676: Synergistic Bacteriostatic and Bactericidal Activity of Fosfomycin Plus Trimethoprim-Sulfamethoxazole Combination Against Carbapenem-Resistant Enterobacteriales, Multidrug-Resistant *P. aeruginosa*, Carbapenem-Colistin-Resistant *A. baumannii,* MRSA, and *Enterococcus spp.*

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ABSTRACT

BACKGROUND: Fosfomycin (F) inhibits the first committed step of peptidoglycan biosynthesis catalyzed by the MurA enzyme which uses phosphoenolpyruvate as a substrate. Trimethoprim and sulfamethoxazole (TS) inhibit successive steps in folate biosynthesis and are commonly used as a fixed combination. In this study we explored the combination (FTS) of F with TS in several gram-negative and gram-positive bacteria of increasing concern.

METHODS: We used the synergy-checkerboard variant of the agar minimal inhibitory concentration (MIC) assay with Mueller-Hinton Agar (glucose-6-phosphate supplemented; CLSI) against a selection of *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii, methicillin-resistant Staphylococcus aureus* (MRSA), and *Enterococcus spp*. clinical isolates to characterize an FTS combination. Component antibiotics, F and TS, and a variety of other antibacterial comparators were evaluated by agar method or broth microdilution. Bactericidal assays of FTS were conducted in broth.

RESULTS: Antibacterial synergy with FTS was exhibited against all *E. coli* (22/22) at antibacterial concentrations below the individual clinical breakpoints for F and TS. Against *K. pneumoniae* isolates, all (13/13) that were resistant to F, and many (10/13) resistant to both F and TS, were susceptible to FTS. All *P. aeruginosa* isolates (18/18) were dually resistant to F and TS. Extrapolating breakpoints for Enterobacteriales, clinically-relevant synergy of FTS was exerted against 11/18 isolates, and susceptibility was achievable for one component in combination against 7/18 isolates. Against *A. baumannii*, susceptibility values below extrapolated breakpoints for FTS were achieved for most isolates (17/19), and for a few (2/19), susceptibility was observed for one component in combination. FTS was active against all MRSA and *Enterococcus spp*. regardless of F or TS susceptibility.

CONCLUSION: The unique synergistic and bactericidal activity of the FTS combination was not impacted by resistance of the tested species to any other antibacterial agent including carbapenem-resistant Enterobacteriales, *P. aeruginosa*, carbapenem-colistin-resistant *A. baumannii,* MRSA, or *Enterococcus spp.*

INTRODUCTION

Lacking timely development of effective novel antibacterials, full and clever exploitation of older therapeutics may partially address the gap resulting from evolution of resistance. A common approach is to administer antibacterial combinations empirically with the hope for adequate spectrum coverage and antibacterial synergy. However, inadequate scientific evidence underlying selection of the combination components often dashes these hopes. In addition, appropriate utilization of combinations of older agents can aid with antibiotic stewardship in slowing rapid develop of newer important therapeutics and their untimely obsolescence. We are applying an approach of targeting bacterial convergent metabolic pathways and biochemical events with an expectation of achieving synergistic therapeutic activity. We have selected the antibacterial fosfomycin which is an older agent often referred to as an "underutilized gem"[1] to combine with trimethoprim-sulfamethoxazole (3, ,5, 6, 7). Trimethoprim-sulfamethoxazole inhibits folate synthesis in bacteria resulting in depletion of metabolite pools from a number of pathways including phosphoenolpyruvate, the substrate of the MurA enzyme which is the target for fosfomycin. We report here the *in-vitro* findings and significance of our studies with this novel fosfomycin-trimethoprim-sulfamethoxazole combination (6).

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RESULTS

Table 1. Susceptibility of bacterial species to fosfomycin (F; FOF), trimethoprim-sulfamethoxazole (TS; SXT), the fosfomycin-trimethoprim-sulfamethoxazole combination (FTS), and comparators.

