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In Vitro Activity of ZTI-01 (Fosfomycin for Injection) against Contemporary Gram-negative and Gram-positive Isolates - A Comparison of Inter-method Testing

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Abstract

Background: ZTI-01 (FOS) is an epoxide IV antibiotic with a novel MOA active against Gram-negative (GN) and –positive (GP) organisms, including MDR organisms. The IV formulation is in clinical development in the USA for the treatment of complicated urinary tract infections. FOS susceptibility (S) testing is unique in that only agar dilution (AD) is recommended as the reference method by both CLSI and EUCAST.

Methods: A total of 354 GN and GP from USA medical centers (2014) were selected. A subset of 106 isolates were MIC tested against FOS by AD +/- 25 µg/mL glucose-6-phosphate [G-6-P], Etest® and disk diffusion. Additionally, all isolates were tested by broth microdilution (BMD) +/- 25 µg/mL G-6-P.

Results: The overall FOS $MIC_{50/90}$ (n=106) was 8/64 µg/mL by reference AD. FOS was active against *Escherichia coli* (n=17; $MIC_{50/90}$, 0.5/1 µg/mL) and Enterococci (n=21; $MIC_{50/90}$, 32/64 µg/mL). *Staphylococcus aureus* MIC values ranged from 1-16 µg/mL (n=9), *Enterobacter* spp. from 8-16 µg/mL (n=12), *Klebsiella* spp. from 4-32 µg/mL (n=9), *Proteus* and *Providencia* spp. from ≤0.12-64 µg/mL (n=11), and 6 *Serratia marcescens* from 4-16 µg/mL. A total of 55/106 (51.9%) Etest® MIC values were +/- one-dilution of the reference AD MIC value. Enterobacteriaceae (EB) Etest® MICs were generally higher than AD reference method. BMD (no G-6-P) MIC values were generally higher (60/106 [56.6%] ≥ 2 dilutions) than AD. Only *E. faecalis* and *E. coli* have CLSI MIC and disk diameter breakpoint criteria. All 17 *E. coli* were categorized as S by disk and MIC. However, two *E. faecalis* were falsely resistant by disk (major error). The significant effect of G-6-P was shown in broth when 354 isolates were tested in the presence of 25 µg/mL G-6-P. A total of 146/230 (63.5%) of EB showed MIC values ≤ 2 dilutions lower when tested with G-6-P.

Conclusions: FOS was highly active against all organisms tested. FOS MICs (reference AD) are lower than Etest® for some organisms. G-6-P lowers MIC values in broth and in agar but appears to be more pronounced for select organism groups. BMD MIC values in the presence of G-6-P correlate within +/- one dilution with reference AD a majority of the time, but differ by > 1 dilution depending on organism group. AD is the recommended reference method, with ETest® MICs typically >1-dilution of the reference value.

Introduction

Fosfomycin (FOS) is an epoxide antibiotic with a mode of action which exhibits bactericidal activity against Gram-negative (GN) and –positive (GP) organisms, including contemporary multidrug resistant organisms. Unlike available antibiotic classes, FOS's unique mechanism of action acts at an early step in bacterial cell wall biosynthesis by irreversibly binding to cytoplasmic enzyme uridine diphosphate N-acetylglucosamine (UDP-GlcNAc) enolpyruvyl transferase (MurA), the enzyme that catalyzes the first step in peptidoglycan biosynthesis, thereby disrupting cell wall synthesis and leading to rapid bacterial cell death. MurA is considered a safe antibiotic target, because human cells do not synthesize a similar enzyme.

The intravenous formulation of FOS (ZT1-01) is currently in clinical development in the USA for the treatment of complicated urinary tract infections (cUTI). A dosage of 6 grams every 8 hours (18g daily) is being evaluated for this therapeutic purpose. An oral formulation (fosfomycin tromethamine), is approved in the USA for single dose (3g) treatment of uncomplicated urinary tract infections. The oral formulation has limited therapeutic utility due to poor bioavailability (37%) and GI intolerance following multiple doses, resulting in the inability to achieve significant concentrations required for deep-seated, or difficult to treat infections. An intravenous formulation could reliably overcome this limitation.

