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Evaluation of Fosfomycin Activity When Combined with Selected Antimicrobial Agents and Tested against Bacterial Isolates Using Checkerboard Methods

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Amended Abstract

Background: ZTI-01 (fosfomycin; FOS) is an intravenous antibiotic under US development to treat complicated urinary tract infections (cUTI). Unlike other classes, FOS covalently binds to MurA, a precursor in bacterial cell wall synthesis. FOS has broad *in vitro* activity against gram-positive and -negative bacteria, including multidrug-resistant (MDR) organisms. Differing mode of action antibiotic combinations are frequently employed to treat concerning MDRs. Optimal FOS combinations producing synergy (SYN) and lacking antagonism (ANT) warrant this investigation.

Methods: Forty strains were evaluated: 5 *Staphylococcus aureus* (SA), 5 *Enterococcus faecalis* (EF), 5 *Pseudomonas aeruginosa* (PSA), 5 *Acinetobacter baumannii* (ACB), and 20 enterics, including clinical and ATCC strains. Interaction between FOS (with 25 µg/mL glucose-6-phosphate) and up to 10 combination agents was investigated by checkerboard broth microdilution methods against each species/group from a total of 16 antimicrobial agents. Summary fractional inhibitory concentration (∑FIC) values were calculated for each FOS/agent combination at the minimum, maximum, and mean. The ∑FIC was used to classify the combined activity as SYN (≤0.5), indifference (INDIF; >0.5 and ≤4), or ANT (>4). Indeterminate (INDET) category was assigned when unable to determine combination effects.

Results: FOS showed no ANT, but showed SYN when combined with multiple agents against isolates from all 5 species/groups. Highest SYN rates were seen when FOS was combined with piperacillin-tazobactam, cephalosporins, meropenem, or penicillin. All agents showed SYN rates of 8.0% to 50.0% when combined with FOS. Among INDIF isolates, 17.7% had ∑FIC >1 and ≤4; 18.5% had ∑FIC =1 (additive); 63.8% had ∑FIC >0.5 and <1 (partial SYN).

Organism (no. tested)	Combination agents	No. of strains by interpretive category (% of total) SYN	INDIF	ANT	INDET
SA (5)	10	16 (32.0)	28 (56.0)	0 (0.0)	6 (12.0)
EF (5)	7	7 (20.0)	27 (77.1)	0 (0.0)	1 (2.9)
PSA (5)	9	7 (15.6)	37 (82.2)	0 (0.0)	1 (2.2)
ACB (5)	8	12 (30.0)	24 (60.0)	0 (0.0)	4 (10.0)
Enteric (20)	10	57 (28.5)	116 (58.0)	0 (0.0)	27 (13.5)
All		99 (26.8)	232 (62.7)	0 (0.0)	39 (10.5)

Conclusions: Nearly 30% of all combinations with FOS were SYN (∑FIC ≤0.5) and 40% demonstrated partial SYN, which indicates combination therapy with FOS may be beneficial. Importantly, no ANT was observed with any of the FOS combinations.

Introduction

- Fosfomycin has been used to treat a variety of infections, including urinary tract, respiratory tract, and skin and skin structure infections and is available in an intravenous and oral formulation
- ZTI-01 (fosfomycin for injection) is administered as 6 grams every 8 hours for 7-14 days and is under development in the US to treat complicated urinary tract infections (https://clinicaltrials.gov/ct2/show/NCT02753946)
- Fosfomycin exhibits a broad spectrum of activity against gram-positive and gram-negative bacteria, including multidrug-resistant (MDR) organisms
- Unlike other antimicrobial classes, fosfomycin demonstrates a unique mode of action where it binds covalently to MurA where it inhibits the synthesis of peptidoglycan by blocking the formation of N-acetylmuramic acid
- Antimicrobial agent combinations with different modes of action are frequently used clinically to treat infections caused by multidrug-resistant organisms
- In the present study, we evaluated the activity of fosfomycin when combined with up to 10 antimicrobial agents from several classes to summarize fractional inhibitory concentration (∑FIC) values for selected isolates of *Staphylococcus aureus, Enterococcus faecalis, Pseudomonas aeruginosa, Acinetobacter baumannii-calcoaceticus* species complex, and *Enterobacteriaceae*