***Escherichia coli* (N=22)**																
	F (FOF)	TS (SXT)	FTS (FOF/SXT)	CR	CAZ	CZA	PIP	TZP	MEM	CIP	TIG	AMK	TOB	GEN	PMB	CST
Range	2->128	0.25/4.75->32/>608	0.5-16/0.015-2/0.3-38	<0.06->64	<0.06->64	<0.06-8	2->64	4->64	<0.06-64	<0.06->64	<0.125-1	4->64	0.5->64	1->64	0.25-1	0.125-0.25
MIC50	8	0.25/4.75	1/0.15/0.3	>64	>64	0.5	>64	32	<0.06	32	0.25	4	32	2	0.25	0.125
MIC90	128	>32/>608	4/0.015/0.3	>64	>64	1	>64	>64	0.125	>64	0.5	8	32	>64	0.5	0.25
***Klebsiella pneumoniae* (N=13)**																
	F (FOF)	TS (SXT)	FTS (FOF/SXT)	CRO	CAZ	CZA	PIP	TZP	MEM	CIP	TIG	AMK	TOB	GEN	PMB	CST
Range	128->128	0.5-9.5->32/>608	1-32/0.015-4/0.3/76	>64	>64	0.25->64	>64	16->64	<0.06->64	<0.06->64	0.125-8	1-64	1->64	1->64	0.5-16	0.125-16
MIC50	>128	27851	32/1/19	>64	>64	1	>64	>64	<0.06	4	2	2	8	2	0.5	0.25
MIC90	>128	>32/>608	32/2/38	>64	>64	2	>64	>64	16	64	8	32	64	>64	8	0.5
***Pseudomonas aeruginosa* (N=18)**																
	F (FOF)	TS (SXT)	FTS (FOF/SXT)	CAZ	CAZ/AVI	PIP	TZP	MEM	CIP	TIG	AMK	TOB	GEN	PMB	CST	
Range	128->128	>32/>608	2-64/0.5-16/9.5/304	1->64	1->64	0.5->64	0.5->64	<0.06->64	<0.06->64	8-32	1->64	0.5->64	2->64	0.25-4	0.5-1	
MIC50	>128	>32/>608	32/1/19	64	2	>64	4	32	16	8	32	>64	2	1	0.5	
MIC90	>128	>32/>608	64/4/76	>64	32	>64	>64	>64	32	16	>64	>64	64	1	1	
***Acinetobacter baumannii* (N=19)**																
	F (FOF)	TS (SXT)	FTS (FOF/SXT)	CAZ	CZA	PIP	TZP	MEM	CIP	TIG	AMK	TOB	GEN	PMB	CST	
Range	64->128	0.25-4.75->32/>608	0.5-64/0.015-4/0.3-76	2->64	2->64	4->64	4->64	0.25->64	0.25->64	1-8	1->64	0.5->64	1->64	0.25-8	0.25-64	
MIC50	128	>32/>608	32/0.03/0.6	>64	64	>64	>64	64	>64	4	>64	>64	>64	0.5	0.5	
MIC90	>128	>32/>608	64/2/38	>64	>64	>64	>64	>64	>64	8	>64	>64	>64	1	1	
***Staphylococcus aureus* (N-18)**																
	F (FOF)	TS (SXT)	FTS (FOF/SXT)	VAN	LZD	TET										
Range	1-16	0.06/1.19-0.25/4.75	0.25-4/0.0078-0.03/0.0078-0.6	0.5-1	0..5-2	<0.06-32										
MIC50	8	0.125/2.38	1/0.03/0.6	0.5	1	0.125										
MIC90	16	0.125/2.38	2/0.03/0.6	1	2	0.125										
***Enterococcus spp.* (N-19)**																
	F (FOF)	TS (SXT)	FTS (FOF/SXT)	AMP	VAN	GEN	LVX	LZD	DAP							
Range	64->128	0.03-32/608	0.06'0.015/0.3-64/2/38	1->8	1->16	4->16	1->4	1->8	0.5-4							
MIC50	128	16/304	16/1/19	>8	>16	8	>4	2	2							
MIC90	128	32/608	32/2/38	>8	>16	>16	>4	2	4							

Abbreviations: AMK, amikacin; AMP. Ampicillin; CAZ, ceftazidime; CIP. Ciprofloxacin; CRO, ceftriaxone; CST, colistin; CZA, ceftazidime-avinactam; DAP, daptomycin; FOF, Fosfomycin; GEN, gentamicin; LVX, levofloxazin; LZD, linezolid; MEM, meropenem; PIP, piperacillin; PMB, polymyxin B; SXT, trimethoprim-sulfamethoxazole; TET, tetracycline; TIG, tigecycline; TOB, tobramycin; TZP, piperacillin-tazobactamVan, vancomycin

Table 2. Susceptibility, bactericidal activity, and synergy of Fosfomycin (F), trimethoprim-sulfamethoxazole (TS), the fosfomycin-trimethoprim-sulfamethoxazole (FTS) combination against individual bacterial strains