Reference MIC susceptibility testing for fosfomycin is recommended by CLSI and EUCAST to be done by agar dilution as the broth MIC test is considered to be unreliable. Supplementation of agar with Glucose-6-Phosphate (G-6-P) is required for testing. G-6-P induces fosfomycin uptake by the hexose-6- phosphate transporter (UhpT), one of two main nutrient transport systems. The Etest® and the 200 µg (fosfomycin)/50 µg G-6-P disks are approved testing methods for fosfomycin in the USA. As agar dilution testing is considered cumbersome and more costly, this study was undertaken to compare reference agar dilution with broth microdilution, Etest® and disk diffusion testing.

Methods

Organisms:

- A total of 354 isolates collected from USA medical centers in 2014 which were isolated from infections including urine/urinary tract, bloodstream, respiratory tract, skin and skin structure and intra-abdominal infections were selected.
- Organisms included: *Escherichia coli* (51 isolates), *Enterococcus faecalis* (50 isolates), *E. faecium* (20 isolates), *Citrobacter koseri* (11 isolates), *C. freundii* (21 isolates), *Enterobacter aerogenes* (21 isolates) *E. cloacae* (21 isolates), *Klebsiella oxytoca* (11 isolates), *K. pneumoniae* (21 isolates), *Proteus mirabilis* (21 isolates), *P. vulgaris* (20 isolates), *Serratia marcescens* (21 isolates), *Providencia* spp. (6 isolates), *Morganella morganii* (5 isolates), *Staphylococcus aureus* (22 isolates; 11 MRSA), CoNS (6 strain), *Pseudomonas aeruginosa* (10 isolates), *Acinetobacter* spp. (11 isolates), *Stenotrophomonas maltophilia* (5 isolates).

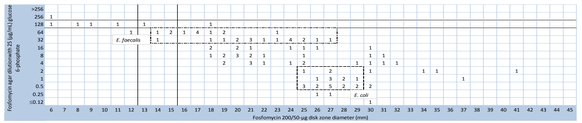
Susceptibility testing:

- Quality control (QC) for susceptibility testing was performed according to CLSI recommendations (M100-S26). QC organisms were tested concurrently with clinical isolates. MIC testing was done for fosfomycin for all isolates (354 isolates) by broth microdilution in cation-adjusted Mueller-Hinton broth with and without G-6-P supplementation.
- MIC testing with fosfomycin was done by agar dilution (AD) for 106 isolates according to CLSI methodology (M07-A10; Mueller-Hinton agar containing 25 µg/mL [G-6-P]). In addition, agar dilution with fosfomycin was performed using Mueller-Hinton agar following CLSI methodology, but without adding the recommended G-6-P.
- MIC testing for fosfomycin for 106 isolates was done by Etest® according to manufacturer's instructions. These strips contained G-6-P, so no G-6-P was added to the Mueller-Hinton agar.
- Disk diffusion for all 354 isolates was done according to CLSI methods (M02-A12) for fosfomycin using Mueller-Hinton agar. The fosfomycin disks were the CLSI recommended 200-µg fosfomycin/50-µg G-6-P (Becton-Dickinson) and disks containing 50-µg fosfomycin/50-µg G-6-P which have been used in Europe (Oxoid).

