**Time Kill Analyses of Concerning Gram-negative Bacteria (GNB) by Fosfomycin Alone and in Combination with Select Antimicrobial Agents**

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**Background**: ZTI-01 (fosfomycin, FOS, for injection) demonstrates broad spectrum activity *in vitro* including multidrug-resistant (MDR) organisms. FOS shows no cross-resistance to other antibiotic classes, and FOS mechanism of action uniquely inhibits an early step in peptidoglycan biosynthesis. Other antibiotic agents in combination with FOS have been proposed to enhance bacterial killing of MDR organisms. Time-kill kinetic analyses (TKK) were performed on select bacteria that demonstrated synergy when tested by checkerboard analysis with FOS and comparator agents.

**Methods**: Broth microdilution for FOS (Mueller-Hinton broth supplemented with 25 µg/mL glucose-6-phosphate) and comparators was performed before performing TKK. TKK employed MIC multiples for FOS, and ¼ and 1X MIC of select comparators, and combinations of FOS and comparator. TKK were sampled for colony counts at $T_0$, $T_2$, $T_4$, $T_8$ and $T_{24}$ hours (h). Two *Klebsiella pneumoniae* isolates (one KPC and one ESBL), two *Pseudomonas aeruginosa* isolates (non-MDR), and one *Acinetobacter baumannii* isolate (MDR) were tested.

**Results**: FOS was bactericidal when tested against a *K. pneumoniae* (KPC-producer) isolate. A >3 $\log_{10}$ reduction in bacterial growth (colony forming units, CFU) occurred by 4h at 2X MIC. By 24h with FOS (0.5, 1, and 2X MIC), bacterial growth increased approximately 2 $\log_{10}$. Piperacillin-tazobactam (PTZ) at ¼ and 1X MIC showed little inhibitory activity. At 24h, bacterial growth was similar to growth control. FOS at 0.5, 1, and 2X MIC in combination with PTZ (¼ and 1X MIC) showed synergy with approximately a 3.8-4.2 $\log_{10}$ reduction at 4h and a 3.4-5.4 $\log_{10}$ reduction at 24h. FOS (1, 2, 4X MIC) showed a slight decrease (1.4-2.2 $\log_{10}$ CFU) at 4h and by 24h, growth was similar to growth control when tested against an ESBL-producing *K. pneumoniae* isolate. For ceftazidime (CAZ) at ¼ and 1X MIC, there was a slight decrease (0.9-2.1 $\log_{10}$ CFU) by 4-8h, and at 24h CFU were similar to growth control. FOS (1, 2, and 4X MIC) in combination with CAZ showed synergy with a 3.8-4.3 $\log_{10}$ reduction by 8h (1X CAZ MIC) and at least a 5.1 $\log_{10}$ reduction at 24h (1/4 and 1X CAZ MIC). FOS activity was shown to be synergistic at 24h when tested at 2, 4 and 8X MIC with either ¼ or 1X CAZ MIC (*P. aeruginosa* #893949) or ¼ or 1X MIC of meropenem (MEM; *P. aeruginosa* #889839). Against *A. baumannii*, FOS was shown to be synergistic at 24h when tested at 0.5, 1 and 2X MIC with MEM (1X MIC).

**Conclusions**: The combination of two cell wall active agents, FOS plus selected β-lactams, provided enhanced killing and *in vitro* synergy against concerning GNB.

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