64 mg/L, respectively.

Conclusions: FOS demonstrated broad-spectrum activity against baseline clinical isolates in a phase 3 cUTI trial, including those with ESBL or carbapenemases. FOS merits further study in infections where resistant GN may occur. Potentially introducing an IV form would warrant a reassessment of susceptibility breakpoints given the current oral formulation's bioavailability limitations.

Introduction

- ZTI-01 (fosfomycin, FOS, for injection) is under US development to treat complicated urinary tract infections (cUTI)
- Fosfomycin has been used for over 40 years to treat a variety of serious infections
- FOS is different than other antimicrobials in that it is thought to inhibit an early step in cell wall synthesis via covalent binding to MurA
- FOS has demonstrated broad *in vitro* activity against gram-negative (GN) and -positive (GP) bacteria, including organisms containing extended-spectrum β-lactamases (ESBL)
- In this study, we investigated the activity of FOS against GN clinical isolates from the phase 3 cUTI trial (ZEUS)

Materials and Methods

- FOS was tested against 465 GN baseline clinical isolates collected in the cUTI clinical trial
- Isolates were susceptibility (S) tested against comparator agents by Clinical and Laboratory Standards Institute (CLSI) reference broth microdilution and FOS by reference agar dilution (with 25 µg/mL glucose-6-phosphate supplementation)
- Existing FDA FOS breakpoints of ≤64 mg/L for the oral formulation were used for comparative assessments
  - CLSI and EUCAST interpretive criteria were used for comparators
- Screen-positive ESBL *Enterobacteriaceae* isolates were characterized by whole genome sequencing and analysis for the presence of known β-lactamase genes

Table 1 Antimicrobial activity of fosfomycin agar tested against the main organisms and organism groups of isolates (mg/L)

Organism/organism group (no. of isolates)	No. of isolates at MIC (mg/L; cumulative %)														MIC_{50}	MIC_{90}
	≤0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	512	>		
Enterobacteriaceae (440)	0 0.0	4 0.9	154 35.9	153 70.7	33 78.2	15 81.6	20 86.1	26 92.0	13 95.0	6 96.4	8 98.2	2 98.6	1 98.9	5 100.0	1	16
Carbapenem-resistant (4)						0 0.0	2 50.0	0 50.0	0 50.0	1 75.0	1 100.0				8	N/A
Escherichia coli (329)	0 0.0	4 1.2	151 47.1	148 92.1	23 99.1	2 99.7	0 99.7	0 99.7	1 100.0						1	1
Non-ESBL-phenotype (275)	0 0.0	3 1.1	129 48.0	123 92.7	19 99.6	1 100.0									1	1
ESBL-phenotype (54)	0 0.0	1 1.9	22 42.6	25 88.9	4 96.3	1 98.1	0 98.1	0 98.1	1 100.0						1	2
Klebsiella pneumoniae (62)			0 0.0	1 1.6	0 1.6	8 14.5	14 37.1	17 64.5	11 82.3	3 87.1	5 95.2	0 95.2	1 96.8	2 100.0	16	128
Non-ESBL-phenotype (29)					0 0.0	6 20.7	9 51.7	6 72.4	5 89.7	1 93.1	2 100.0				8	64
ESBL-phenotype (33)			0 0.0	1 3.0	0 3.0	2 9.1	5 24.2	11 57.6	6 75.8	2 81.8	3 90.9	0 90.9	1 93.9	2 100.0	16	128
Proteus mirabilis (20)		0 0.0	2 10.0	2 20.0	7 55.0	2 65.0	2 75.0	2 85.0	1 90.0	1 95.0	0 95.0	0 95.0	0 95.0	1 100.0	2	32
Non-ESBL-phenotype (17)		0 0.0	1 5.9	2 17.6	6 52.9	2 64.7	2 76.5	2 88.2	1 94.1	1 100.0					2	32
ESBL-phenotype (3)		0 0.0	1 33.3	0 33.3	1 66.7	0 66.7	0 66.7	0 66.7	0 66.7	0 66.7	0 66.7	0 66.7	0 66.7	1 100.0	2	N/A
Enterobacter cloacae species complex (14)			0 0.0	1 7.1	1 14.3	2 28.6	2 42.9	5 78.6	0 78.6	1 85.7	1 92.9	0 92.9	0 92.9	1 100.0	16	128
Pseudomonas aeruginosa (21)				0 0.0	1 4.8	1 9.5	0 9.5	0 9.5	4 28.6	9 71.4	3 85.7	1 90.5	1 95.2	1 100.0	64	256
Acinetobacter baumannii-calcoaceticus species complex (2)										0 0.0	2 100.0				128	N/A

Shaded column represents CLSI breakpoints for *E. coli* for oral formulation, for information