Results

- When tested by reference AD, the MIC_{90} for all organisms was two-fold lower than when tested by Etest® (64 µg/mL compared to 128 µg/mL (**Table 1**). By AD, the MIC_{90} for *E. coli* was 1 µg/mL and for *E. faecalis* it was 64 µg/mL (**Table 1**). By Etest®, the MIC_{90} for *E. coli* was 4 µg/mL and for *E. faecalis* it was 128 µg/mL (**Table 1**). A total of 41/106 (38.7%) of MIC values were ≥ 2 dilutions higher when tested with Etest®.
- When testing by AD, MIC values for Enterobacteriaceae and *S. aureus* were generally higher in the absence of G-6-P (**Table 2**). For the Enterobacteriaceae, 45/64 (70.3%) of isolates exhibited MIC values ≥ 3 dilutions higher when tested in the absence of G-6-P. A total of 8/9 (88.9%) *S. aureus* exhibited MIC values ≥ 2 dilutions higher when tested in the absence of G-6-P.
- Broth microdilution (0 µg/mL G-6-P) MIC values for Enterobacteriaceae were generally higher than for reference AD (**Table 3**). A total of 51/64 (79.7%) of MIC values were ≥ 2 dilutions higher than AD (**Table 3**). A total of 6/9 (66.7%) of *S. aureus* exhibited MIC values ≥ 2 dilutions higher (**Table 3**). Overall, only 46/106 (43.4%) of isolates exhibited broth microdilution MIC values that were within one-dilution of the AD MIC value (**Table 3**).
- Broth microdilution (25 µg/mL G-6-P) MIC values for Enterobacteriaceae were generally higher than when tested in AD with G-6-P (**Table 4**). A total of 16/64 (25.0%) of Enterobacteriaceae MIC values were ≥ 2 dilutions higher than AD (**Table 4**). Overall, 86/106 (81.1%) of isolates exhibited broth microdilution MIC values that were within one-dilution of the AD MIC value (**Table 4**).
- The addition of G-6-P (25 µg/mL) in broth microdilution generally lead to lower MIC values (**Table 5**). A total of 146/230 (63.5%) of Enterobacteriaceae showed MIC values at least 2 dilutions lower than in the absence of G-6-P (**Table 5**).
- **Figure 1** shows the correlation of reference AD MIC values and 200/50-µg disk zone diameters for fosfomycin. Only *E. faecalis* and *E. coli* have CLSI MIC and disk diffusion zone diameter breakpoints. All 17 *E. coli* were categorized as susceptible by disk and MIC. There were no major or minor errors. However, two *E. faecalis* were miscategorized as falsely resistant by the disk method (major error).

Figure 1. Scatter plot of fosfomycin MIC results generated by agar dilution method (25 µg/mL glucose-6-phosphate) vs. fosfomycin zone diameter using 200-µg disk (50 µg/mL glucose-6-phosphate).[a,b]



a. Calculated error rates.

All isolates					*E. coli* only					*E. faecalis* only				
		Error Rates					Error Rates					Error Rates		
Category	# Isolates	Very Major	Major	Minor	Category	# Isolates	Very Major	Major	Minor	Category	# Isolates	Very Major	Major	Minor
I+2	0	0	-	0	I+2	0	0	-	0	I+2	0	0	-	0
I+1 to I-1	20	0	1 (5.0)	8 (40.0)	I+1 to I-1	0	0	0	0	I+1 to I-1	2	0	0	1 (50.0)
I-2	86	-	0	4 (1.2)	I-2	17	-	0	0	I-2	13	-	0	0

b. The majority of data points for *E. coli* or *E. faecalis* were located in the respective box.

Table 1. Cumulative frequency distribution of fosfomycin MIC results generated by agar dilution (AD) with 25 µg/mL glucose-6-phosphate and Etest® methods.

Method/ Organism Group	No. of Isolates	MIC in µg/mL (cumulative %): ≤0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	>256	MIC_{50}	MIC_{90}
Agar Dilution																
All isolates[a]	106	1 (0.9)	2 (2.8)	16 (17.9)	8 (25.5)	7 (32.1)	13 (44.3)	12 (55.7)	9 (64.2)	18 (81.1)	13 (93.4)	6 (99.1)	1 (100.0)		8	64
E. coli	17			11 (64.7)	5 (94.1)	1 (100.0)									0.5	1
E. faecalis	15									13 (86.7)	2 (100.0)				32	64
Etest																
All isolates[a]	106	1 (0.9)	1 (1.9)	4 (5.7)	14 (18.9)	14 (32.1)	3 (34.9)	4 (38.7)	7 (45.3)	20 (64.2)	20 (83.0)	8 (90.6)	6 (94.3)	4 (100.0)	32	128
E. coli	17			1 (5.9)	9 (58.9)	5 (88.2)	2 (100.0)								1	4
E. faecalis	15									5 (33.3)	8 (86.7)	1 (93.3)	1 (100.0)		64	128