	MIC (μg/ml)[a]; MBC (μg/ml)[b]				
	F; FOF	TS; SXT	FTS; FOF-SXT	FIC[c]	Interpretation[d]
Bacteria					
***Escherichia coli* ATCC 25922**	8; 32	0.5/9.5; 2/38	0.03/0.125/2.38; 0.12/0.5/9.5	0.25	Synergy
***Escherichia coli* 854535**	64; 256	8/152; 16/304	16/2/38; 64/8/152	0.5	Synergy
***Escherichia coli* 928017**	64; 256	0.25/4.75; 2/38	16/0.03/0.6; 64/0.25/4.75	0.38	Synergy
***Pseudomonas aeruginosa* 924190**	256; >512	4/76; 16/304	32/1/19; 64/4/76	0.38	Synergy
***Pseudomonas aeruginosa* 985543**	128; 512	2/38; 16/304	128/2/38	0.5	Synergy
***Staphylococcus aureus* ATCC 29213**	32; 128	0.5/9.5; 2/38	0.25/0.125/2.38; 1/0.5/9.5	0.26	Synergy
***Staphylococcus aureus* ATCC 33591**	64; 256	2/38; 8/152	8/0.25/4.75; 32/1/19	0.25	Synergy
***Staphylococcus aureus* USA-300 CDC**	64; 256	0.5/9.5; 2/38	16/0.125/2.38; 16/0.5/9.5	0.5	Synergy
***Staphylococcus aureus* 918019**	32; 128	0.5/9.5; 2/38	16/0.5/9.5; 16/0.5/9.5	0.38	Synergy
***Staphylococcus aureus* 959797**	16; 64	2/38; 8/152	2/0.25/4.75; 8/1/19	0.25	Synergy
***Enterococcus faecalis* ATCC 29212**	64	1/19; 16/304	16/0.25/4.75; 64/4/76	0.5	Synergy
***Enterococcus faecalis* MGH-01 (VanA)**	256	16/304; 32/608	64/2/38; 128/4/76	0.38	Synergy
***Enterococcus faecalis* MGH-06**	128	4/76; 16/304	32/0.5/9.5; 64/1/19	0.38	Synergy

Note: [a]MIC, minimal inhibitory concentration; [b]MBC, minimal bactericidal concentration; [c]FIC, fractioanal inhibitory concentration; and Inter[retation, FIC of ≤ 0.5 μg/ml is defined as synergy.

MATERIALS & METHODS

Bacterial reference strains and clinical isolates were kindly provided by the American Type Culture Collection (ATCC; Manassas, VA USA), International Health Management Associates (IHMA; Schaumberg, IL, USA), the U.S. Center for Disease Control, and the Massachusetts General Hospital. Frozen cultures were aseptically prepared from bacteria cultured in Trypticase soy broth (BD; Becton-Dickinson, Baltimore, MD USA), resuspended in the same medium containing 30% glycerol, frozen on dry ice, and stored frozen at -80ºC. To propagate bacteria for susceptibility assay, a small amount of frozen culture was transferred onto a Mueller-Hinton Agar plate, streaked for isolation, incubated at 35ºC, and typical colony morphology was observed. Overnight cultures were propagated either in cation-adjusted Mueller-Hinton II Broth or on Mueller-Hinton Agar (BD). Innocula were prepared by diluting bacteria from the overnight culture to the equivalent of a 0.5 McFarland standard via turbidimetric method. Antibiotic susceptibility was determined via agar or broth microdilution method according to a guideline assay (1). The medium was supplemented with glucose-6-phosphate (25 mg/Liter). For evaluation of the fosfomycin-trimethoprim-sulfamethoxazole combination, the synergy checkerboard variant of the agar dilution method was utilized(2). Antibiotics were evaluated either alone, or the combination of the different components was supplemented into the medium. Trimethoprim-sulfamethoxazole was utilized at a fixed ratio of 1:19. Concentration ranges for fosfomycin were 0-128 ug/ml, and for trimethoprim-sulfamethoxazole of 0-32/608 ug/ml. Bacterial growth was observed visibly after incubation according to the guideline assay. The minimal inhibitory concentration (MIC) was recorded as the lowest concentration of antibiotics inhibiting bacterial growth. Antibacterial synergy was determined based on the fractional inhibitory concentration (FIC) value (2). An FIC of ≤0.5 was indicative of synergy. In the comparator data table where a breakpoint is available, isolates that are resistant are highlighted in red (2, 3, 4). The minimal bactericidal concentration (MBC) was determined by conducting the MIC assay in cation-adjusted Mueller-Hinton broth supplemented with Glucose-6-phosphate and comparing plate counts from antibiotic exposed bacteria with the inocula counts, with the MBC defined as the lowest antibiotic concentration able to effect a 3-log or greater reduction in viable bacteria after 24-hours incubation.

CONCLUSION

- The novel combination of **fosfomycin-trimethoprim-sulfamethoxazole exerted potent antibacterial synergy and bactericidal activity** against the bacterial isolates tested (5).
- **Clinically relevant *in-vitro* susceptibility** to the unique combination was achievable against most isolates of multidrug-resistant bacteria, **including from those resistant and intrinsically resistant** species that are not susceptible to either fosfomycin, trimethoprim-sulfamethoxazole, or dually resistant to both components individually of the combination.
- All bacterial isolates regardless of resistance to cephalosporins, beta-lactam/beta-lactamase inhibitor combination, carbapenem, aminoglycosides, quinolones, or colistin were susceptible to the fosfomycin-trimethoprim-sulfamethoxazole combination.
- Early development work is underway to determine an **optimal dosing regimen** based on pharmacodynamics and to develop a **co-formulation** of the promising combined therapeutic.