Results

- The most common organism isolated was *Escherichia coli* (n=329) followed by *Klebsiella pneumoniae* (n=62) and *Pseudomonas aeruginosa* (n=21)
- FOS was very active against *Enterobacteriaceae* ($MIC_{50/90}$, 1/16 mg/L; 96.4% ≤64 mg/L)
  - For 329 *E. coli,* 100.0% were S to FOS ($MIC_{50/90}$, 1/1 mg/L), and for 62 *K. pneumoniae*, the FOS $MIC_{50/90}$ values were 16/128 mg/L (87.1% ≤64 mg/L) (Table 1)
  - The FOS $MIC_{50/90}$ values for 14 *Enterobacter cloacae* complex and 20 *Proteus mirabilis* were 16/128 and 2/32 mg/L, respectively
  - For 21 *P. aeruginosa*, higher FOS $MIC_{50/90}$ values were observed (64/256 mg/L)
- Table 2 shows the activity of FOS and comparators against the enterics
  - The least active agents were tetracycline with 60.7%S and trimethoprim-sulfamethoxazole with 69.3%S (CLSI)
  - The most active agents tested were FOS, imipenem, meropenem, and amikacin with ≥95%S (EUCAST)
- The most common ESBL was CTX-M (42/49 *E. coli* and 27/28 *K. pneumoniae*), with CTX-M-15 (belonging to Group 1) the most common variant
- Table 3 shows the activity of FOS and comparators for the isolates with characterized ESBL for the 3 most common organisms: *E. coli, K. pneumoniae,* and *P. aeruginosa*
  - The FOS $MIC_{50/90}$ values for 49 *E. coli* were 1/2 mg/L, for 28 *K. pneumoniae* were 16/128 mg/L, and for 3 *P. mirabilis* the MIC_{50} value was 2 mg/L
  - For 11 *P. aeruginosa* the $MIC_{50/90}$ was 64/128 mg/L
- Carbapenemase genes NDM-1 and OXA-48 were detected in 2 *K. pneumoniae* isolates, which had FOS MIC values of 8 and 64 mg/L, respectively
- 1 *K. oxytoca* had KPC-2, with an MIC value of 8 mg/L

Table 3 Summary of MIC results obtained against main baseline pathogens that met the MIC-based screening criteria

Pathogen	Genotype (n)	Agent	MIC (mg/L)			
			50%	90%	Min.	Max.
E. coli	All (49)	Fosfomycin	1	2	0.25	32
		Piperacillin-tazobactam	2	32	1	>64
		Aztreonam	32	>32	1	>32
		Ceftazidime	16	>64	≤0.5	>64
		Meropenem	≤0.06	≤0.06	≤0.06	0.12
	CTX-M (42)	Fosfomycin	1	2	0.25	32
		Piperacillin-tazobactam	2	32	1	>64
		Aztreonam	32	>32	1	>32
		Ceftazidime	16	>64	≤0.5	>64
		Meropenem	≤0.06	≤0.06	≤0.06	0.12
K. pneumoniae	All (28)	Fosfomycin	16	128	1	>512
		Piperacillin-tazobactam	32	>64	1	>64
		Aztreonam	>32	>32	8	>32
		Ceftazidime	>64	>64	1	>64
		Meropenem	≤0.06	8	≤0.06	>8
	CTX-M (25)[a]	Fosfomycin	16	128	1	>512
		Piperacillin-tazobactam	16	>64	1	>64
		Aztreonam	>32	>32	8	>32
		Ceftazidime	>64	>64	1	>64
		Meropenem	≤0.06	2	≤0.06	>8
P. aeruginosa	All (11)	Fosfomycin	64	128	32	128
		Piperacillin-tazobactam	64	64	16	>64
		Ceftazidime	>64	>64	2	>64
		Meropenem	4	>8	1	>8

[a] Analysis excludes carbapenemase-producing isolates.

Table 2 Activity of fosfomycin agar and comparator antimicrobial agents when tested against 440 isolates of *Enterobacteriaceae*

Antimicrobial agent	MIC_{50} (mg/L)	MIC_{90} (mg/L)	Range (mg/L)	CLSI[a]			EUCAST[a]		
				%S	%I	%R	%S	%I	%R
Fosfomycin agar	1	16	0.25 to >512				95.0		5.0
Meropenem	≤0.06	≤0.06	≤0.06 to >8	98.9	0.2	0.9	99.1	0.5	0.5
Imipenem	0.12	0.25	≤0.06 to >8	95.9	2.3	1.8	98.2	1.4	0.5
Amikacin	2	4	≤0.5 to >64	96.8	0.5	2.7	95.9	0.9	3.2
Gentamicin	0.5	>8	≤0.06 to >8	87.5	0.7	11.8	86.1	1.4	12.5
Levofloxacin	≤0.06	>8	≤0.06 to >8	72.7	2.3	25.0	67.7	3.0	29.3
Tetracycline	2	>32	≤0.25 to >32	60.7	1.4	38.0			
Trimethoprim-sulfamethoxazole	≤0.25	>32	≤0.25 to >32	69.3		30.7	69.3	1.1	29.5
Ceftazidime	≤0.5	64	≤0.5 to >64	80.9	2.0	17.0	78.2	2.7	19.1
Aztreonam	≤0.25	>32	≤0.25 to >32	79.1	3.0	18.0	77.5	1.6	20.9
Piperacillin-tazobactam	2	16	≤0.5 to >64	90.0	3.6	6.4	88.2	1.8	10.0
Ceftriaxone	0.06	>2	≤0.015 to >2	76.8	1.6	21.6	76.8	1.6	21.6
Nitrofurantoin	16	128	4 to >128	75.2	6.4	18.4			

[a] Criteria as published by CLSI 2018. CLSI fosfomycin oral breakpoints used for information, and EUCAST 2018.
Organisms include: *Citrobacter amalonaticus/farmeri* (1), *C. freundii* species complex (1), *Enterobacter cloacae* species complex (14), *Escherichia coli* (329), *Klebsiella oxytoca* (4), *K. pneumoniae* (62), *Morganella morganii* (2), *Proteus mirabilis* (20), *Providencia rettgeri* (2), *Raoultella ornithinolytica* (2), *Serratia marcescens* (3)

Conclusions

- FOS demonstrated broad-spectrum activity against baseline clinical isolates in a phase 3 cUTI trial, including those with ESBL or carbapenemases
- FOS merits further study in infections where resistant GN may occur
- Potentially introducing an IV form of FOS would warrant a reassessment of susceptibility breakpoints given the current oral formulation's bioavailability limitations

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