a. Includes 17 *Escherichia coli*, 8 *Citrobacter* spp., 12 *Enterobacter* spp., 9 *Klebsiella* spp., 1 *Morganella morganii*, 10 *Proteus* spp., 1 *Providencia rettgeri*, 6 *Serratia* spp., 5 *Pseudomonas aeruginosa*, 5 *Acinetobacter* spp., 9 *Staphylococcus aureus*, 2 coagulase-negative staphylococci, 15 *E. faecalis*, and 6 *E. faecium*.

Table 2. Fosfomycin MIC results generated by agar dilution (AD) without glucose-6-phosphate compared to reference AD with 25 µg/mL glucose-6-phosphate.

Organism	Total	Fosfomycin AD (0 µg/mL G-6-P) vs Fosfomycin AD (25 µg/mL G-6-P): -1[a]	0	1	2	3	4	5	6	7
Enterobacteriaceae	64[b]	2	7	10		4	7	13	14	7
Escherichia coli	17						1	2	9	5
other	47	2	7	10		4	6	11	5	2
Pseudomonas aeruginosa	5	1	4							
Acinetobacter spp.	5		5							
Staphylococcus aureus	9			1	4	4				
Coagulase-negative staphylococci	2		2							
Enterococcus faecalis	15		8	7						
Enterococcus faecium	6	1	3	2						
Total	106	4	29	20	4	8	7	13	14	7

a. Number of two-fold dilution steps greater than or less than reference agar dilution with 25 µg/mL glucose-6-phosphate.
b. Number of isolates. Enterobacteriaceae include 17 *Escherichia coli*, 8 *Citrobacter* spp., 12 *Enterobacter* spp., 9 *Klebsiella* spp., 1 *Morganella morganii*, 10 *Proteus* spp., 1 *Providencia rettgeri*, 6 *Serratia* spp.

Table 3. Fosfomycin MIC results generated by broth microdilution method (0 µg/mL glucose-6-phosphate) compared to agar dilution (AD) method (25 µg/mL glucose-6-phosphate).

Organism	Total	Fosfomycin BMD MIC (0 µg/mL G-6-P) vs Fosfomycin AD MIC (25 µg/mL G-6-P): -1[a]	0	1	2	3	4	5	6	7
Enterobacteriaceae	64[b]		5	8	6	6	9	12	11	7
Escherichia coli	17				2	2	4	2	4	3
other	47		5	8	4	4	5	10	7	4
Pseudomonas aeruginosa	5		4		1					
Acinetobacter spp.	5		1	2	2					
Staphylococcus aureus	9		1	2	1	4		1		
Coagulase-negative staphylococci	2	1		1						
Enterococcus faecalis	15	1	11	3						
Enterococcus faecium	6		4	2						
Total	106	2	26	18	10	10	9	13	11	7

a. Number of two-fold dilution steps greater than or less than reference agar dilution with 25 µg/mL glucose-6-phosphate.
b. Number of isolates. Enterobacteriaceae include 17 *Escherichia coli*, 8 *Citrobacter* spp., 12 *Enterobacter* spp., 9 *Klebsiella* spp., 1 *Morganella morganii*, 10 *Proteus* spp., 1 *Providencia rettgeri*, 6 *Serratia* spp.

Table 4. Fosfomycin MIC results generated by broth microdilution method (25 µg/mL glucose-6-phosphate) compared to agar dilution method (25 µg/mL glucose-6-phosphate).