Materials and Methods

- Isolates tested included 35 gram-negative and gram-positive clinical isolates collected in 2015 as part of the global SENTRY Antimicrobial Surveillance Program and 5 ATCC strains
- Susceptibility testing for fosfomycin and combination agents was performed using broth microdilution checkerboard synergy methods as described in Clinical Microbiology Procedure Handbook, 4th Edition
  - Cation-adjusted Mueller-Hinton broth was supplemented with 25 mg/L glucose-6-phosphate following CLSI recommendations for testing fosfomycin alone and in combinations
- Checkerboard synergy panels were produced by JMI Laboratories (North Liberty, Iowa, USA) and stored at less than -70°C until use
- Formula used to calculate the summary fractional inhibitory concentration (∑FIC) value was:

$$\sum FIC = FIC_A \left(\frac{\text{MIC of agent A in combination}}{\text{MIC of agent A alone}}\right) + FIC_B \left(\frac{\text{MIC of agent B in combination}}{\text{MIC of agent B alone}}\right)$$

- The ∑FIC value for each well adjacent to growth in the checkerboard synergy panel was calculated and used to identify the ∑FIC minimum, ∑FIC maximum, and ∑FIC mean for each antimicrobial combination
- The FIC index categorical interpretations define synergy as when the value is ≤0.5, indifferent when the FIC index is >0.5 to ≤4.0, and antagonistic when the FIC index is >4.0
  - Alternatively, the FIC index can be defined as partial synergy when the FIC index is >0.5 to <1, additive when the FIC index is 1, and indifferent when the FIC index is >1 to ≤4.0
- Some combination interactions could not be determined due to the presence of off-scale MIC test results and a categorical result of indeterminate was recorded

Results

- Fosfomycin antimicrobial combinations against *S. aureus*
  - Among the 5 isolates (3 MRSA) and 10 antimicrobial agents, a total of 50 combinations with fosfomycin were tested and 32% showed synergy (Table 1)
  - Meropenem and linezolid exhibited synergy when tested with fosfomycin in 4/5 isolates
  - High rates of synergy also were seen when fosfomycin was combined with minocycline (3/5), piperacillin-tazobactam (2/5), and rifampin (2/5)
  - No antagonism was observed among combinations tested against *S. aureus*
- Fosfomycin antimicrobial combinations against *E. faecalis*
  - Synergy was observed in 20% of the 35 combinations tested with fosfomycin against the 5 *E. faecalis* isolates
  - Piperacillin-tazobactam showed synergy for 3/5 isolates while penicillin showed synergy for 2/5 isolates (Table 1)
  - For 19/35 (54%) antimicrobial combinations FIC_{min} was >0.5 to <1 and 5/35 (14%) combinations had FIC_{min} = 1, suggesting partial synergy or additive activity when combined with fosfomycin
  - Average FIC_{min} values for piperacillin-tazobactam, meropenem, and levofloxacin for the 5 *E. faecalis* isolates tested were 0.56, 0.59, and 0.70, respectively (Table 4)
- Fosfomycin antimicrobial combinations against *P. aeruginosa*
  - Fosfomycin tested in checkerboard synergy combinations with 9 antimicrobials showed synergy in 16% of the 45 total combinations
  - The most common synergistic combinations for fosfomycin were with ceftazidime (2/5 isolates) and minocycline (2/5; Table 2)
  - FIC_{min} >0.5 to <1 (partial synergy) was observed in 24/45 (53%) combinations, including 5/5 isolates for piperacillin-tazobactam (average FIC_{min} = 0.66; Table 2 and Table 4)
  - No antagonism was observed among combinations tested against *P. aeruginosa*
- Fosfomycin antimicrobial combinations against *A. baumannii-calcoaceticus* species complex
  - 30% of the 40 antimicrobial/isolate combinations tested with fosfomycin against *A. baumannii-calcoaceticus* species complex isolates showed synergy
  - Synergy was observed for piperacillin-tazobactam in 3/5 isolates and for amikacin, gentamicin, ceftazidime, and colistin in 2/5 isolates (Table 2)
  - Average FIC_{min} values for piperacillin-tazobactam, meropenem, and levofloxacin for the 5 *A. baumannii-calcoaceticus* species complex isolates was 0.51, 0.61 and 0.78, respectively (Table 4)
- Fosfomycin antimicrobial combinations against *Enterobacteriaceae*
  - Twenty isolates from 4 species groups were tested in checkerboard configurations with 10 antimicrobials where 29% of combinations showed synergistic interactions with fosfomycin
  - Most frequent interaction of synergy was seen for piperacillin-tazobactam 12/20 isolates followed by meropenem and ceftazidime, 8/20 isolates and then levofloxacin and gentamicin, 6/20 isolates (Table 2)
  - Average FIC_{min} values for the 4 groups of *Enterobacteriaceae* isolates showed variability among piperacillin-tazobactam, meropenem, and levofloxacin with results between 0.44-0.54, 0.47-0.70, and 0.53-0.84, respectively (Table 4)
  - No antagonism was observed among combinations tested against *Enterobacteriaceae*

