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P1625 Novel mechanisms of fosfomycin resistance in Escherichia coli

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Background: Fosfomycin resistance results from chromosomal mutations (in *murA*, *glpT*, *uhpT*, *uhpA*, *cyaA*, *ptsI*) or acquisition of plasmid-mediated genes (*fosA*, *fosB*, *fosC*, *fosX*). Because these known mechanisms may not be found in some resistant strains, the aim of this study was to decipher the genetic basis of fosfomycin resistance in a panel of *E. coli* isolates.

Materials/methods: Mechanisms of fosfomycin resistance were studied using 4 mutants obtained in vitro from the reference strain CFT073 (MIC, 1 mg/L), and using a set of 20 clinical isolates (11 susceptible, 9 resistant). MICs of fosfomycin were determined by the agar dilution method as recommended by the CLSI. All genomes were sequenced by Nextseq (Illumina) and bioinformatic analysis was done using the CLC Genomics Workbench software (Qiagen). Single-nucleotide allelic replacement was carried out using the suicide vector pDS132 in order to confirm the role of novel mutations.

Results: Two first-step isogenic mutants were obtained (MICs, 128 mg/L), which exhibited unique mutations in two novel genes: G469R in *uhpB* (M3); F384L in *uhpC* (M4). Corresponding second-step mutants (MICs, 256 mg/L) presented additional mutations: R282V in *galU* (M7 from M3); Q558X in *lon* (M8 from M4). The introduction of *uhpB* or *uhpC* mutations by site-directed mutagenesis conferred a 64-fold increase in MICs of fosfomycin whereas single mutations in *galU* or *lon* were only responsible for a 2-fold increase. Whereas all 11 fosfomycin-susceptible clinical isolates (MICs, 0.5-8 mg/L) were devoid of mutations, several were detected in all fosfomycin-resistant clinical isolates (MICs, 64-256 mg/L): 1 in *uhpA*, 3 in *uhpB*, 5 in *uhpC*, 1 in *uhpT* and 2 in *glpT*. Note that none strain harbored a plasmid-mediated gene. The role of 3 other *uhpC* mutations (T72I, Q210X, del_459-532) was also confirmed by site-directed mutagenesis in CFT073, each leading to a 128-fold increase in fosfomycin MICs.

Conclusions: In this study, we have identified novel chromosomal mutations both selected in vitro and in vivo and experimentally demonstrated their role in fosfomycin resistance. Mutations in *uhpB* and *uhpC* appear to be more frequent than those in already known genes.