ZTI-01 (Fosfomycin (FOS) for injection) activity against carbapenem-resistant and ceftazidime-avibactam resistant Enterobacteriaceae (CRE) clinical isolates with diverse resistance mechanisms

M. Hong Nguyen, Liang Chen, Binghua Hao, Ryan Shields, B Kreiswirth, Cornelius J Clancy

University of Pittsburgh

Background: Ceftazidime-avibactam (C-A) is an important advance against CRE infections, but lacks activity against class B- and C-carbapenemases. We recently reported C-A resistance (R) due to *bla*$_{KPC-3}$ mutations in ~10% of KPC-*K. pneumoniae* (KPC-Kp)-infected patients treated at our center, and showed that C-A activity was reduced against KPC-Kp with *ompK36* porin mutations. FOS is a cell wall synthesis inhibitor with bactericidal activity against Enterobacteriaceae. We evaluated *in vitro* activity of FOS against 50 clinical CRE isolates in which whole genome sequencing (WGS) was performed.

Methods: FOS susceptibility was tested using agar dilution in Mueller-Hinton supplemented with 25 µg/mL glucose-6-phosphate. Existing breakpoints for oral FOS were used for comparison. MIC>64 µg/mL defined FOS-R. WGS was performed using Illumina MiSeq.

Results: 50 isolates were tested: 42 Kp (34 KPC, 3 NDM-1, 4 class C-carbapenemase (2 OXA-46, 1 each OXA-181 and OXA-232), and 1 with ESBL and ompK36 mutation), 5 *Enterobacter* spp (all KPC, 1 also NDM-1), 2 *E. coli* (1 NDM-1 and 1 NDM-6), and 1 *K. oxytoca* (KPC-2 and VIM-1). 14 isolates were C-A R: 5 NDM-1, 1 NDM-6, 1 VIM-1, 2 wild-type KPC-3, 5 mutant KPC-3 (D179Y substitution (n=2), D179Y/T243M (n=2) and V240G (or KPC-8, n=1)). 72% (36) and 40% (20) had *ompK35* and *ompK36* mutations, respectively. FOS MIC_{50} and MIC_{90} were 2 and 512 µg/mL, respectively. 94% (47) of isolates were susceptible to FOS, including all C-A R isolates. Median FOS MIC against C-A R isolates was 8 µg/mL (range: 1-64). 3 isolates harbored genes encoding FOS-modifying enzyme fosA2, 1 of which was FOS R (MIC 256 µg/mL). The R isolate also had a SNP in FOS transporter gene *glpT.* The second FOS-R isolate harbored *fosA3* (MIC ≥1024 µg/mL). The third FOS-R isolate (OXA-181+, CTXM-15+ Kp; MIC 128 µg/mL) had unclear mechanisms of R. SNPs were found within *glpT* (3 isolates, including 1 mentioned above), FOS transporter gene *uhpT* (3 isolates), and regulator genes *ptsI* (1 isolate, which also had *uhpT*) and *uhpA* (1 isolate). None of these isolates was FOS R. All isolates had wild-type genes encoding FOS target MurA and regulator CyrA. No isolates had *fosC* or *fomA*/*B* kinase genes.

Conclusions: FOS is currently in development for treatment of cUTI and IV breakpoints are not established. Utilizing existing oral breakpoints, FOS was highly active against CRE *in vitro*, including isolates that were C-A R, isolates with class B- and C-carbapenemases, and KPC-Kp with *ompK36* porin mutations. Therefore, FOS was active against CRE isolates for which there are currently no active β-lactams.