Table 1 Summary of categorical interactions for antimicrobial agents when tested in checkerboard combinations with fosfomycin for gram positive isolates

	S. aureus						*E. faecalis*					
	SYN	PS	INDIF[a] ADD	INDIF[b]	ANTAG	INDET	SYN	PS	INDIF[a] ADD	INDIF[b]	ANTAG	INDET
Antimicrobial agent												
Gentamicin		4		1			—[c]	—	—	—	—	—
Ceftriaxone	1	1	1			2	—	—	—	—	—	—
Penicillin	—	—	—	—	—	—	2	2	1			
Piperacillin-tazobactam	2		1			2	3	2				
Meropenem	4					1	1	4				
Levofloxacin		4		1				4	1			
Minocycline	3	1	1					3	2			
Linezolid	4	1						2		3		
Rifampin	2	1	1			1	—	—	—	—	—	—
Trimethoprim-sulfamethoxazole		5					—	—	—	—	—	—
Vancomycin		3	1	1			1	2	1			1
Total	16 32%	20 40%	5 10% 56%	3 6%	0 0%	6 12%	7 20%	19 54%	5 14% 77%	3 9%	0 0%	1 3%

[a] INDIF (>0.5 to ≤4) category broken down into PS, partial synergy (>0.5 to <1); ADD, additive (1); INDIF, indifferent (>1 to ≤4)
[b] INDIF (>1 to ≤4)
[c] -, not tested

Table 2 Summary of categorical interactions for antimicrobial agents when tested in checkerboard combinations with fosfomycin for gram-negative isolates

	P. aeruginosa						*A. baumannii-calcoaceticus* species complex						*Enterobacteriaceae*					
	SYN	PS	INDIF[a] ADD	INDIF[b]	ANTAG	INDET	SYN	PS	INDIF[a] ADD	INDIF[b]	ANTAG	INDET	SYN	PS	INDIF[a] ADD	INDIF[b]	ANTAG	INDET
Antimicrobial agent																		
Amikacin	—	3	1	1			2	1	2				—[c]	—	—	—	—	—
Gentamicin		1	3	1			2	3					6	11	2	1		
Aztreonam	1	3				1	—	—	—	—	—	—	5	5	1			9
Ceftazidime	2	3					2	1		1		1	8	9	1			2
Piperacillin-tazobactam		5					3		1			1	12	5	2			1
Meropenem	1	2	1	1			1	2	1			1	8	9	1			2
Levofloxacin	1	3	1					3	1	1			6	8	4	2		
Minocycline	2	3					—	—	—	—	—	—	4	7	6	1		2
Tigecycline	—	—	—	—	—	—	—	2	2	1			5	9	1	5		
Colistin		1		4			2	1		1		1	1	5		8		6
Trimethoprim-sulfamethoxazole	—	—	—	—	—	—	—	—	—	—	—	—	2	4	2	7		5
Total	7 16%	24 53%	6 13% 56%	7 16%	0 0%	1 2%	12 30%	13 33%	7 17% 60%	4 10%	0 0%	4 10%	57 29%	72 36%	20 10% 58%	24 12%	0 0%	27 13%

[a] INDIF (>0.5 to ≤4) category broken down into PS, partial synergy (>0.5 to <1); ADD, additive (1); INDIFF, indifferent (>1 to ≤4)
[b] INDIF (>1 to ≤4)
[c] -, not tested

Table 3 Summary of categorical interactions for antimicrobial agents when tested in checkerboard combinations with fosfomycin for *Enterobacteriaceae*

