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A novel beta-lactam enhancer Gram-negative antibiotic, zidebactam (ZID) - phenotypic and genotypic characteristics of ZID-selected mutants of Enterobacteriaceae - expressing Class C, KPC and NDM

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Background: ZID is a novel pan-Gram-negative PBP2 binding β-lactam enhancer antibiotic, which in combination with FEP triggers rapid cidality against diverse MDR pathogens. FEP-ZID provides therapeutically relevant activity against MBL-expressing Enterobactericeae, *P. aeruginosa* and OXA-carbapenemase-expressing *A. baumannii*. ZID being an antibacterially active partner it is important to characterize the phenotypic and genotypic profile of ZID-selected mutants.

Material/methods: Single step mutants of *E. coli* (EC) -M50 (CMY2, DHA) and M44 (NDM-1, TEM) and *K. pneumoniae* H521 (KPC2, SHV, TEM) were selected by inoculating 100 µL from 10^8 log CFU/mL suspension. Selection was undertaken at 2x and 8x/16x parent ZID MIC. After 48h of incubation, 3 mutants/strain/selecting concentration were selected and enrinched on selecting concentrations of ZID. FEP-ZID MIC determination (1:1) was undertaken for mutants as per CLSI. Time-kill studies were performed (starting inoculum : 6.54-6.9 $\log_{10}$ CFU/mL) with mutants selected under higher drug pressure (8x/16x ZID MIC). During the time-kill study, FEP and ZID-triggered-specific morphological changes were observed microscopically. Whole-genome-sequencing (Illumina® platform) of one mutant of each strain was undertaken.

Results: Standalone ZID MICs increased for all the ZID selected mutants. However, FEP-ZID MICs and cidal concentrations remained unchanged for H 521 mutants whereas a smaller 2-4fold increase in the FEP-ZID MICs was noted for EC M50 and EC M44 mutants. Microscopic observations showed that ZID minimal spheroplasting concentrations (MSC) for mutants were similar to parent (Table). However, standalone FEP failed to cause elongation even at 32 µg/mL (16x parent elongation concentration). Despite this, for mutants, FEP and ZID when combined at MIC concentration, showed elongated cells with pronounced blebs (2h post-incubation) indicating the evidence of concurrent PBP2 and PBP3 binding suggesting a drop in the FEP PBP3-binding threshold in the presence of ZID PBP2 binding. Time-kill studies showed that for ZID mutants with elevated FEP-ZID MICs, FEP-ZID

brought about 2.1-2.5 log_{10} kill corroborating well with microscopic observations. MICs of IPM increased for EC M50, but remain unchanged for KP H521. Surprisingly, for NDM EC M44, a significant drop in IPM MICs was noted. PCR analysis revealed an increase in the NDM amplicon size by 200 base pairs. PFGE analysis showed clonal relatedness between parents and mutants.

Conclusions: ZID-resistant mutants remain susceptible to FEP-ZID as concurrent PBP2-PBP3 binding leads to rapid cidality which was supported by the observation that FEP-ZID efficiently induced cell elongation with bleb formation just at mutant MIC concentrations.

Activity of FEP-ZID against ZID-selected mutants

Parent/mutant ZID selecting concentrations (x MIC)		MIC (µg/mL)			
		ZID		FEP-ZID (1:1)	IPM
		MIC	MSC		
EC M44	Parent	0.12	0.12	0.12	16
	ZID 2x/8x	>256	0.12	1/0.5	1
KP H521	Parent	0.5	0.25	0.5	8
	ZID 2x/16x	>256	0.25	0.5	8
EC M50	Parent	0.25	0.12	0.25	0.25
	ZID 2x/16x	>256	0.12	0.5	2