Organism	Total	Fosfomycin BMD MIC (25 µg/mL G-6-P) vs Fosfomycin AD MIC (25 µg/mL G-6-P): -2[a]	-1	0	1	2	3	4
Enterobacteriaceae	64[b]		2	24	22	9	6	1
Escherichia coli	17			8	7	2		
other	47		2	16	15	7	6	1
Pseudomonas aeruginosa	5			4		1		
Acinetobacter spp.	5			1	2	2		
Staphylococcus aureus	9	1	4	3	1			
Coagulase-negative staphylococci	2		1		1			
Enterococcus faecalis	15		1	12	2			
Enterococcus faecium	6			5	1			
Total	106	1	8	49	29	12	6	1

a. Number of two-fold dilution steps greater than or less than reference agar dilution with 25 µg/mL glucose-6-phosphate.
b. Number of isolates. Enterobacteriaceae include 17 *Escherichia coli*, 8 *Citrobacter* spp., 12 *Enterobacter* spp., 9 *Klebsiella* spp., 1 *Morganella morganii*, 10 *Proteus* spp., 1 *Providencia rettgeri*, 6 *Serratia* spp.

Table 5. Fosfomycin MIC results generated by broth microdilution method (25 µg/mL glucose-6-phosphate) compared to broth microdilution method (0 µg/mL glucose-6-phosphate).

Organism	Total	Fosfomycin BMD MIC (25 µg/mL G-6-P) vs Fosfomycin BMD MIC (0 µg/mL G-6-P): -9[a]	-8	-7	-6	-5	-4	-3	-2	-1	0	1	2
Enterobacteriaceae	230[b]	1	1	4	23	26	44	32	15	33	46	4	1
Escherichia coli	51		1	3	9	8	6	11	4	6	3		
other	179	1		1	14	18	38	21	11	27	43	4	1
Pseudomonas aeruginosa	10										10		
Acinetobacter spp.	11									1	10		
Stenotrophomonas maltophilia	5									1	4		
Staphylococcus aureus	22						3	6	10	3			
Coagulase-negative staphylococci	6									1	5		
Enterococcus faecalis	50									4	45	1	
Enterococcus faecium	20									1	19		
Total	354	1	1	4	23	26	47	38	25	44	139	5	1

a. Number of two-fold dilution steps greater than or less than broth microdilution without glucose-6-phosphate.
b. Number of isolates. Enterobacteriaceae include 17 *Escherichia coli*, 8 *Citrobacter* spp., 12 *Enterobacter* spp., 9 *Klebsiella* spp., 1 *Morganella morganii*, 10 *Proteus* spp., 1 *Providencia rettgeri*, 6 *Serratia* spp.

Conclusions

- ZTI-01 (fosfomycin for injection) was active against all isolates tested.
- Agar dilution in Mueller-Hinton supplemented with 25 µg/mL G-6-P is the reference MIC method for testing fosfomycin. MIC values are generally higher when G-6-P is not included in the agar medium. The greatest differences were seen with Enterobacteriaceae.
- Etest® MIC results correlated with reference agar dilution MICs (+/- one dilution) 51.9% of the time when tested against a collection of 106 bacterial isolates. There was a bias toward elevated fosfomycin MIC values for Etest® for the organism groups tested; generally one to two dilutions higher. The exception was *S. aureus*. The MIC_{90} for a collection of 106 organisms was 64 µg/mL when tested by reference AD and 128 µg/mL when tested by Etest®.
- Cation-adjusted Mueller-Hinton broth microdilution supplemented with 50 µg/mL G-6-P correlated with reference agar dilution MICs (+/- one dilution) 81.1% of the time when tested against a collection of 106 bacterial isolates. There was a bias toward higher fosfomycin MIC values with broth microdilution; generally one to two dilutions higher.
- Correlation of fosfomycin reference agar MIC values and the CLSI recommended 200/50-µg disk showed that *E. coli* were categorized correctly without major or minor errors, however, for *E. faecalis* major errors did occur.

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