	Enterobacter spp.						*E. coli*						*K. pneumoniae*						*P. mirabilis*					
	SYN	PS	INDIF[a] ADD	INDIF[b]	ANTAG	INDET	SYN	PS	INDIF[a] ADD	INDIF[b]	ANTAG	INDET	SYN	PS	INDIF[a] ADD	INDIF[b]	ANTAG	INDET	SYN	PS	INDIF[a] ADD	INDIF[b]	ANTAG	INDET
Antimicrobial agent																								
Gentamicin	2	3				1	4						1	3	1				2	1	1	1		
Aztreonam	5						4	1						1			4							5
Ceftazidime	4				1	3	2							4					1	3	1			
Piperacillin-tazobactam	2	3				3	1	1					4		1					3	1			1
Meropenem	3	2				1	3				1			4	1					4				1
Levofloxacin	2	3				1	1	2	1				2	2		1			1	2	2			
Minocycline		4			1		3	1	1						4				1	4	1			
Tigecycline	2	2	1			2	2		1					2	1	2				3		1		
Colistin		2	2		1	1	2		2					1		4								5
Trimethoprim-sulfamethoxazole	1	1	1			1		2	2		1			1		2		2		2		2		1
Total	21	20	1	4	0	4	13	22	6	7	0	2	7	18	8	9	0	8	16	12	5	4	0	13
	42%	40%	2% 50%	8%	0%	8%	26%	44%	12% 70%	14%	0%	4%	14%	36%	16% 70%	18%	0%	16%	32%	24%	10% 42%	8%	0%	26%

[a] INDIF (>0.5 to ≤4) category broken down into PS, partial synergy (>0.5 to <1); ADD, additive (1); INDIF, indifferent (>1 to ≤4)
[b] INDIF (>1 to ≤4)

Table 4 Average minimum, maximum, and mean calculated FIC values for selected antimicrobial agents when tested in checkerboard combinations

	No.	Piperacillin-tazobactam FIC			Meropenem FIC			Levofloxacin FIC		
Organism group	strains	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.	Mean
S. aureus	5	0.59	0.89	0.70	0.38	0.53	0.46	0.79	1.51	1.07
E. faecalis	5	0.56	1.15	0.86	0.59	1.01	0.78	0.70	1.38	1.04
P. aeruginosa	5	0.66	1.73	1.23	0.84	1.56	1.14	0.73	1.33	0.96
A. baumannii-calcoaceticus species complex	5	0.51	1.43	0.80	0.61	0.94	0.78	0.78	1.61	1.08
Enterobacteriaceae	20	0.51	1.11	0.74	0.53	1.16	0.86	0.69	1.45	1.00
Enterobacter spp.	5	0.51	1.36	0.81	0.47	1.08	0.86	0.53	1.28	0.86
E. coli	5	0.54	1.19	0.77	0.56	1.05	0.81	0.84	1.24	1.06
K. pneumoniae	5	0.44	1.10	0.71	0.70	1.69	1.15	0.65	1.70	1.03
P. mirabilis	5	0.53	0.72	0.63	0.39	0.74	0.57	0.73	1.58	1.04

Conclusions

- Fosfomycin tested in combination with a variety of currently used antimicrobial agents demonstrated synergy for 26.8% of the 370 organism/combinations tested
- More than 82% of isolates categorized as indifferent by ASM guidelines, showed partial or additive activity (63.8% had an FIC result >0.5 and <1, suggesting partial synergy and 18.5% had an FIC =1, suggesting additive activity)
- No instances of antagonism were observed among the 370 organism/antimicrobial agent combinations tested
- The highest rates of synergy across isolates were seen when fosfomycin was combined with piperacillin-tazobactam (50%), ceftazidime (40%), meropenem (37.5%), rifampin (40%), linezolid (40%), or penicillin (40%); other agents showed synergy rates of 8.0% to 25.7% when combined with fosfomycin
- For *Pseudomonas* isolates, highest rates of synergy and partial synergy were ceftazidime and minocycline combinations with fosfomycin at rates of 40% and 60%
- For *Acinetobacter* isolates, highest rates of synergy, partial synergy, or additivity were piperacillin-tazobactam, gentamicin, and amikacin combinations with fosfomycin at rates of 60/40/40%, 0/60/20%, and 20/0/40%, respectively
- The high percentage of synergy or partial synergy/additive combinations observed suggests enhanced activity may occur when fosfomycin is used in combination therapy

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